

ISSFAL 2010



9th Congress of the International Society for the Study of Fatty Acids & Lipids *incorporating the 8th International Congress on Essential Fatty Acids and Eicosanoids and the 5th PUFA in Maternal and Infant Health meeting*



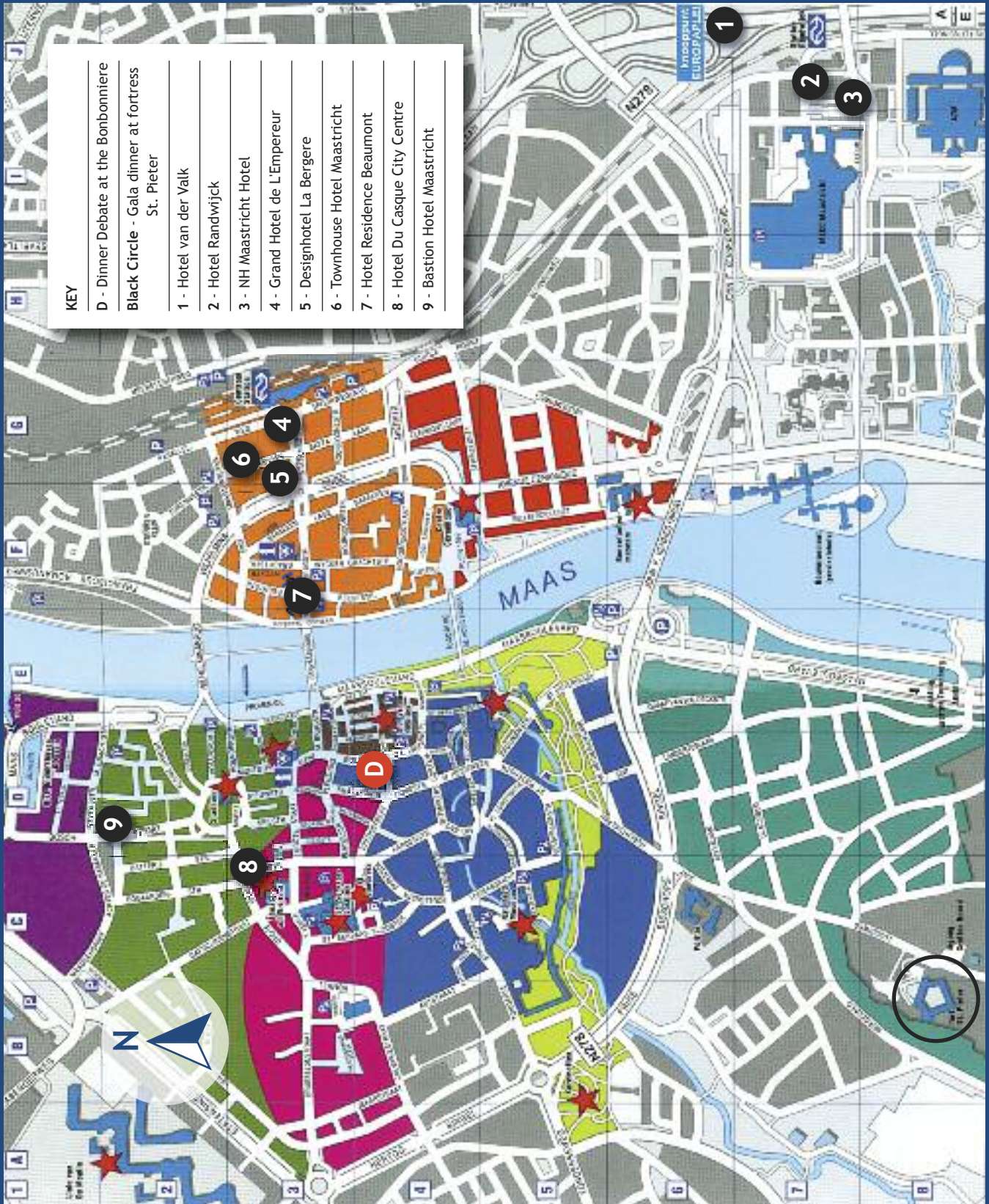
MAY 29 - JUNE 2, 2010

MECC CONGRESS CENTRE
Maastricht, The Netherlands



PROGRAM & ABSTRACTS

Maastricht Map



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ISSFAL 2010

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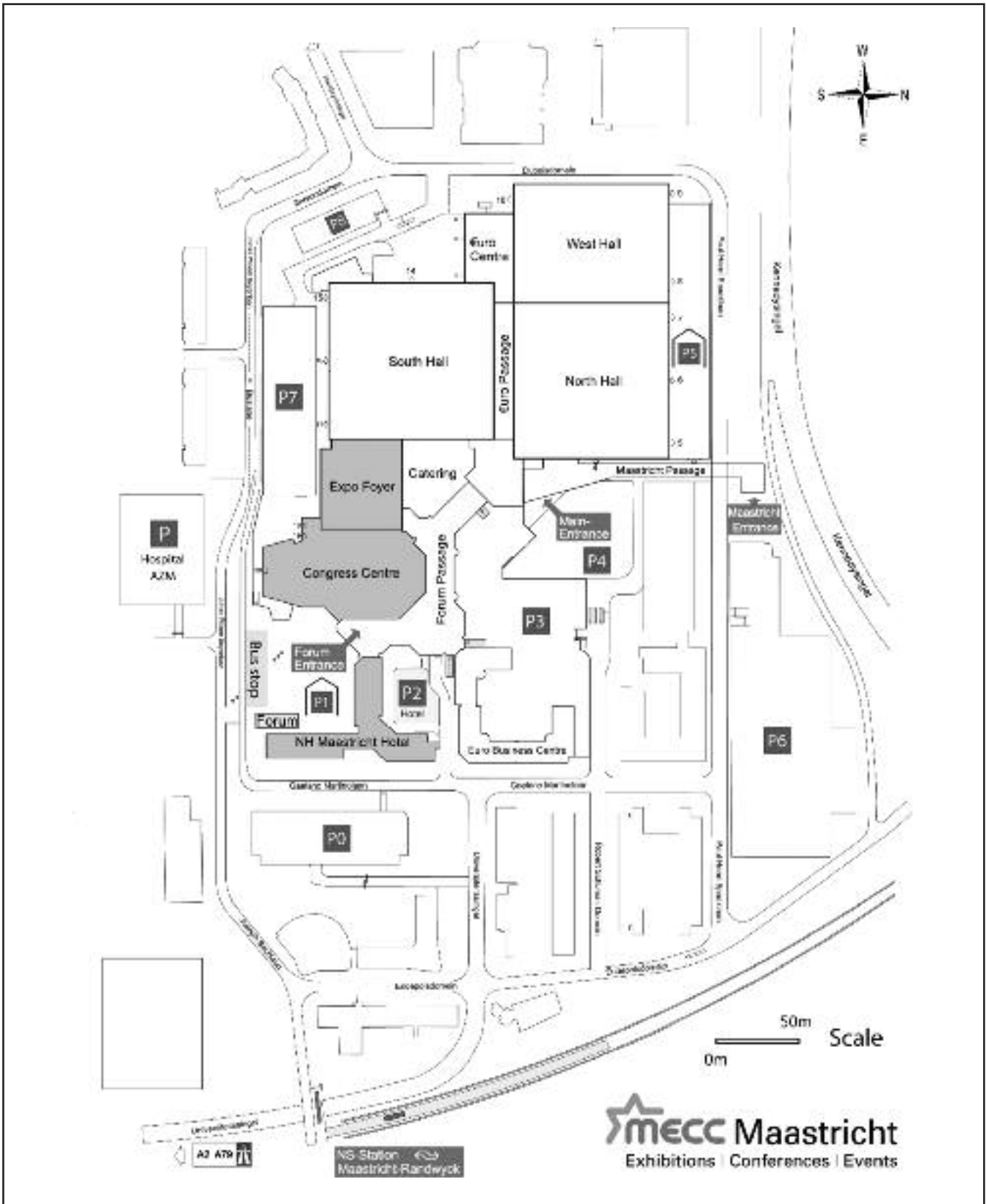
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Welcome!

Dear Participant,

We welcome you to Maastricht and the 9th biennial ISSFAL Conference: 'Lipids in Metabolic Health and Disease'.

ISSFAL 2010 will provide the most recent advances in the study of fatty acids and lipids in health and disease. The scientific program is intense and includes lectures of the winners of the Alexander Leaf Distinguished Scientist Award and of the Early Career Award, 14 plenary lectures by invited scientists, as well as 21 concurrent sessions on selected topics that are grouped in three tracks: Lipids & Health, Lipids & Nutrition, and Biochemistry of Lipids. Most concurrent sessions are introduced by an invited speaker, followed by short oral communications. In addition, we have programmed over 275 scientific abstracts into three days of poster presentations (Sunday through Tuesday).

Continuing the tradition of previous ISSFAL meetings we have encouraged the participation of students and new investigators. Thanks to the generosity of the ISSFAL Board and of our sponsors, we have been able to provide 40 free registrations to these new colleagues, as well as some significant travel support. Nine of these new investigators will be awarded a laptop computer in recognition for their outstanding contributions. Finally, on Wednesday an Award Session is scheduled for the Top New Investigator Award Winners, one each for the three tracks. To select these winners we ask each participant to cast one's ballot before Tuesday evening (forms in your conference bag).

We have been careful to set aside time for formal and informal scientific discussions and for socializing. Thus, the program includes on Saturday evening a Welcome Reception, on Monday evening a Dinner Debate (separate tickets required), and on Tuesday evening a Gala Dinner in a unique environment.

Maastricht is a city to explore. Your delegate badge serves as a free bus pass on the city bus network, so please allow yourself the opportunity to discover why Maastricht has become a beloved city for many.

The organization of ISSFAL 2010 Maastricht would not have been possible without the generosity of our sponsors. Please take every opportunity to let the representatives of our sponsors know they are appreciated.

We hope you have a stimulating and fruitful conference!

Yours sincerely,

The ISSFAL 2010 Maastricht Organizers



Jan Glatz



Renate de Groot



Patrick Schrauwen



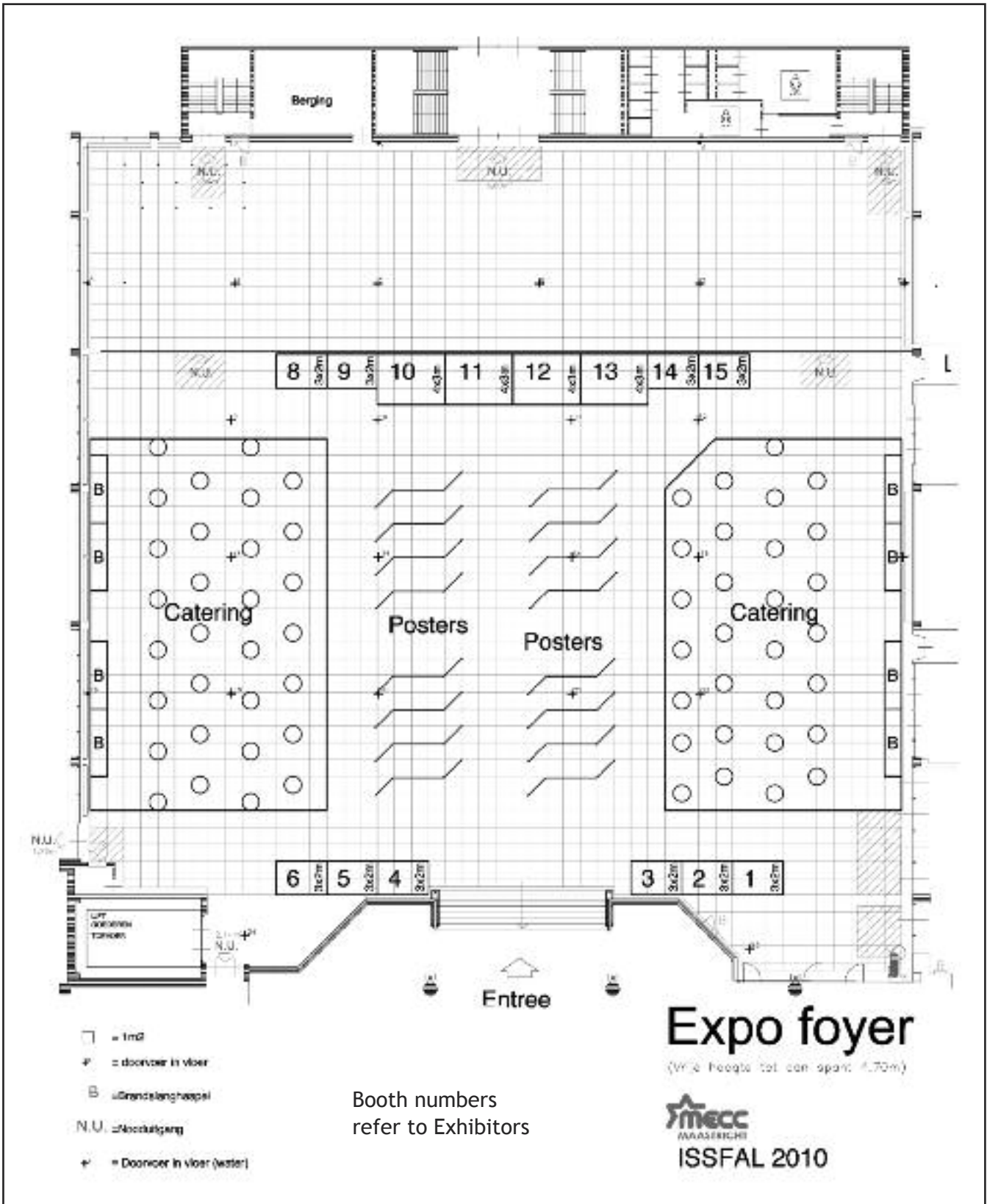
Matthijs Hesselink



Kim Willems



Ray Rice



Social Program

Venue Information

Registration Desk

The registration Desk is located on level 1 at the entrance of the MECC. Once you have registered you will be provided with a name tag and delegate bag.

Opening times:

Saturday May 29	16.00 - 20.30
Sunday May 30	08.00 - 16.00
Monday May 31	08.00 - 16.00
Tuesday June 1	08.00 - 16.00
Wednesday June 2	08.00 - 14.00

MECC Maastricht, Forum 100, 6229 GV Maastricht
043 - 3838383



Catering

We will provide you with coffee, tea and lunch at the venue (*included in the registration fee*). This service will be available to you in the poster and exhibition area adjacent to the main lecture hall.

Local Public Transportation

Your conference name tag will also serve as a bus pass, which will take you anywhere in Maastricht free of charge. Please show your name tag to the driver upon boarding. Using public transportation is a very common way of traveling in The Netherlands and is considered safe and reliable. The city buses generally run every 10 minutes.

No Smoking Policy

Smoking is prohibited in all buildings, restaurants and cafés throughout The Netherlands.

Internet

An Internet Café will be set up in the MECC where you are welcome to check your e-mail throughout the duration of the conference. The Café will be open during conference hours only i.e. from Sunday morning till Wednesday afternoon. For everyone who brings their own laptop, free WIFI is available throughout the building.

Welcome Reception - Saturday May 29

The Welcome Reception will be held at the conference venue MECC from 19.30 - 21.00 h. We will provide you with food and drinks (*included in the registration fee*).

Dinner Debate - Monday May 31

A dinner debate will be organized from 19.00 - 23.00 h in La Bonbonnière. The dinner debate is only accessible to participants who have registered for this event (*not included in the registration fee, don't forget to bring your voucher*). The former municipal theater La Bonbonnière is a distinguished multifunctional party and conference venue in the heart of Maastricht. It is one of the most striking buildings, close to the 'Onze Lieve Vrouweplein' (Our Lady's Square). For more than 200 years this exceptional theatre building has been the cultural meeting place for Maastricht's citizens. There is a possibility to choose from two different menus, on a first come first serve basis. Drinks will be included.

La Bonbonnière, Achter de Comedie 1, 6211 GZ Maastricht
043 - 3500935

Gala Dinner - Tuesday June 1

The Gala Dinner will be held at Fort Sint Pieter from 19.00 - 23.00 h (*included in the registration fee, don't forget to bring your voucher*). The Fortress Sint Pieter, built in 1701-1702, is located on the 'Sint Pietersberg' (Saint Pietershill), the highest point of Maastricht. Nowadays the fortress has the unique combination of history and tremendous views over the city and its surroundings. It is the perfect location to meet with other participants and to enjoy our 'landenbuffet' (typical food from different countries). Drinks will be included as well as transportation to and from the venue. A bus will pick you up at 18.45 h at one of the following locations:

- MECC
- Central Railway Station
- Market Square
- Apple Park



Departure from Fort Sint Pieter will be at 23.00 h and you will be dropped off at any of the above locations. If you wish to leave the dinner earlier, we advise you to take a taxi or city bus.

Fort Sint Pieter, Luikerweg 80, 6212 NH Maastricht
043 - 3217133

Sponsors

ISSFAL would like to thank the following sponsors:

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Welcome Reception and Gala Dinner



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The support that the ISSFAL 2010 has received from sponsors, exhibitors and other supporters is critically important in keeping the cost of registration at a reasonable level, and also to enable the award of 40 free registrations (worth over 10,000 euros) to New Investigator Award winners, thus encouraging good investigators into, and to remain in, the field of fatty acid research.

The meeting organisers and the Society generally appreciate this support, and urges delegates to take every opportunity to express this appreciation to the representatives of sponsors, exhibitors and other supporters that they come into contact with during the meeting and afterwards.

Sponsors and Exhibitors

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Amarin is committed to improving the lives of patients suffering from central nervous system (CNS) and cardiovascular diseases. Our goal is to be a leader in the research, development and commercialization of novel drugs that address unmet patient needs.

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Lecture Sponsor

CARIM, in close collaboration with the Cardiovascular Center of the University Hospital Maastricht, aims to develop an internationally recognized center of excellence in cardiovascular medicine. Since the '80's we hold a strong position in the international cardiovascular research arena, especially in the field of translational molecular medicine. We study basic mechanisms as well as early diagnosis and individual risk stratification of cardiovascular diseases, allowing faster translation of new research concepts to clinical

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Lecture Sponsor

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Contributor

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GROW*Lecture Sponsor*

The School for Oncology and Developmental Biology (GROW) harbours research and education on (epi)genetic, cellular and environmental factors underlying normal embryonic and perinatal development, as well as carcinogenesis. Within GROW basic scientists and clinicians closely collaborate in translational research. More information about the School can be obtained at our website: <http://www.grow-um.nl>

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Mead Johnson Nutritionals*Platinum Sponsor - Stand 11*

Mead Johnson Nutritionals is a world leader in nutrition, dedicated to helping provide infants and children around the world with the best start in life. Consistent with our support of breastfeeding as the gold standard in infant nutrition, we pattern our infant formulas after human milk. Our science-based products also include nutritional drinks and vitamins. For more information go to www.meadjohnson.com or contact MJMedicalAffairs@bms.com.

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Nederlandse Zuivel Organisatie

Dinner Debate Contributor

The Dutch Dairy Association is the sector organisation of the Dutch dairy processing industry. The objective of the NZO is to strengthen the economic and social position of the Dutch dairy companies. The NZO conducts a nutrition and health program (including scientific research) to ensure a high quality inflow of information on dairy products and its translation and communication to relevant target groups.

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Nestlé

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Exhibitor - Stand 6

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Seahorse Bioscience Europe

Exhibitor - Stand 5

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what is now a consolidated presence, such as the cardiovascular, metabolic and central nervous system areas.

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Contributor

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TNO

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It is the Unilever mission to add Vitality to life. We meet everyday needs for nutrition, hygiene and personal care with brands that help people feel good, look good and get more out of life. The Unilever Food and Health Research Institute brings this mission to life with science and technology innovations in foods. One of our science-based food brands is Becel/Flora, with a history of more than 45 years in the dietary management of risk factors of cardiovascular disease.

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Equazen offers a range of nutritional supplements with specific combination of EPA, DHA, and GLA. Equazen products have been tested in clinical trials and showed efficacy in supporting cognitive function of children and adolescents. Vifor Pharma is dedicated to furthering the scientific understanding of PUFAs and their health benefits: www.equazen.com

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New Investigators

We are pleased to announce the 40 New Investigator Award winners, which originate from 14 different countries. NIA winners are awarded a free registration for this meeting, as well as the opportunity to apply for an ISSFAL travel award.

Ten such travel awards were made for this Congress, as a result of the funding of \$10,000 provided by the ISSFAL Board of Directors.

In addition to this, nine New Investigator Award winners will be awarded a laptop computer in recognition for their outstanding contribution and their posters will be on display from Sunday till Tuesday.

Three New Investigator Award winners (one per track) will be invited to present their work orally in the Awards Session, at the Closing Ceremony. We ask for your help in deciding the three winners, by voting on the ballot form, which you will find in your delegate bag.

The awards session will be held on Wednesday June 2nd from 13.30-14.30 h, just before the Closing Ceremony. The investigators (or a surrogate) must be present to receive the computer. New Investigators planning to leave before the close of the conference should designate someone who can pick up their prize for them and share the name of that individual with Chairman of the Award Committee Matthijs Hesselink.

Nominees for Top NIA

Giuseppe Astarita, (Daniele Piomelli), University of California, USA
Mariela Bernabe, (Mardia Lopez-Alarcon), University of Mexico, Mexico
Caroline Childs, (Parveen Yaqoob), University of Southampton, United Kingdom
Claudia Coomans, (Hans A. Romijn), University of Leiden, The Netherlands
Melissa Gregory, (Michael J. James), Flinders University, Australia
Philippa Jackson, (David Kennedy), Northumbria University, United Kingdom
Remko Kuipers, (Frits A.J. Muskiet), University of Groningen, The Netherlands
Jennifer Lambert, (M. Tom Clandinin), University of Alberta, Canada
Kuan-Pin Su, (Carmine Pariante), China Medical University & Hospital, Taiwan

NIA winners

Anita Røyneberg Alvheim, (Livar Frøyland), University of Bergen, Norway
Hildur Hronn Arnardottir, (Jon J. Jonsson), University of Iceland, Iceland
Alexander Bartelt, (Jörg Heeren), University of Hamburg, Germany
Vincent de Boer, (Ronald J.A. Wanders), University of Amsterdam, The Netherlands
Chuck Tzu Chen, (Richard P. Bazinet), University of Toronto, Canada
Camilla Damsgaard, (Lotte Lauritzen), University of Copenhagen, Denmark

Shalinee Dhayal, (Noel Morgan), Universities of Exeter and Plymouth, United Kingdom
Lieke van den Elsen, (Philip Calder), University of Utrecht, The Netherlands
Alex Kitson, (Ken Stark), University of Waterloo, Canada
Michel Lucas, (Alberto Ascherio), Harvard School of Public Health, USA
Waleed Marei, (Ali A. Fouladi-Nashta), Royal Veterinary College, United Kingdom
Liandr  van der Merwe, (Andrew M. Prentice), London School of Hygiene & Tropical Medicine, United Kingdom
Catherine Milte, (Natalie Sinn), University of South Australia, Australia
Daniella Mizurini, (Maria das Graças), Federal University of Rio de Janeiro, Brasil
Beverly Muhlhausler, (Maria Makrides), University of Adelaide, Australia
Z Nikolakopoulou, (E. Kenneth Parkinson), University of London, United Kingdom
Paul Noakes, (Philip Calder), University of Southampton, United Kingdom
Patricia Nunes, (Arend Heerschap), University of Nijmegen, The Netherlands
Sungwhan Oh, (Charles N. Serhan), Harvard Medical School, USA
Sarah Orr, (Richard P. Bazinet), University of Toronto, Canada
Ashley Patterson, (Ken Stark), University of Waterloo, Canada
Carolina Molt -Puigmart , (Carmen Lopez Sabater), University of Barcelona, Spain
Julia Schumann, (H. Fuhrmann), University of Leipzig, Germany
Clare Smith, (Tony Postle) University of Southampton, United Kingdom
Ana Francisca Soares, (John Griffith Jones), University of Coimbra, Portugal
Laura Steinbusch, (Jan F.C. Glatz), Maastricht University, The Netherlands
Virginia Stone, (Noel Morgan), Universities of Exeter and Plymouth, United Kingdom
Wei-Chun Tu, (Robert A. Gibson), University of Adelaide, Australia
Heidi Urwin, (Parveen Yaqoob), University of Reading, United Kingdom
JC Woodall, (Tony Postle) University of Southampton, United Kingdom
Jinping Zhao, (Hope Weiler), McGill University, Canada

We offer congratulations to all NIA winners and look forward to their active participation in future ISSFAL meetings.

Achievement Awards



ALEXANDER LEAF DISTINGUISHED SCIENTIST AWARD FOR LIFETIME ACHIEVEMENT

Presenting: Saturday, May 29th - 18.30-19.30

Lecture Title: My life with DHA.

Norman Salem, Jr., PhD

University of Rochester School of Medicine, Rochester, NY (USA)

Dr. Salem is currently the Chief Scientific Officer and Vice President of Research for Martek Biosciences Corporation. He manages the Clinical, Discovery, Analytical, Microbial Biotechnology and Fermentation research groups at Martek. Martek research activities focus on DHA, ARA and other essential fatty acids as well as on other bioactive compounds and processes. Areas of active research interest include infant and child development, aging and cognition, inflammatory processes and diseases, maternal health and nutrition and sports nutrition.

He previously served as the Chief of the Laboratory of Membrane Biochemistry & Biophysics within the Intramural Research program of the National Institutes on Alcohol Abuse and Alcoholism at the National Institutes of Health where he worked for 30 years. While at the NIH, he worked on studies of DHA (docosahexaenoic acid) composition, metabolism and biological function with an emphasis on the nervous system. His laboratory was an internationally recognized center for studies of the mechanisms underlying the critical nature of DHA in the nervous system and for the scope of work involving clinical and behavioral studies ranging to organ, cellular, biomembrane and molecular biological studies.

His professional affiliations include the Society for Neuroscience, the American Society for Biochemistry & Molecular Biology, the American Society for Neurochemistry, the International Society for the Study of Fatty Acids and Lipids, and the American Oil Chemists Society. He is an author of about 240 publications. Dr. Salem serves on the editorial board of *Lipids* and *Nutritional Neuroscience*. He is the Past President of the ISSFAL Society and recipient of the Supelco/Nicholas Pelick Award from the American Oil Chemists Society. He was named recently as the recipient of the 2010 ISSFAL Lifetime Achievement Award. He received his B.S. degree in physics from Miami University and his Ph.D. in Neurobiology from the University of Rochester School of Medicine.



EARLY CAREER AWARD

Presenting: Wednesday, June 2nd - 13.00-13.30

Lecture Title: Emerging Opportunities for an Integrated Systems Approach to the Study of Nutrient-Disease Relationships: Technical Application and Findings from Investigations on the Impact of Highly Unsaturated Fatty Acids in Health and Disease of the Retina.

John Paul SanGiovanni

John Paul SanGiovanni recently received the Director's Award at the U.S. National Institutes of Health (NIH). He serves in the Clinical Trials Branch of the U.S. National Eye Institute. Dr. SanGiovanni's primary scholarship interest is in understanding the role of dietary factors in development and prevention of visual system disorders. His primary research interest is in examining the relationship of long-chain polyunsaturated fatty acids (LCPUFAs) and associated bioactive molecules with pathogenic processes implicated in retinal vascular diseases. He leads analysis teams for the Age-Related Eye Disease Study (AREDS) in projects examining the relationship of dietary factors with age-related macular degeneration (AMD) and cataract. In addition to this work, Dr. SanGiovanni is conducting large-scale genomic studies on the role of lipid-associated genes and mitochondria in pathogenesis of AMD. He served as Project Officer of AREDS and now co-directs AREDS2, a 4000-person, 5-year phase III randomized clinical trial on the safety and efficacy LCPUFAs in the prevention of sight-threatening AMD.

He is conducting mechanistic studies on diet and retinal angiogenesis in a number of cellular and animal models. Dr. SanGiovanni has participated as a technical expert for a number of government-directed projects, including an evidence report on omega-3 fatty acid intake and eye health from the Agency for Healthcare Research and Quality and a research symposium on the role of omega-3 fatty acids in health and disease planned by the National Center for Complementary and Alternative Medicine. Prior to his appointment at NIH in 2000, Dr. SanGiovanni held positions at Harvard Medical School, the International Nutrition Foundation and The United Nations University

Dr. SanGiovanni earned his doctorate and masters degrees at the Harvard School of Public Health. His dissertation research was on the relationship of diet with visual resolution acuity in infancy. Prior to completing his degrees he conducted psychophysical research at The Children's Hospital (Boston) and Harvard Medical School Department of Ophthalmology.

Plenary Speakers



Philippe Bougnoux, MD is a medical oncologist, specialized in breast and gynaecologic cancers. He performed his trainings in medicine in Tours and in immunology at the Pasteur Institute in Paris. After a 3 years post-doctoral staying as a Fogarty fellow at the National Cancer Institute in Bethesda, MD, he became professor of cancer biology at the university of Tours, and chief of the cancer outpatient unit at the university cancer centre Henry S. Kaplan. He is also heading the Inserm research Unit 921 « Nutrition, growth and Cancer » and coordinates a consortium of research units in chemistry and biology on marine-derived anticancer agents within the canceropôle of the western part of France.

His research interests are to understand how diet and lipid nutrients influence the molecular alterations which result in malignant tumors and how they integrate to delay breast cancer occurrence or individual response to anticancer agents. He does translational research in the field of dietary lipids in relation to breast cancer prevention and treatment. He is currently carrying out clinical trials of dietary intervention with omega-3 polyunsaturated fatty acids to enhance the sensitivity of tumors to radiation or chemotherapy.



Patricia Bozza, MD is a Senior Scientist at the Instituto Oswaldo Cruz, Brazil. Research in Dr. Bozza's laboratory focuses on molecular and cellular mechanisms of leukocyte activation and inflammatory mediator generation in host defense and other forms of inflammation. The principal areas of investigation are the biogenesis and functions of lipid droplets in inflammation and host pathogen-interactions; mechanisms of formation and function of lipid mediators in inflammatory and infectious process; and sepsis pathogenesis and biomarkers. Patricia Bozza received an M.D. in 1990 from the School of Medical Sciences, State University of Rio de Janeiro, and a Ph.D. in Pharmacology in 1993 from the Instituto Oswaldo Cruz in Rio de Janeiro, Brazil. She was named a Pew Latin American Fellow in 1994 and she went on to do postdoctoral work at the Beth Israel Hospital, Harvard Medical School (1994-1997).

From her studies she received the Young Researcher Travel Award, from the International Union of Pharmacology (IUPHAR, 1994) and Young Researcher Aspirin Award (Bayer) in 1995. Patricia returned to Brazil to join the Instituto Oswaldo Cruz as an Associated Scientist in 1997. Patricia was named International Scholar from the Howard Hughes Medical Institute (2002-2006) and Chair of the Pew Program in Biomedical Sciences Regional (Brazil) Committee in 2004 (2004-2008). She was elected councilor of the Brazilian Society in Immunology (2009-2011). Her Laboratory is part of the Brazilian network of Laboratories of Excellence since 2003. In 2002 she was appointed as Scholar and later as Senior Scholar of the Brazilian National Research Council and was honored as a Scientist of the State of Rio de Janeiro (FAPERJ), and received the Scopus Award Brazil in 2008 (Elsevier). Patricia is Academic Editor of PlosOne. Patricia has published over 120 papers in peer-reviewed international journals.



Stephen Cunnane, PhD obtained a PhD in Physiology at McGill University in Montreal in 1980. He then held a post-doctoral fellowships in Aberdeen and London and joined the Department of Nutritional Sciences at the University of Toronto in 1986. While in Toronto his research focused on developing a better understanding of the metabolism of polyunsaturated fatty acids, especially during early development. In 2003, Dr. Cunnane was awarded a Tier 1 Canada Research Chair at the Research Center on Aging, and holds a full professor in the departments of Medicine and Physiology and Biophysics at the Université de Sherbrooke.

The theme of his current research uses PET imaging and other isotope methods aims to learn more about energy metabolism in the aging brain, and whether polyunsaturates help maintain cognitive function during aging via effects on fuel availability to the brain. Dr. Cunnane has sat on grant selection committees of Canada's main science funding agencies and is on the editorial boards of three journals. He was the chief organizer of ISSFAL 2002 and was on the ISSFAL Executive Board from 2000-2008. He is the author *Survival of the Fattest: The Key to Human Brain Evolution* (2005), and co-editor of the recently published book - *Human Brain Evolution - Influence of Freshwater and Marine Food Resources* (2010). Both books describe the pivotal role of nutritional and metabolic changes in hominids as prerequisites for evolution of the cognitively advanced human brain. He has also written a book on zinc nutrition, co-edited two books on flaxseed in human nutrition, and published 250 peer-reviewed research papers.



Robert Gibson, PhD is a Senior NHMRC Research Fellow, a Fellow of the Nutrition Society and Professor; Food Science and Nutrition, School of Agriculture, Food and Wine, University of Adelaide. He has been actively involved in nutrition research in cells, animals and humans for over 25 years and has published over 200 papers in this area. He has designed and conducted many randomised controlled trials involving nutrition interventions in the perinatal period. The trials were designed to test the effects of interventions with iron, selenium, probiotics, nucleotides and long chain polyunsaturated fatty acids (PUFA) on infant biochemistry, growth, physiology and developmental outcome. One RCTs has recently been published in JAMA - DINO: a National DHA-dose response study on mental development in 650 preterm infants. DOMInO: a National DHA in pregnancy study on postnatal depression and child development in 2500 women/infants is complete and will be published in 2010. Prof Gibson lives close to the McLaren Vale wine district south of Adelaide that is famous for red wines.



Jan Glatz, PhD is Professor of Cardiac Metabolism at the Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, the Netherlands. He studied chemistry and biochemistry (Nijmegen and Utrecht) and received his PhD degree from Nijmegen University in 1983. Following a post-doc period in Human Nutrition at Wageningen University, he joined Maastricht University in 1986 to be trained as molecular physiologist and study cardiac lipid metabolism with special reference to the functioning and significance of fatty acid-binding proteins. His current interest is in membrane substrate transporters and their application for so-called metabolic modulation therapy. He is a member of the Editorial Board of several journals, including Obesity and PLEFA. In addition, he has organized several international conferences including the 5th conference of the Society for Heart and Vascular Metabolism (SHVM, 2007) and the 49th International Conference on the Bioscience of Lipids (ICBL, 2008).



Ingrid Helland, PhD got her medical Degree at the University of Bergen in 1986, and worked at the Forensic Institute, University of Oslo, from 1988 to 2000. She then spent one year at the Department of Neuropathology, Rikshospitalet, Oslo, before she started specializing in Paediatrics. She finished her PhD at the University in Oslo in 2002 with a work on “Fatty Acids, Mothers and Newborns”. From 2000 she has been working at the Department of Paediatrics, Oslo University Hospital, and from 2003 as a Paediatric Neurologist.



Gerard Hornstra, PhD studied Medicine at Erasmus University Rotterdam and Leiden University. He received his professional training in the field of Experimental Nutrition at the Unilever Research Laboratories in Vlaardingen. In 1981 he obtained a PhD degree in Medicine at Maastricht University on a thesis, entitled ‘Dietary fats and arterial thrombosis’. In 1980, Prof. Hornstra joined Maastricht University where he investigated health aspect of essential fatty acids, with special emphasis on cardiovascular disease, pregnancy and early human development. After his retirement, early 2003, Prof. Hornstra continued to supervise and advise PhD students. In addition, he works as a private research consultant in the area of dietary lipids in health and disease. His special scientific interest concerns the importance of essential and other fatty acids in cardiovascular disease, maternal nutrition, and infant development.



Johnathan Napier, PhD studied Agricultural Sciences at the University of Nottingham and carried out research on plant biochemistry for his PhD from King’s College, London. He worked at as post-doc in the Department of Plant Sciences, University of Cambridge (1989-1992), looking at the mechanism of chloroplast protein import and the assembly of the thylakoid ATP synthase complex. In 1992 he joined Long Ashton Research Station (University of Bristol) as a junior project leader and established a program looking at trafficking and deposition of seed storage reserves. This lead to his on-going interests in understanding and manipulating plant seed oil composition. In 2003 he relocated to Rothamsted Research, where he is currently a Principle Investigator and leader of the Plant Lipid Metabolism and Signalling group.

Dr. Olga Sayonova will present Dr. Napier’s paper.



Christopher B. Newgard, PhD is the Director of the Sarah W. Stedman Nutrition and Metabolism Center and the W. David and Sarah W. Stedman Distinguished Professor of Pharmacology and Cancer Biology at the Duke University Medical Center. Prior to coming to Duke in 2002, Dr. Newgard was the Gifford O. Touchstone Jr. and Randolph G. Touchstone Distinguished Professor, Department of Biochemistry and Co-Director of the Touchstone Center for Diabetes Research, University of Texas Southwestern Medical Center, Dallas.

Dr. Newgard's research focuses on application of an interdisciplinary approach for understanding of diabetes and obesity mechanisms involving gene discovery, metabolic engineering, and comprehensive tools of metabolic analysis ("metabolomics") such as mass spectrometry-based metabolic profiling and NMR-based metabolic flux analysis. Dr. Newgard has authored over 200 peer-reviewed and review articles, and has been the recipient of several awards, including the Kayla Grodsky Award for Outstanding Basic Science Research from the Juvenile Diabetes Foundation (1999), the Outstanding Scientific Achievement (Lilly) Award from the American Diabetes Association (2001), a Merit Award from the NIH (2001), the Solomon Berson Prize of the American Physiological Society (2003), and a Freedom to Discover Award in Metabolic Research from Bristol-Meyers Squibb (2006).



Stanley I. Rapoport, MD is Chief of the Brain Physiology Section at the National Institute on Aging (NIH) in Bethesda, Maryland (USA). He received his M.D. degree from Harvard Medical School, and did post-doctoral research at Uppsala University in Sweden. While at the NIH, Dr. Rapoport developed an osmotic method to reversibly open the blood-brain barrier to allow drugs into the brain to treat brain tumors, and directed a clinical program that used positron emission tomography (PET) to characterize brain metabolism in human aging and Alzheimer disease. In recent lipid studies, he developed methods and models to quantify the kinetics brain fatty acid metabolism in unanesthetized rodents and in humans, in relation to rates of liver PUFA synthesis and PUFA nutrition.

He demonstrated rapid energy-dependent recycling of arachidonic and docosahexaenoic acids in brain phospholipids, and is elucidating the roles of both PUFAs in neurotransmission. He reported arachidonic acid metabolism is upregulated in brain from Alzheimer disease and bipolar disorder patients, in relation to neuroinflammation and excitotoxicity, which may contribute to progression in both disorders. He is showing that the mood stabilizers approved for treating bipolar disorder commonly downregulate brain

arachidonic acid metabolism in awake rodents, which may account for their therapeutic efficacy. Dr. Rapoport's recent clinical studies have employed PET to image brain arachidonic acid and docosahexaenoic acid metabolism in human subjects, in relation to age, functional and pharmacological activation, and neuroinflammation.



Gerald Reaven, MD received his degree from the University of Chicago in 1953, and postgraduate training at the University of Chicago, University of Michigan, and Stanford. He joined the Stanford University School of Medicine faculty in 1961, where he remains as Professor of Medicine (Active Emeritus), Division of Cardiovascular Medicine. Dr. Reaven has served as Head of the Division of Endocrinology and Metabolic Diseases (1974-1977), the Division of Gerontology (1977-1990), and Division of Endocrinology, Gerontology, and Metabolism (1990-1995).

Dr. Reaven has published over 600 peer-reviewed research articles in scientific journals, as well as authoring or co-authoring numerous textbook chapters and other scholarly works. His research contributions have been widely recognized, and he has received the highest awards for research from the Department of Veteran Affairs (William S. Middleton Award for Outstanding Achievement in Medical Research, 1987), the American Diabetes Association (Banting Award for Distinguished Scientific Achievement, 1988), the British Diabetes Association (Banting Memorial Lecture, 1990), the European Association for the Study of Diabetes (Claude Bernard Lecture, 1994), and the Endocrine Society (Fred Conrad Koch Award, 2006). In addition, Dr. Reaven has received the Elliot Proctor Joslin Memorial Lecture (1990), the Nordisk-McGill Lecturer in Diabetes (1990), the Joseph Kirby Lilly, Distinguished Service Award (1995), Novartis Award for Longstanding Achievement in Diabetes (2000), the Renold Medal of the American Diabetes Association (2002), the Frontier in Science Award from the American Association of Clinical Endocrinologists (2003), the National Institutes of Health Directors Astute Clinician Lectureship (2004), Ellen Browning Scripps Medal (2004), The Dewitt Goodman Memorial Lecture, Columbia Univ School of Med (2004), the Priscilla White Lectureship on Metabolism, Joslin Diabetes Center (2005), the Presidential Lectureship at the Canadian Hypertension Society (2005), and the Fred Conrad Koch Award from the Endocrine Society for Research of Special Distinction in Endocrinology (2006).



Michael Roden, MD obtained his degree and certifications in Internal Medicine, Endocrinology and Clinical Pharmacology at the University of Vienna, Austria. He completed post-doc fellowships at the Pharmacological Institute, University of Vienna, and as a Max-Kade Fellow at Yale University, New Haven, CT. Then he held a tenure position as Associate Professor of the Division of Endocrinology and Metabolism, Department of Medicine 3 at the Medical University of Vienna/General Hospital of Vienna. Subsequently, he became Head of the 1. Medical Department, Coordinator of the University Teaching Hospital, Hanusch Hospital, and Director of the Karl-Landsteiner Institute of Endocrinology and Metabolism, Vienna. Since 2008, he is Full Professor of Medicine, Chair and Head of the Department of Metabolic Diseases at Heinrich-Heine University, and Director of the German Diabetes Center, Düsseldorf, Germany.

Roden combined multitracer techniques and multinuclear magnetic resonance spectroscopy to non-invasively assess metabolic pathways in humans. He contributed concepts to nutrient-induced insulin resistance, metabolic and mitochondrial function. He received many international awards including the International Novartis Award for Innovative Patient-Oriented Research (2004), the European Society for Clinical Investigation-Award (2006) and the Oskar-Minkowski Prize by the European Association for the Study of Diabetes (EASD, 2006). He has published more than 200 papers in peer-reviewed journals and his work is/was supported by EU, EFSD/EASD, JDRF, German and Austrian Science Foundations. He was President of the Austrian Diabetes Association and is President of the Central European Diabetes Association/FID. Currently he serves as on the editorial boards of Endocrinology, Am J Physiol and Eur J Clin Invest.



Walter Wahli, PhD studied biology at the University of Berne (PhD in 1977). He worked as a post-doc researcher at the *Department of Embryology, Carnegie Institution of Washington* in Baltimore, and was visiting associate at the *National Cancer Institute, NIH*, in Bethesda (1978-1980). He became Professor and Director of the *Institut de Biologie Animale* of the University of Lausanne in 1980 and was Research Vice-Rector from 1999 to 2003. He founded the *Center for Integrative Genomics*, which he directed from 2002 to 2005. Walter Wahli has been a member of the Swiss National Science Foundation's research council since 1987 and presided over the Biology and Medicine Division from 2004 to 2006. He is a member of the Swiss Science and Technology Council, which is the advisory body to the Swiss Federal Council in all matters relating to science policy.

His research focuses on the genetic control of energy metabolism, for which he received the Otto-Naegeli Prize (2002), the European Federation of Lipid Research Award (2002) and the Hartmann Müller Prize (2008). His main interest is in the functions of PPARs, which are nuclear receptors that act as lipid sensors to modulate gene expression. These receptors are implicated in metabolic and inflammatory regulations with a far-reaching medical impact. PPARs exhibit an isotype-specific tissue expression pattern, which can account for the variety of cellular functions they regulate. This diversity of functions is also reflected by the broad range of ligands that can be accommodated within their ligand binding pocket. These ligands are naturally occurring lipids, which include diverse fatty acids, leukotrienes and prostaglandins.



Rudi Zechner, PhD is a professor of biochemistry at the Institute of Molecular Biosciences, University of Graz, Austria. He received his Ph.D. in chemistry from the University of Graz, spent 3 years as a postdoctoral fellow in the Laboratory of Human Genetics and Metabolism (Prof. J. Breslow) at the Rockefeller University, New York, and started his own lab in Graz in 1989. His research focuses on enzymes involved in the synthesis and catabolism of lipids with special emphasis on metabolic lipases.

Conference Program

ISSFAL 2010 Maastricht

Lipids in Metabolic Health and Disease

Saturday May 29

16.00 - 18.00	Registration at MECC congress venue
18.00 - 18.30	Welcome address (Philip Calder, Jan Glatz)
18.30 - 19.30	Plenary: Alexander Leaf Distinguished Scientist Award for Lifetime Achievement Lecture <i>Chair: Philip Calder, Jan Glatz</i> Norman Salem (Bethesda USA) <i>My life with DHA</i>
19.30 - 21.00	Welcome reception (sponsored by Nu-Mega/GTC Nutrition)

Tracks:

Lipids and Health

Biochemistry of Lipids

Lipids and Nutrition

Sunday May 30

08.30 - 10.00	Plenary: Lipids and Health 1: Metabolic syndrome <i>Chairs: Patrick Schrauwen, Sander Kersten</i>
08.30 - 09.15	(PL1) <i>Netherlands Heart Foundation Lecture</i> Insulin resistance and the many faces of the metabolic syndrome: why a cluster is truly a cluster (Gerald Reaven, Stanford, USA)
09.15 - 10.00	(PL2) Ectopic fat, NASH and the metabolic syndrome (Michael Roden, Düsseldorf, Germany)
10.00 - 10.30	Break

Concurrent sessions 1 - 3			
10.30-12.00	Metabolic syndrome - molecular aspects <i>Chairs: Joris Hoeks, Bret Goodpaster</i>	Brain fatty acid uptake and metabolism <i>Chairs: Jan Glatz, Richard Bazinet</i>	Dietary lipids and lipoproteins <i>Chairs: Eric Murphy, Barbara Meyer</i>
10.30 - 11.00	Bret Goodpaster (USA) The role of mitochondria in lipid-induced insulin resistance within skeletal muscle	<i>CARIM Lecture</i> Jim Hamilton (USA) Fatty acid transport in membranes: new approaches give new insights	Barbara Meyer (Australia) Long Chain Omega-3 PUFA intakes and health
11.00 - 11.15	J. Hoeks (Netherlands) Prolonged fasting-induced insulin resistance, lipid accumulation and mitochondrial (dys)function in human skeletal muscle	Grant Hatch (Canada) Characterization of fatty acid transport across human blood brain barrier microvessel endothelial cells	C. Calzada (France) Oxidatively modified high density lipoproteins inhibit human platelet aggregation
11.15 - 11.30	P. Xia (Australia) Role of cIAP1 in ER stress - mediated lipotoxicity: a determinative factor		A. Bartelt (Germany) Brown adipose tissue is a major determinant of plasma clearance and organ uptake of triglyceride rich lipoproteins
11.30 - 11.45	J. Fiamoncini (Brazil) Increased peroxisomal fatty acid oxidation in liver contributes to the protection against obesity and glucose intolerance by fish oil diets	C.T. Chen (Canada) Rapid disappearance of eicosapentaenoic acid from rat brain phospholipids: an <i>in vivo</i> intracerebroventricular study	A. Funke (Netherlands) LDLR knock-out mice fed a cholesterol diet show increased susceptibility to the development of insulin resistance
11.45 - 12.00	R. Colas (France) Low-density lipoproteins from patients with a metabolic syndrome or type-2 diabetes activate platelets	H.Y. Kim (USA) Metabolism of docosahexaenoic acid in hippocampal development	F. Borthwick (Canada) Dietary n-3 PUFA improves post prandial metabolism in the insulin resistant Jcr:la-cp rat by lowering enterocytic apob48 production and lymphatic cholesterol

12.00 - 12.15		M.P. Agbaga (USA) ELOVL4 protein elongates n3 and n6 long chain fatty acids to very long chain polyunsaturated fatty acids	
12.00 - 14.00	Lunch with Poster Session 1		
Concurrent sessions 4 - 6			
14.00 - 15.30	Brain function I- Depression <i>Chairs: Tom Brenna, Joseph Hibbeln</i>	Lipid, genes and (epi)genetics <i>Chairs: Willem Voncken, Arild Rustan</i>	Maternal and Infant nutrition I - Body composition <i>Chairs: Maria Makrides, Marius Smuts</i>
14.00 - 14.15	<i>EURON Lecture</i> Joseph Hibbeln (USA) Suicide death among us military: a case control study and reduction of relapse among chronic alcoholics: a randomized placebo controlled trial	<i>GROW Lecture</i> Andre van Assche (Belgium) Developmental programming in maternal diabetes and in experimental maternal overfeeding and underfeeding	C. Smith (UK) Effects of exposure to high fat in utero on phospholipid profiles in a mouse model of the metabolic syndrome
14.15 - 14.30			L. Hellgren (Denmark) Late gestation undernutrition and post-natal diet, programmes hepatic lipid composition and fatty acid profiles in adult sheep
14.30 - 14.45	O. Schiepers (Netherlands) Fish consumption, not fatty acid status, is related to quality of life in a healthy population	Arild Rustan (Norway) The impact of eicosapentaenoic acid on regulation of fatty acid metabolism, metabolic flexibility	B. Muhlhausler (Australia) Maternal omega-3 supplementation alters fat distribution in the offspring

14.45 - 15.00	J. Assies (Netherlands) Plasma and erythrocyte fatty acid patterns in patients with recurrent depression: a matched case control study	and gene expression in human skeletal muscle cells	M. van Eijsden (Netherlands) Maternal n-6 and n-3 lcpufa status is related to body composition and glycaemic control in the offspring: the abcd cohort
15.00 - 15.15	W. Oddy (Australia) Fatty acid intake and depression in adolescents	A. Jans (Netherlands) Impact of different dietary fat quantity and quality on skeletal muscle fatty acid handling in subjects with the metabolic syndrome	L. Schram (Denmark) Determinants of infant n-3 lcpufa status and its associations with blood pressure and lipid profile
15.15 - 15.30	M. Lucas (USA) Dietary omega-3 and omega-6 intake and the risk of clinical depression: results from the nurses' health study	W.C. Tu (Australia) Omega-3 long chain fatty acid synthesis in rats is regulated more by substrate levels than gene expression	L. Lauritzen (Denmark) Fish oil-supplementation has little effect on growth and body composition in late infancy
15.30 - 16.00	Break		
16.00 - 17.30	Plenary: Biochemistry of Lipids 1: Lipids, genes, and metabolomics <i>Chairs: Michel Lagarde, Annemie Schols</i>		
16.00 - 16.45	(PL 3) New insights in the role of PPARs (Walter Wahli, Lausanne, Switzerland)		
16.45 - 17.30	(PL 4) <i>NUTRIM Lecture</i> Metabolomics (Chris Newgard, Durham, USA)		

Monday May 31

08.30 - 10.00	Plenary: Diet and Nutrition: Maternal and infant nutrition <i>Chairs: Renate de Groot, Susan Carlson</i>		
08.30 - 09.15	(PL 5) FA status and supplementation (Gerard Hornstra, Maastricht, The Netherlands)		
09.15 - 10.00	(PL6) Consequences of FA supplementation (Ingrid Helland, Oslo, Norway)		
10.00 - 10.30	Break		
Concurrent sessions 7 - 9			
10.30 - 12.00	Metabolic syndrome - obesity/diabetes <i>Chairs: Ellen Blaak, Sebastiano Banni</i>	Lipid signaling/Lipid mediators <i>Chair: Jan Kopecky</i>	Maternal and Infant nutrition II - Mental development <i>Chairs: Peter Howe, Renate de Groot</i>
10.30 - 11.00	Matthijs Hesselink (Netherlands) Ectopic fat storage and insulin sensitivity	Jan Kopecky (Czech Republic) Multiple mechanisms of action of n-3 fatty acids on metabolism - possible impact for the treatment of metabolic syndrome	Karen Simmer (Australia) LCPUFA supplementation and infant development
11.00 - 11.15	A. Alvheim (Norway) Dietary linoleic acid promotes hyperactive hepatic 2-arachidonoyl-glycerol and contributes to diet-induced obesity	P. Chen (France) Inhibition of platelet aggregation by a new class of fatty acid oxygenated products called poxotrans, including a protectin d1 isomer	U. Ramakrishnan (USA) Effect of prenatal supplementation with docosahexanoic acid on child size and development at 18 mo: randomized placebo-controlled trial in Mexico
11.15 - 11.30	P. Flachs (Czech Republic) N-3 fatty acids augment beneficial effects of mild calorie restriction in mice fed high-fat diet - role of sirt1	T. Hornemann (Switzerland) Accumulation of two atypical sphingolipids underlies the pathology in hereditary sensory neuropathy hsn1	D. Hoffman (USA) Cognitive function in 18-month old term infants of the diamond study: a dha dose-response randomized, controlled clinical trial

11.30 - 11.45	E. Newberry (USA) Reduced hepatic and serum fatty acid amides in l-fabp-/- mice alter feeding behavior and protect against obesity	G. Dramane (France) Identification of different isoformes of pla2 in mouse cd36 positive lipid gustatory cells: implication in calcium signalling	T. Karlsson (Sweden) LCPUFA supplemented to mothers during pregnancy and breast-feeding improves cognitive performance in their children four years later—an RCT study
11.45 - 12.00	P. Nunes (Netherlands) Creatine depletion hampers glucose and lipid metabolisms of AGAT-/- mice	V. Stone (UK) The cytoprotective actions of oleoylethanolamide in pancreatic beta-cells require fatty acid amide hydrolase activity and are not mediated via GPR119	C. Milte (Australia) Effects of EPA versus DHA on literacy and behaviour in children with adhd and learning difficulties
12.00 - 14.00	Lunch with Poster Session 2		
Concurrent sessions 10 - 12			
14.00 - 15.30	Brain function II - Other aspects <i>Chairs: Olga Schiepers, Hee-Yong Kim</i>	Cardio biochemistry <i>Chairs: Peter McLennan, Vera Schrauwen</i>	Standardizing protocol <i>Chairs: Clemens von Schacky, Simon Dyall</i>
14.00 - 14.15	N. Osumi (Japan) The effects of arachidonic acid and docosahexaenoic acid on neural stem/progenitor cells	P. McLennan (Australia) Fish oil feeding induces nutritional preconditioning in the rat heart by upregulating endogenous antioxidants and reducing oxidative damage	Simon Dyall (UK) Methodological issues and inconsistencies in omega-3 fatty acid research
14.15 - 14.30	B. Levant (USA) Effects of dietary n-3 fatty acids and brain dha content on activity and response to novelty in the developing rat	M. Bernard (France) The cardioprotective effect of docosahexaenoic acid occurs through myocardial physiological and metabolic changes in rat heart on digitalis drug treatment	

14.30 - 14.45	R. Gow (UK) The relationship between measures of depression, anxiety and anger/disruptive behaviour and omega-3/6 fatty acids in adolescents with and without ADHD	W. Schunck (Germany) Effect of dietary omega-3 fatty acids on the endogenous cyp eicosanoid profile	B. de Roos (UK) Participating in a controlled dietary fat study, rather than the nature of intervention, regulates the plasma proteome in healthy men
14.45 - 15.00	Y. Matsuoka (Japan) Omega-3 fatty acids for secondary prevention of posttraumatic stress disorder following accidental injury: an open-label pilot study	L. Steinbusch (Netherlands) Balanced substrate supply is essential for cardiac protection of the compromised heart	A. Patterson (Canada) Long term adherence following advice to increase eicosapentaenoic and docosahexaenoic acid intakes using different dietary strategies
15.00 - 15.15	Y. Kiso (Japan) Arachidonic acid (ARA)-enriched triacylglycerol supplementation was more effective in improving cognitive function of elderly men with low serum ARA level	D. Abdurrachim (Netherlands) Myocardial lipid accumulation and diabetic cardiomyopathy status studied in vivo in a diabetic mouse model using 1H-MRS and MRI	T. Gilhooly (UK) Percentage of n-3 HUFA: a simple measure of HUFA balance
15.15 - 15.30	O. van de Rest (Netherlands) Fish fatty acids and mental health in older people	C. van den Brom (Netherlands) Myocardial sphingolipid metabolism in Zucker diabetic fatty rats	F. Marangoni (Italy) Whole blood fatty acids, not only n-3, are associated with dietary habits and cardiovascular risk factors in an Italian population
15.30 - 16.00	Break		
16.00 - 17.30	Plenary: Lipids and Health 2: brain function <i>Chairs: Mehar Manku, Ray Rice</i>		
16.00 - 16.45	(PL 7) Mechanisms of altered brain function and aging (Stanley Rapoport, Bethesda, USA)		
16.45 - 17.30	(PL 8) DHA and cognitive decline during aging (Stephen Cunnane, Quebec, Canada)		
19.00 - 23.00	Dinner Debate (limited attendance) Less fat or different fat? (held in the Bonbonniere, Maastricht) (Supported by The Dutch Dairy Association)		

Tuesday June 1

08.30 - 10.00	Plenary: Biochemistry of Lipids 2: Lipid droplets and transport <i>Chairs: Sven Olofsson, Francesco Visioli</i>		
08.30 - 09.15	(PL9) Fatty acid chaperones and transporters (Jan Glatz, Maastricht, The Netherlands)		
09.15 - 10.00	(PL10) Lipid droplets, lipases (Rudi Zechner, Graz, Austria)		
10.00 - 10.30	Break		
Concurrent sessions 13 - 15			
10.30 - 12.00	Lipids and inflammation <i>Chairs: Ronit Sverdlov, Bruce Levy</i>	Diabetes, lipid droplets, mitochondria <i>Chairs: Patrick Schrauwen, Sven Olofsson</i>	Clinical nutrition <i>Chairs: Peter Zock, Konstantin Mayer</i>
10.30 - 11.00	Bruce Levy (USA) Pro-resolving actions of resolvin E1 in airway inflammation, injury and infection	Sven Olofsson (Sweden) The formation of lipid droplets; possible role in the development of insulin resistance/type 2 diabetes	Konstantin Mayer (Germany) Lipids in Critical Care Medicine
11.00 - 11.15	S. Oh (USA) Novel 18s-E-series resolvins: stereoselective biosynthesis and pro-resolving actions	J. Jocken (Netherlands) Dysbalance in skeletal muscle lipase content and activity in obesity and type 2 diabetes	J. Faber (Netherlands) Supplementation with a fish-oil enriched sip-feed leads to EPA incorporation into white blood cells and enhanced immune responses within one week
11.15 - 11.30	S. Orr (Canada) Protective effects of brain dha in a mouse model of neuroinflammation	A. Tingaud-Sequeira (France) In vivo dynamics of zebrafish adipocyte lipid droplets in response to nutritional status and pharmaceuticals regulating lipid metabolism	J. Woodall (UK) Modulation by lipid nutrition of inflammatory cell phosphatidylcholine compositions in preterm infants

11.30 - 11.45	J. Schumann (Germany) Impairment of NFκB activity by unsaturated fatty acids	D. Stringer (Canada) Differential effects of conjugated linoleic acid (CLA) isomers on hepatic lipid droplet size and lipid droplet proteins in obese rats	A. Daak (UK) The effect of omega-3 fatty acids on prevention of vaso-occlusive crisis in homozygous sickle cell disease
11.45 - 12.00	L. van den Elsen (Netherlands) Polyunsaturated fatty acids alter the phenotype of human mast cells in vitro	S. Timmers (Netherlands) Increased TAG and DAG content paradoxically parallel the insulin sensitizing effect of DGAT1 overexpression in rat skeletal muscle	R. Metcalf (Australia) Fish oil and the incidence of post-cardiac surgery atrial fibrillation: a double-blind randomised controlled trial
12.00-13.30	Lunch with Poster Session 3		
13.30-14.00	ISSFAL members meeting		
Concurrent sessions 16 - 18			
14.00-15.30	Cardiovascular Health <i>Chairs: Matthijs Hesselink, William Stanley</i>	XXXdomics <i>Chairs: Edwin Mariman, Kim Ekroos</i>	Maternal and Infant nutrition III - Allergy and Immune function <i>Chairs: Berthold Koletzko, Gerard Hornstra</i>
14.00 - 14.30	William Stanley (USA) n-3 PUFAs for the prevention and treatment of heart failure	Kim Ekroos (Finland) Lipidomics in health & disease	Berthold Koletzko (Germany) Red blood cell levels of docosahexaenoic acid and other polyunsaturated fatty acid levels in pregnant women are modified by single nucleotide polymorphisms of the fads gene cluster: results from the ALSPAC study

14.30 - 14.45	A. Georgiadi (Netherlands) Induction of cardiac angptl4 by dietary fatty acids is mediated by PPARb and protects against oxidative stress	R. Atmeh (Jordan) Lipidomics and proteomics of some high density lipoprotein subclasses in healthy human subjects	M. Makrides (Australia) Allergy and respiratory outcomes from the dino (DHA for the improvement of neuro-developmental outcome in preterm infants) trial
14.45 - 15.00	R. Block (USA) Eicosapentaenoic acid plus docosahexaenoic acid enhances aspirin's effect on platelet function	P. Babin (France) Identification of on-off switch lipid genes in the intestine of a model system	N. D'Vaz (Australia) PUFA levels at 6 months of age after oil supplementation from birth predict symptoms of atopic dermatitis, cough and wheeze
15.00 - 15.15	M. Pedersen (Denmark) The effect of dietary fish oil on metabolic risk markers and body composition in slightly overweight teenage boys	S. Ducheix (France) A nutrigenomic approach reveals that LXR is required for hepatic steatosis induced by essential fatty acid deficiency	P. Noakes (UK) Salmon in pregnancy study (sips): effects of increased oily fish intake during pregnancy on neonatal immune responses and clinical outcomes
15.15 - 15.30	V. Schrauwen-Hinderling (Netherlands) Systolic function is improved in overweight type 2 diabetic subjects after physical activity training without changes in cardiac lipid content		H. Urwin (UK) The effects of salmon consumption during pregnancy on breast milk fatty acid composition and immune function
15.30 - 16.00	Break		
16.00 - 17.30	Plenary: diet and nutrition 2: Clinical nutrition (ESPEN) <i>Chairs: Ellen Blaak, Birgitta Strandvik</i>		
16.00 - 16.45	(PL11) Lipids as modulator of inflammatory response (Patricia Bozza, Rio de Janeiro, Brazil)		
16.45 - 17.30	(PL12) Fatty acids as amplifiers of anti-cancer therapy (P. Bougnoux, Tours, France)		
19.00 - 23.00	Gala Dinner (Fort St. Pieter, Maastricht, bus transport arranged) <i>(Sponsored by Nu-Mega/GTC Nutrition)</i>		

Wednesday June 2

8.30 - 10.00	Plenary: Hot topics <i>Chairs: Margaret Craig-Schmidt, Ingeborg Brouwer</i>		
8.30 - 9.15	(PL 13) Alternative omega-3 fatty acid sources (Olga Sayanova, Harpenden, UK)		
9.15 - 10.00	(PL14) Results of the DOMInO Trial on DHA in pregnancy (Robert Gibson, Adelaide, Australia)		
10.00 - 10.30	Break		
	Concurrent sessions 19 - 21		
10.30 - 12.00	Lipids, ageing and longevity <i>Chairs: Tomohito Hamazaki, Riekelt Houtkooper</i>	Hepatic lipid metabolism <i>Chairs: Joost Luiken, Sander Kersten</i>	Novel topics <i>Chairs: Wim Saris, Helen Roche</i>
10.30 - 11.00	Riekelt Houtkooper (Switzerland) Integrating metabolism by NAD+ sensors	Sander Kersten (Netherlands) Transcriptional regulation of hepatic fatty acid metabolism	Helen Roche (Eire) Interactions between dietary fat and inflammatory genes on the metabolic syndrome - Insights from the LIPGENE Study
11.00 - 11.15	I. Denis (France) N-3 polyunsaturated fatty acid (PUFA) deficiency aggravates the age-related impairment of astroglial function in rat brain	V. de Boer (Netherlands) SIRT4 suppresses hepatic fatty acid oxidation by signalling through PPARα	H. Dabadie (France) A myristic acid enriched cream improves the metabolic syndrome: the Semyramis study
11.15 - 11.30	K. Yurko-Mauro (USA) Cognitive benefits of docosahexaenoic acid in age related cognitive decline	M. Rossmeisl (Czech Republic) N-3 fatty acids as phospholipids are superior over triacylglycerols in ameliorating hepatic steatosis and other disorders induced by high-fat feeding	S. Banni (Italy) Krill oil improves metabolic syndrome by modifying endocannabinoid biosynthesis

11.30 - 11.45	C. Von Schacky (UK) Association of marine omega-3 fatty acid levels with total mortality and telomeric aging in patients with coronary heart disease	A. Soares (Portugal) Hepatic de novo lipogenesis is promptly altered in the stz diabetic rat	E. Jackson (USA) Vegetarian source of omega-3 fatty acids by metabolic engineering of <i>yarrowia lypolytica</i>
11.45 - 12.00	A. Petroni (Italy) Dietary medicament for the treatment of neurodegenerative disorders	A. Kitson (Canada) Tissue specific sex differences in the expression of genes involved in docosahexaenoic acid biosynthesis in rats	J. Kralovec (Canada) Strategies for production and structural assessment of modern omega-3 based nutraceuticals and medicinals
12.00 - 13.00	Meet The Professor lunch (New Investigator's Awardees only)		
12.00 - 13.00	Lunch		
13.00 - 14.30	Plenary: Award Session Chairs: Philip Calder, Matthijs Hesselink		
13.00 - 13.30	(PL15) Early Career Award - John Paul San Giovanni <i>Emerging Opportunities for an Integrated Systems Approach to the Study of Nutrient-Disease Relationships: Technical Application and Findings from Investigations on the Impact of Highly Unsaturated Fatty Acids in Health and Disease of the Retina.</i>		
13.30 - 14.15	(PL16) New Investigator Awards (one oral presentation for each track, to be selected on June 1 from the nominees below) <i>Nominees:</i> Track 1: Lipids and Health M. Bernabe (Mexico) Evidence of beneficial effects of enteral docosahexaenoic acid on cytokine production and clinical outcomes in surgical neonates P. Jackson (UK) DHA-rich fish oil modulates cerebral haemodynamics in a dose response manner in healthy young adults K. Su (Taiwan) The effects of polymorphisms in phospholipase a2 and cyclo-oxygenase 2 genes on interferon-alpha-induced depression and polyunsaturated fatty acids levels		

	<p>Track 2: Biochemistry of Lipids</p> <p>Giuseppe Astarita (USA) Deficient liver biosynthesis of docosahexaenoic acid correlates with cognitive impairment in alzheimer's disease</p> <p>M. Gregory (Australia) Cloning and functional characterisation of the fatty acyl elovl2 and elovl5 from rat</p> <p>C. Coomans (Netherlands) Brain insulin signaling promotes fatty acid uptake by adipose tissue</p> <p>Track 3: Lipids and Nutrition</p> <p>J. Lambert (Canada) Islet transplant patients do not respond to hypolipidemic dietary intervention - role of hepatic de novo lipogenesis</p> <p>C. Childs (UK) The DHA content of rat liver phospholipids are significantly higher when an alpha-linolenic acid rich diet is provided during pregnancy.</p> <p>R. Kuipers (Netherlands) The relation between DHA and AA is synergistic at low DHA status and antagonistic at high DHA status</p>
14.15 - 14.30	Presentation of the Awards
14.30 - 15.00	Invitation to Vancouver, Canada for ISSFAL 2012; Closing ceremony and adjourn

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With leading food category positions in spreads, dressings, savoury products and ice cream, researching fatty acids has been a core activity of Unilever for decades: from the introduction of linoleic acid in spreads in the sixties, the removal of trans fatty acids from our spreads in the nineties, to the improved fatty acid profiles across our portfolio through the Nutrition Enhancement Programme; from our education on essential fats for children to facilitating discussions on the importance of an optimal fat quality of the diet through our partnership agreement with the International Union of Nutritional Sciences (IUNS) (www.IUNS.org/features.htm).

In 2009, 50 nutrition intervention studies were undertaken, 390 peer-reviewed articles were published and at least 250 new patents were applied for. We particularly value our interactions with experts from academia, health authorities, governments and non-governmental organisations and look forward to meeting you at the 2010 ISSFAL in Maastricht.

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Abstracts - Saturday May 29

Plenary: Alexander Leaf Award Lecture

18.30 - 19.30

My life with DHA

N Salem Jr

Martek Biosciences Corporation, Columbia, United States of America

My personal discovery of docosahexaenoic acid (DHA) came in 1972 while a graduate student. I realized immediately that it was an extraordinary molecule and the fact that the brain concentrated it must be related to some special function of the neural aminophospholipids in which it was concentrated. This led to a PhD thesis in which the DHA molecular species of bovine brain phosphatidylserine was isolated and their biophysical properties in liposomal membranes examined. Covalent labeling studies of membranes showed a preferential association of DHA-PS with membrane proteins. The initial fascination with DHA was to become a lifelong pursuit.

The next phase of my work on DHA centered on oxygenated metabolites including various lipoxygenase and auto-catalytic structures. This period in the 1980's was also the beginning of our studies into the EFA modifying effects of alcohol abuse. In attempting to understand the variability of animal experiments involving alcohol exposure, it was realized that the EFA composition of the diet was crucial. Alcohol abuse seemed to be the one thing able to knock DHA out of the brain in adult mammals. Writing a review in the late 1980's gave me time to read thru Michael Crawford's work on DHA metabolism and this proved to be a great stimulus for study of EFA metabolism. We began work with ALA metabolism to DHA in animals and in human infants and adults. Slowly, over the ensuing years, the variables of n-6 fatty acid competition and the relative efficacies of short chain and long chain n-3 and n-6 PUFAs in modulating tissue EFA composition began to be appreciated. We demonstrated ALA to DHA conversion in humans of all ages but an important conclusion was that this metabolism was very limited and preformed DHA was required to impact tissue DHA content.

Over the past 20 years, we have sought to define clearly that dietary n-3 fatty acid deficiency led to losses of nervous system DHA and functional deficits, defined mainly by behaviorally means. Today this proposition is on solid ground and supports the concept that DHA is an essential nutrient. This was bolstered by description by my NIH colleagues of important underlying mechanisms including effects on G-couple receptor function and on neuronal apoptosis and differentiation. These mechanisms in turn were related to unique biophysical and conformational properties of DHA-PLs. Our approach then began to transition to a study of DHA supplementation in an attempt to understand what benefits raising organ levels of DHA might have. Recent studies have shown a benefit of DHA supplements for maintenance of cognition during normal aging and have suggested possible protection from neurological diseases.

Abstracts - Sunday May 30

Plenary: Lipids and Health 1: Metabolic syndrome

08.30 - 09.15

Netherlands Heart Foundation lecture

Insulin resistance and the metabolic syndrome; why a cluster is truly a cluster

G Reaven

Stanford University School of Medicine, Stanford, United States of America

In 1936 Himsworth and colleagues, using a newly-developed method to quantify insulin action, concluded that “a primary deficiency of insulin is one, and than not the commonest cause, of the diabetic syndrome.” Although this view was largely ignored for more than 30 years, we now know that insulin resistance: 1) occurs more commonly in family members of patients with type 2 diabetes (2DM; 2) predicts the development of 2DM; and 3) is present in the vast majority of patients with 2DM. Although insulin resistance is characteristic of patients with 2DM, most insulin resistant individuals compensate by secreting enough insulin to prevent development of 2DM. Unfortunately, insulin resistant/hyperinsulinemic individuals are at increased risk to develop some degree of glucose intolerance, hypertension, and a high triglyceride (TG) and low high-density lipoprotein cholesterol (HDL-C) concentration. This cluster of related abnormalities, initially designated as Syndrome X, constitutes a major contributor to cardiovascular disease. The Adult Treatment Panel III (ATP III) and the International Diabetes Federation (IDF) used the components of Syndrome X, plus excess adiposity, to propose a new diagnostic category—the Metabolic Syndrome (MetS). The ATP III stated that the MetS “is a grouping of ASCVD risk factors, but one that probably has more than one cause,” whereas the IDF version requires that abdominal obesity be present, arguing that “central obesity is an early step in the etiological cascade leading to the full metabolic syndrome.” The two groups have recently “harmonized” the MetS, and although it is still claimed that the “cause” is abdominal obesity, it no longer is required to make the diagnosis. This presentation will challenge these points of view by providing evidence that: 1) insulin resistance accounts for the clustering of glucose intolerance, elevated blood pressure, and a high TG and low HDL-C concentration; and 2) the clustering of these can occur in the absence of abdominal obesity.

09.15 - 10.00

Ectopic fat, NASH, and the metabolic syndrome

M Roden

Heinrich-Heine University Düsseldorf, Düsseldorf, Germany

Depending on the respective definition, the so-called metabolic syndrome comprises of parameters indicating increased fat mass, vascular dysfunction and abnormalities of lipid and glucose metabolism. These alterations variably relate to impaired insulin sensitivity, i.e. insulin resistance. Current paradigms postulate that (i) abdominal obesity causes imbalanced release of adipocytokines and free fatty acids (FFA) which initiate inflammatory processes or that (ii) postprandial overflow of nutrients such as FFA to liver and muscle induces intracellular disturbance of signalling and metabolic pathways all together leading to insulin resistance. This would lead to lipid deposition in muscle (intramyocellular lipids, IMCL) and liver (hepatocellular lipids, HCL). Elevation of HCL is also termed steatosis or non-alcoholic fatty liver (NAFL) and bears an increased of inflammatory liver disease or non-alcoholic steatohepatitis (NASH) which predisposes for cirrhosis and hepatocellular carcinoma. More recently, evidence accumulated that insulin resistance associates with impaired mitochondrial oxidative phosphorylation (ATP synthesis) in muscle and in liver. We demonstrated that FFA elevation markedly inhibits insulin-stimulated ATP synthesis prior to changes in IMCL suggesting that nutrients primarily inhibit insulin signaling and subsequently reduce mitochondrial function. Furthermore, we found that patients with type 2 diabetes show impaired hepatic energy metabolism without alterations in IMCL, but along with elevated HCL. This suggests that decreased liver mitochondrial function could be a primary abnormality at least in some insulin resistant states and may contribute to the development of NAFL and NASH.

Concurrent Session - 1

Lipids and Health : Metabolic syndrome - molecular aspects

10.30 - 11.00

Invited contribution

The role of mitochondria in lipid-induced insulin resistance within skeletal muscle

B Goodpaster

University of Pittsburgh School of Medicine, Pittsburgh, United States of America

Skeletal muscle insulin resistance (IR) has been linked with the etiology of type 2 diabetes in both aging and obesity. The exact causes of IR in humans, however, remain elusive. During the past decade the potential link between impaired mitochondria function and skeletal muscle IR has been extensively investigated and disputed. On one hand, the literature is replete with studies supporting an association between mitochondria content or function with insulin resistance of aging, obesity, or type 2 diabetes. On the other hand, animal models or cell systems have been inconsistent in delineating a causal relationship between mitochondria and IR. Most studies - both model systems and human - examining the potential role of mitochondria in skeletal muscle insulin resistance have taken the simplistic view that mitochondria function can be generally characterized. The alternative view presented here is that there are many different facets of mitochondria function, each of which may or may not be implicated in alterations in energy demand, oversupply, and insulin resistance. Studies from model systems and humans have provided sufficient evidence that specific components of mitochondria performance may play an important role in skeletal muscle insulin resistance. Finally, evidence from human intervention studies will be presented to support or refute a role for mitochondria in insulin resistance.

11.00 - 11.15

Prolonged fasting-induced insulin resistance, lipid accumulation and mitochondrial (dys)function in human skeletal muscle

J Hoeks, N van Herpen, M Hesselink, P Schrauwen

Maastricht University Medical Centre, Maastricht, The Netherlands

Background: Skeletal muscle mitochondrial dysfunction has been linked to the development of insulin resistance and type 2 diabetes mellitus (T2DM). We have suggested that this mitochondrial dysfunction may result from lipotoxicity: fat accumulation in skeletal muscle could lead to impaired mitochondrial function.

Objective: Interestingly, prolonged fasting also results in intramyocellular lipid (IMCL) accumulation and insulin resistance. Whether this also leads to reduced mitochondrial function is unknown and aim of the present study.

Procedure: 12 healthy males (age 23.6±1.0 yrs, BMI 22.6±0.5 kg/m², VO₂max 48.5±1.6 ml/min/kg (mean ± SEM) fasted for 60h or received a control diet, while in a respiration chamber. Afterwards, insulin-sensitivity was assessed using a hyperinsulinemic-euglycemic clamp and muscle biopsies were taken. Blood was sampled after 12, 36 and 60 hours.

Mitochondrial DNA copy number was determined by real-time PCR while mitochondrial function was quantified *ex vivo* in permeabilised muscle fibers using high-resolution respirometry. IMCL levels were assessed by Oil Red O staining.

Results: In the fed condition FFA levels after 12, 36 and 60 hours were 222±17, 247±25 and 387±37 µmol/l. During fasting FFA levels progressively increased from 212±25 to 1054±187 (36h) and to 1925±106 µmol/l (60h). Insulin sensitivity (SI-index) in fasted subjects dropped to ~55% of fed values (8.8±0.8 vs. 4.8±0.4, p<0.001). Mitochondrial density was similar in both conditions. However, both ADP-stimulated oxygen consumption and maximally uncoupled respiration were significantly lower after fasting (State 3: 94.6±7.4 vs. 79.2±4.4 and State U: 170.6±9.3 vs. 132.2±9.7 pmol/(s*mg)/(mtDNA copy numbers*10000, p<0.05), indicating a reduced intrinsic mitochondrial capacity after fasting. IMCL levels are currently under investigation.

Conclusion: 60 hours of fasting increased plasma FFA levels and decreased insulin sensitivity and intrinsic mitochondrial capacity. This may suggest that high FFA levels contribute to reduced mitochondrial function, supporting the hypothesis that mitochondrial dysfunction as observed in T2DM originates from lipotoxicity.

11.15 - 11.30

Role of cIAP1 in ER stress-mediated lipotoxicity: a determinative factorP Xia¹, Y Qi²¹Centenary Institute, Newtown, Australia²University of Sydney, Sydney, Australia

Background: Endoplasmic reticulum (ER) stress response has been broadly implicated in human disease. For instance, overload of free fatty acids induce ER stress and lead to pancreatic β -cell apoptosis, namely lipotoxicity, which is recognized as fundamental basis for the development of type 2 diabetes. However, how ER stress induces apoptosis is poorly understood.

Objective: The study was designed to identify a key factor that may determinate cell's fate, i.e., adaptation to ER stress or apoptotic death, under the lipotoxic conditions.

Procedure and Results: Exposure of pancreatic β -cells to palmitic acid (PA; C16:0) resulted in overt ER stress response and apoptotic cell death in a dose-dependent manner. Remarkably, knockdown of the stress-inducible transcription factor CHOP expression by its siRNA significantly inhibited PA-induced apoptosis, suggesting a critical requirement of CHOP for ER stress-mediated cell death. Interestingly, PA treatment induced a significant increase in ubiquitin-proteasomal degradation of cIAP1 independent of caspase 3 activation. Notably, the lipotoxicity of PA was significantly inhibited by overexpression of cIAP1, whereas it was aggravated by cIAP1-targeted siRNA. In addition, exposure to PA induced more cell death in cIAP1^{-/-} mouse embryonic fibroblasts than in the wild type cells, suggesting a protective role of cIAP1 against ER stress-induced cell death. Furthermore, palmitoleic acid (POA; C16:1) had a similar effect to PA in CHOP upregulation, whereas POA caused neither cIAP1 degradation nor cell death. In stark contrast, in cIAP1 knockdown or knockout cells, POA was capable of mimicking PA to induce ER stress-mediated cell death.

Conclusion: Our findings for the first time suggest that in addition to CHOP induction, cIAP1 is a key determinative factor for cell survival or death under ER stress conditions.

11.30 - 11.45

Increased peroxisomal fatty acid oxidation in liver contributes to the protection against obesity and glucose intolerance by fish oil dietsJ Fiamoncini¹, N Turner², S Hirabara³, T Lima¹, A Marçal¹, S Leslie², G Cooney², R Curi¹¹Institute of Biomedical Sciences - University of São Paulo, São Paulo, Brazil²Garvan Institute of Medical Research, Sydney, Australia³Universidade Cruzeiro do Sul, São Paulo, Brazil

Objective: In this study, we investigated how different concentrations of fish oil and lard can modulate glucose and lipid homeostasis and affect insulin resistance. The role of peroxisomes in these effects was also evaluated.

Procedure: Swiss mice were fed for 10 weeks on diets containing either fish oil at 4% (NFO) or 45% (HFO) and lard at 4% (NL) or 45% (HL). Glucose and lipid metabolism were studied in liver and muscle.

Results: Fish oil fed animals weighed ~10 % less than corresponding lard-fed animals, and also displayed smaller epididymal and retroperitoneal fat pad ($p < 0.05$). Despite the lean phenotype, fish oil-fed mice showed lower oxygen consumption when compared with the lard fed groups ($p < 0.01$). NFO and HFO exhibited a better response during a Glucose Tolerance Test than lard fed animals (average area under the curve = 375 and 564 for NFO and HFO, vs. 703 and 867 for NL and HL, respectively). Triacylglycerol content and palmitate oxidation rate were not different in muscle across the 4 groups. In contrast, fish oil fed animals exhibited 60% lower triacylglycerol levels in liver than lard-fed counterparts. Furthermore, despite lower complete oxidation of palmitate in liver homogenates from NFO and HFO animals, a ~50% higher level of acid soluble metabolites were generated in these groups. This suggests that peroxisomal fat oxidation was increased. The activity of the key peroxisomal enzyme, acyl-CoA oxidase and other enzymes involved in beta-oxidation were also substantially enhanced in the liver of fish oil fed animals.

Conclusion: The increased acid soluble metabolite production from palmitate and the increased activity of peroxisomal enzymes in the liver of fish oil fed groups, suggest that inefficient peroxisomal metabolism of fatty acids in liver is an important contributor to the lower fat accumulation and better glucose tolerance in n-3 PUFA fed mice.

11.45 - 12.00

Low-density lipoproteins from patients with a metabolic syndrome or type-2 diabetes activate platelets

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Background: Metabolic syndrome (MetS), a pre-diabetic state, and type-2 diabetes mellitus (T2DM), are associated with an increased cardiovascular risk. Platelet activation and dyslipidemia are two major factors favoring atherothrombosis. Our team previously reported that T2DM patients showed platelet hyperactivation associated with increased oxidative stress. We also showed that low-density lipoproteins (LDL) from T2DM patients activated platelets. The mechanism underlying platelet activation remains undetermined.

Objective: To determine the lipidomic profile of LDL from MetS and T2DM patients, compared to LDL from healthy volunteers, and to explore their role in platelet activation.

Procedure: LDL were isolated from plasma of patients and healthy volunteers. Their lipid and fatty acid compositions as well as their redox status were determined. Their impact on platelet arachidonic acid signaling cascade was assessed.

Results: Compared to LDL from healthy volunteers, LDL from MetS and T2DM patients contained significantly decreased cholesteryl ester (CE) and increased triacylglycerol (TG) levels. Concentrations of linoleic acid, the main polyunsaturated fatty acid, decreased in phosphatidylcholine, phosphatidylethanolamine, CE and TG. Concentrations of hydroxylated fatty acids and malondialdehyde, indices of lipid peroxidation, increased in LDL from patients. Ethanolamine plasmalogens decreased in T2DM LDL. No change in α -tocopherol was observed. While control LDL had no effects on platelets, LDL from both MetS and T2DM patients activated platelets from healthy volunteers, as shown by an increased phosphorylation of p38 MAPK and cPLA₂, and an increased formation of thromboxane A₂.

Conclusion: Our results show that the occurrence of T2DM and the presence of a MetS itself in subjects is associated with lipid changes and increased oxidative stress in LDL, which lead to significant platelet activation. They suggest that lipid alterations in LDL may be part of the mechanism underlying platelet hyperactivation in T2DM and MetS.

Concurrent Session - 2

Biochemistry of Lipids : Brain fatty acid uptake and metabolism

10.30 - 11.00

Invited contribution: CARIM lecture

Fatty acid transport in membranes: new approaches give new insights

J Hamilton

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The plasma membrane presents a complex interface in which fatty acids can interact with the lipid bilayer and proteins. Continuing investigations of both model and biological membranes sometimes yield confounding hypotheses of how a plasma membrane. Development of new methodologies that help clarify details of the interactions both lipid and protein components with fatty acids will help clarify the molecular mechanisms of membrane transport. Recently, our lab has evaluated a new fluorescence probe for detecting interactions of fatty acids and with the polar interface in a phospholipid bilayer, which permits measuring the incorporation of fatty acids specifically into one leaflet of the lipid bilayer. This method has the potential for examine how fatty acid binding is influenced by membrane proteins. In addition, we have defined conditions for solubilizing fatty acids with cyclodextrin and achieving both delivery and extraction of fatty acids. Cyclodextrin permitted delivery of the highly water-insoluble very long chain saturated fatty acids (VLCFA) to both model membranes and the plasma membrane of cells in vitro. Our fluorescence assays establishing that these fatty acids bind to and diffuse across the lipid bilayer rapidly. Since VLCFA are markers if not causative agents for X-linked neurological diseases, their passage from the blood to neural cells can, like other fatty acids, be achieved without a specialized transport system. Our hypothesis of transport of fatty acids through the blood brain barrier will be discussed.

11.00 - 11.30

Invited contribution**Characterization of fatty acid transport in human blood brain barrier microvessel endothelial cells**

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The blood-brain barrier (BBB) formed by brain capillary endothelial cells provides a protective barrier between the systemic blood and the extracellular environment of the central nervous system. We examined the mechanism of fatty acid transport across primary human brain microvessel endothelial cells (HBMEC). Permeability of radiolabelled fatty acids was determined using confluent cells grown on Transwell® inserts in both the absence or presence of bovine serum albumin in the basolateral media, and following inhibition of various fatty acid transporters. Passage of [1-¹⁴C]oleate across confluent HBMEC monolayers was significantly enhanced when albumin was present in the basolateral media indicating that a fatty acid acceptor is a requirement for transport across HBMEC monolayers. Passage of [1-¹⁴C]oleate from apical to basolateral side was much greater than passage from basolateral to apical side indicating directionality of transport. The non-specific fatty acid uptake inhibitor phloretin significantly decreased [1-¹⁴C]oleate passage into the basolateral medium indicating involvement of fatty acid transport proteins. Passage of various radiolabelled]fatty acids across HBMECs was dependent on both fatty acid chain length and degree of unsaturation. RNAi knockdown of fatty acid transport protein-1 (FATP-1) or fatty acid translocase/CD36 significantly decreased [1-¹⁴C]oleate transport across the HBMEC monolayer from either apical as well as basolateral sides. RNAi knock down of specific fatty acid transport proteins had variable effects on movement of specific radiolabelled fatty acids across HBMECs. Purified mouse brain capillaries and human brain grey matter exhibited similar levels of mRNA expression of FATP-1, FATP-4 and fatty acid binding protein-5 (FATP-5), FATP-7 as major fatty acid transporter proteins. Our results suggest that transport of fatty acids across HBMEC is, in part, a transcellular process mediated by fatty acid transport proteins.

11.30 - 11.45

Rapid disappearance of eicosapentaenoic acid from rat brain phospholipids: an *in vivo* intracerebroventricular study

CT Chen, Z Liu, RP Bazinet

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Background: Eicosapentaenoic acid (EPA) is currently being investigated for its therapeutic potential in neurodegenerative diseases. Although it is known that the concentration of EPA is low within the brain, its metabolic fate in the brain remains largely uncharacterized. Previously, we demonstrated that *in situ*, EPA was 2.5-fold more readily β -oxidized compared to docosahexaenoic acid (DHA) upon entry from the plasma through the blood-brain barrier (BBB) which is the candidate site of fatty acid oxidation, into the brain. In addition, non- β -oxidized EPA was found to be esterified into brain phospholipids.

Objective: To characterize the metabolism of EPA in the brain, *in vivo*, upon bypassing the BBB.

Procedures: Ten μ Ci of ¹⁴C-radiolabeled EPA or palmitate was administered via intracerebroventricular infusion in rats.

Brains were collected from 4 to 128 days post-infusion. Brain total lipids were isolated and fractionated by thin-layer chromatography. High-performance liquid chromatography, liquid scintillation counting and gas chromatography-FID/MS were used to quantify and to identify radiolabeled fatty acids.

Results: Total phospholipid radioactivity from ¹⁴C-EPA-infused brains was 6-fold lower than ¹⁴C-Palmitate-infused brains four days post-infusion ($P < 0.05$). Four days post-infusion, 56% of radioactivity in ¹⁴C-palmitate-infused brain was confirmed to be palmitate; whereas 8.9% of radioactivity in ¹⁴C-EPA-infused brain was EPA and the remainder was palmitate (26%), n-3 docosapentaenoate (25%) and DHA (27%). The half-life of esterified ¹⁴C-palmitate and ¹⁴C-EPA was calculated to be 32 ± 4 (2% loss per day) and 5 ± 0.2 days (15% loss per day), respectively.

Conclusion: The rapid loss of EPA upon entry into the brain and from phospholipids may explain the low levels of EPA in brain phospholipids.

11.45 - 12.00

Metabolism of docosahexaenoic acid in hippocampal development

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Background: We demonstrated that docosahexaenoic acid (DHA, 22:6n-3), which is essential for proper brain development and function in animals and humans, promotes neurite growth and synaptogenesis in hippocampal neurons (1). It is well-recognized that DHA can be transformed to bioactive lipid mediators. Accordingly, DHA metabolites may be involved in hippocampal development promoted by DHA

Objective: To identify DHA metabolites involved in hippocampal neuronal development.

Procedures: Rat or mouse E-18 hippocampal cultures were supplemented with uniformly labeled ¹³C-DHA for 3 or 7 days in the serum-free Neurobasal medium containing 2% B27 supplements, and the formation of DHA metabolites is monitored by tandem mass spectrometry in relation to neurite growth, synaptogenesis and synaptic function.

Results: In developing hippocampal neuronal culture, DHA promoted neurite growth and synaptogenesis which were further promoted by a fatty acid amide inhibitor URB597, suggesting an involvement of an amide form of DHA. Active metabolism of DHA to N-docosahexaenylethanolamine (DEA) was observed in the E-18 hippocampal neuronal culture and its level increased in the presence of URB597. Synthetic DEA promoted neurite growth, synaptogenesis and synaptic protein expression in cultured hippocampal neurons at 10-100nM ranges.

Conclusion: Our data demonstrated that DEA is an active component for DHA-mediated neurite growth, synaptogenesis and synaptic protein expression involved in synaptic function. The novel DEA-dependent mechanism offers new molecular targets for the regulation of neurodevelopment and function.

1. Cao D, Kevala K, Kim J, Moon H, Jun S, Lovinger D and Kim HY. Docosahexaenoic Acid Promotes Hippocampal Neuronal Development and Synaptic Function, *J Neurochem.*, 111, 510-521, 2009

12.00 - 12.15

ELOVL4 protein elongates n3 and n6 long chain fatty acids to very long chain polyunsaturated fatty acids

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Background: We have previously shown that Elongation of Very Long Chain Fatty acids-4 (ELOVL4) protein is involved in biosynthesis of very long chain polyunsaturated fatty acids (VLC-PUFA, C28-C38). These fatty acids are found in the retina, brain, testis and sperm. Their absence due to mutations in *ELOVL4* gene has been associated with Stargardt-like macular dystrophy (STGD3). Fatty acids from red blood cell membranes of a family of STGD-3 showed a significant inverse relationship between the degree of retinal phenotype and 22:6n3. While patients with severe retinal degeneration had average 20:5n3 and 22:6n3 levels, patients with higher levels of 20:5n3 and 22:6n3 had less severe retinal phenotypes. Previous studies demonstrated that in the retina, 20:5n3 is the preferred substrate for biosynthesis of VLC-PUFA.

Objectives: We hypothesized that reduced levels of VLC-PUFA may be the underlying cause of retinal phenotype seen in STGD-3 patients. We sought to determine the efficiency of elongation of C20-C22 PUFA by ELOVL4 protein.

Procedures: ELOVL4 protein was expressed in PC-12, Y-79, and 661W cells. GFP-expressing and non-transduced cells were used as controls. The transduced cells were treated with 20:5n3, 22:6n3, and 20:4n6, or with equimolar concentrations of 20:5n3 and 22:6n3, all precursors of VLCFAs (> 26 carbons). Cells were collected, total lipids extracted, converted to fatty acid methyl esters, and analyzed by gas chromatography-mass spectrometry (GC-MS).

Results: Both transduced and control cells internalized and elongated the fatty acid precursors. Only ELOVL4-expressing cells elongated 20:5n3, 22:6n3, and 20:4n6 to C28 - C38 VLC-PUFAs. In each fatty acid treatment group, C34-C36 VLC-PUFA were the predominant VLC-PUFA in ELOVL4 expressing cells.

Conclusions: We have shown that ELOVL4 efficiently elongates C20-C22 polyunsaturated fatty acids to VLC-PUFA. We propose that these steps are important in the retina for synthesis of VLC-PUFAs (C28-C38) that are esterified into phosphatidylcholine in rod outer segment membranes.

Concurrent Session - 3

Lipids and Nutrition : Dietary lipids and lipoproteins

10.30 - 11.00

Invited contribution

Long chain omega-3 polyunsaturated fatty acids intakes and health

B Meyer

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Long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) intakes are generally very low yet their attributed healthy benefits are enormous. Western countries' intakes are approximately 200 mg per day whereas Japan has the highest intakes of 1200 mg per day, although Japanese living Hawaii consume approximately 360mg per day. The most studied research area for optimal health is pregnancy and cardiovascular disease, but there is also evidence for other disease states including inflammatory disease and behavioural disorders. Various professional bodies and government organisations have recommendations for LC n-3 PUFA intakes per day and these recommendations include: European Commission PERILIP and Early Nutrition Programming Project recommend at least 200mg of DHA for pregnant women; International Society of Fatty Acids and Lipids recommend 500mg per day; the American Heart Association Nutrition Committee recommend the consumption of 2 fish meals per week, preferably oil fish, which is equivalent to 500mg per day; the Australian National Health & Medical Research Council Suggested Dietary Target intakes are 610mg per day for men and 430mg per day for women; the Australian National Heart Foundation recommend 500mg per day for cardiovascular health and 1000mg per day for people with existing cardiovascular disease. However, the actual intakes of LC n-3 PUFA in Western countries fall short of these recommendations and these will be discussed in terms of pregnancy, children diagnosed with ADHD, depression and cardiovascular disease. Consumption of enrichment of foods with LC n-3 PUFA to meet the recommended intakes as well as assessment of LC n-3 PUFA intakes using a validated electronic PUFA questionnaire will also be discussed.

11.00 - 11.15

Oxidatively modified high-density lipoproteins inhibit human platelet aggregation

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Background: Many studies have shown that high-density lipoproteins (HDL) possess anti-atherogenic properties, which include antioxidant and anti-thrombotic properties. There are few studies on the functional properties of oxidized HDL, which are present in pathologies associated with oxidative stress.

Objective: To determine the role of HDL, either oxidized *in vivo* or *in vitro*, in platelet function.

Procedure: Abetalipoproteinemia (ABL) is a metabolic rare disease characterized by the lack of very low- and low-density lipoproteins and fat malabsorption. We used blood from these patients as a means to determine the effects of HDL, the only lipoproteins present in their plasma, on platelets. We also used *in vitro* copper-oxidized HDL to investigate their impact on platelet aggregation.

Results: ABL patients showed enhanced oxidative stress, as assessed by very low concentrations of tocopherols in plasma and platelets, increased platelet malondialdehyde levels and two-fold increased urinary 8-iso-PGF_{2α} isoprostane concentrations. Platelets isolated from plasma of ABL patients were hyperaggregable. By contrast, platelet-rich plasma from ABL patients aggregated in the same way as platelet-rich plasma from control subjects. HDL from ABL patients, but not control HDL, fully inhibited platelet aggregation by binding to scavenger receptor SR-BI. As expected, HDL from ABL patients were oxidized as shown by decreased α-tocopherol levels (-44%), increased hydroxy-octadecadienoic acid/linoleic acid ratio (x2) and increased malondialdehyde concentrations (x7.8), compared with control HDL. In addition, copper-oxidized HDL inhibited aggregation of platelets from healthy volunteers. The strongest inhibition was provided by the highest concentration of copper sulphate added to HDL and the least by control HDL.

Conclusion: Oxidized HDL may protect platelets from hyperaggregation in a pathology associated with a severe oxidative stress. The inhibitory effects of oxidized HDL on platelet aggregation were also found *in vitro*. Altogether, our results strengthen a recent new concept indicating that HDL upon oxidation may possess anti-thrombotic properties.

11.15 - 11.30

Brown adipose tissue is a major determinant of plasma clearance and organ uptake of triglyceride-rich lipoproteins

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⁴1. Medizinische Klinik, Asklepios Klinik St. Georg, Hamburg, Germany

Objective: Elevated serum triglycerides represent an independent risk factor for developing cardiovascular disease. Chylomicrons and VLDL as triglyceride-rich lipoproteins (TRL) transport triglycerides to peripheral tissues for storage and energy supply. Recently it has been shown that adults have substantial amounts of functional brown adipose tissue (BAT). Murine as well as human BAT has a high energy demand especially when it is activated upon cold exposure. Here we investigate the role of TRL metabolism for energy supply of BAT upon cold exposure in mice.

Procedure: C57BL/6J mice were kept for 24 hours in a cold room (10 °C) or at room temperature. Clearance and organ uptake of radiolabelled TRL were determined in control and cold-exposed mice. TRL organ uptake was visualised by magnetic resonance imaging (MRI) using nanocrystals embedded into the TRL core.

Results: After an oral fat gavage postprandial serum triglyceride peak is diminished in cold-exposed mice. Accordingly we observe a massive acceleration of TRL turnover and a concomitant 20-fold increase in organ uptake of TRL components into BAT of cold-exposed mice. Taken together, we observe an accelerated TRL plasma clearance with an enormous shift in TRL uptake from liver to BAT in cold-exposed mice.

Conclusion: BAT is a novel determinant of plasma clearance and organ uptake of TRL especially upon cold exposure. Therefore, BAT activation by cold-exposure or other means may be an interesting target in diseases associated with elevated serum triglycerides and elevated risk for cardiovascular diseases.

11.30 - 11.45

LDLR knock-out mice fed a cholesterol diet show increased susceptibility to the development of insulin resistance

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Background: LDLR^{-/-} mice are a validated model to study the role of cholesterol-induced inflammation in relation to atherosclerosis. Although mutations in the LDL-receptor contribute to insulin resistance, it is not known what the role is of cholesterol-induced inflammation in the development of (hepatic) insulin resistance. As LDLR^{-/-} mice show a human-like lipoprotein profile, these mice could elucidate the mechanism by which dietary cholesterol contributes to the development of (hepatic) insulin resistance.

Objective: Therefore, we aim to reveal the role of dietary cholesterol in the development of hepatic insulin resistance to understand how addition of cholesterol in the diet can alter metabolic and genetic factors involved in glucose and lipid metabolism in LDLR^{-/-} mice.

Procedure: Whole body and hepatic insulin resistance were studied in male LDLR^{-/-} mice fed 3 different diets, i.e., chow, high-fat cholesterol (HFC; 0.2% cholesterol) and high-fat non cholesterol (HFNC; 0% cholesterol) for 2 and 15 weeks respectively. Biochemical parameters in plasma and liver were measured and hepatic mRNA expression levels for inflammatory genes were determined.

Results: Although body weight was not different, plasma and hepatic cholesterol levels were increased in HFC-fed mice compared to chow- and HFNC-fed mice. Hepatic inflammatory gene expression levels were elevated in HFC-fed mice. In addition, these mice developed both peripheral and hepatic insulin resistance, as indicated by GTT and immunoblot for Akt-phosphorylation. Although, HFNC mice did show slightly elevated gene expression levels for inflammatory markers, insulin resistance was not observed. These data suggest that dietary cholesterol contributes to hepatic inflammation and may accelerate (hepatic) insulin resistance in LDLR^{-/-} mice.

Conclusion: Our data clearly demonstrate that LDLR^{-/-} mice fed a cholesterol diet are more susceptible to develop hepatic insulin resistance and provide insight into the mechanisms by which dietary cholesterol contributes to the development of (hepatic) insulin resistance.

11.45 - 12.00

Dietary n-3 PUFA improves post-prandial metabolism in the insulin resistant JCR:LA-*cp* rat by lowering enterocytic apoB48 production and lymphatic cholesterol

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Background: Post-prandial dyslipidaemia occurs in obesity and insulin resistance (IR), and is associated with increased risk of developing cardiovascular disease. Dietary *n*-3 polyunsaturated fatty acids (PUFA) are proposed to modulate plasma lipids, lipoprotein metabolism and the inflammatory state; however, results remain inconsistent during conditions of IR. The JCR:LA-*cp* rat develops post-prandial dyslipidaemia concomitant with complications of IR and the metabolic syndrome. We recently reported acute dietary *n*-3 PUFA improves fasting, post-prandial lipid metabolism (*via* apolipoprotein(apo)-B48) and IR in JCR:LA-*cp* rats.

Objective: To assess the impact of increased dietary *n*-3 PUFA on chylomicron production and markers of the insulin-signaling cascade in primary enterocytes, as well as corresponding secretion of chylomicron-cholesterol into mesenteric lymph, in JCR:LA-*cp* rats.

Procedure: Male JCR:LA-*cp* rats were fed either a control, isocaloric, lipid balanced diet (LBD) or a LBD with 5% *n*-3 PUFA (fish oil derived EPA/DHA) for 3 weeks. Primary enterocytes were isolated and fractionated (Weiser method) and an adapted western immune-blot method used to determine distribution and concentration of apo-B48 protein. The activity of Jun N-terminal (JNK) was assessed *via* western-blot to target phosphorylated-JNK protein. Secretion of chylomicron-derived cholesterol was determined by cannulating the mesenteric lymph duct.

Results: Enterocyte-specific apo-B48 protein was decreased (43.8%; **p*<0.05), in rats fed 5% *n*-3 PUFA relative to rats fed the control diet. Enterocytic phosphorylated-JNK protein was significantly lower (58.2%; ***p*<0.01) in rats fed 5% *n*-3 PUFA *versus* control. Additionally, rats fed the 5% *n*-3 PUFA diet secreted less cholesterol (30%; **p*<0.05) into mesenteric lymph.

Conclusion: Data suggests that *n*-3 PUFA may improve post-prandial dyslipidemia by reducing apo-B48 production in the enterocyte as well as subsequent secretion of lymphatic chylomicron-cholesterol in the JCR:LA-*cp* rat. Preliminary data supports the hypothesis that *n*-3 PUFA may act directly on the enterocyte by down-regulating JNK activity, and improving impaired insulin signaling in the JCR:LA-*cp* rat.

Concurrent Session - 4

Lipids and Health : Brain function I - Depression

14.00 - 14.30

Invited contribution: EURON lecture

Suicide Death Among US Military: A Case Control Study and Reduction of Relapse Among Chronic Alcoholics: A Randomized Placebo Controlled Trial

J Hibbeln

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Background Deficiencies in neural active omega-3 essential fatty acids from typical US Military diets or from excessive alcohol consumption may increase risk of depression and suicide.

Study 1: Objective Determine if deficiencies of serum highly unsaturated omega-3 essential fatty acids (*n*-3 HUFA) are associated with increased risk of suicide death among US military suicide deaths (*n*= 800) and controls (*n*=800) matched for age, date of collection, sex, rank and year of incident (2002-2008). **Methods** Total fatty acid composition of *n*=1,600 serum fatty acids were quantified using high through put analyses. **Results** Risk of suicide death was 62% greater among men with low DHA status and 54% greater having seen wounded, dead or killed coalition personnel (Odds Ratio [OR] =1.62, 95% CI 1.12-2.34, *p*<0.01, comparing DHA % above [*n*=1,389] and below 1.75 [*n*=141] and OR = 1.54, 95% CI; 1.12-2.12, *p*< 0.007,

respectively, in adjusted analyses). **Conclusion** Most male US military personnel (95.0%) had serum DHA below 2.0%, a potential target for protection from a 62% higher risk of death by suicide.

Study 2: Objective Determine if 2 gm/ d of EPA+ DHA reduce aggression among recovering alcoholics, compared to placebo. **Methods** after 30 d inpatient treatment, severe alcoholics (n=97) were randomized to placebo or agent for 16 weeks as outpatients. Serum, cerebrospinal fluid (CSF), psychometric testing for depression and aggression were collected at baseline and after treatment. **Results** CSF fatty acid analyses indicated that n=7 active subjects were non-compliant. Comparing the (n=28) active compliant to the (n=46) placebo/non compliant completers, no treatment effects were found for CSF 5-HIAA and HVA, metabolites of serotonin and dopamine respectively, measures of aggression, hostility depression or impulsivity. Compliant subjects had fewer drinking days (mean 3.2 95%CI 2.5-4.9) vs. (mean 17.4 95%CI 12.6-19.8), a effect size of 0.84 (Hedges g, p<0.0005). **Conclusion**, restoration of n-3 HUFA status is a promising treatment for recovering alcoholics.

14.30 - 14.45

Fish consumption, not fatty acid status, is related to quality of life in a healthy population

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Background: Long-chain polyunsaturated fatty acids (LCPUFA) have frequently been implicated in depressed mood and poor mental well-being. By causing functional and psychosocial impairment, depressive symptoms have a considerable impact on quality of life in the general population. The direct relationship between LCPUFA status and quality of life, however, has scarcely been investigated.

Objective: In a population-based sample, this study examined the cross-sectional associations between plasma phospholipid LCPUFA concentrations and fish consumption on the one hand, and the mental and physical health components of quality of life on the other.

Procedure: Plasma phospholipid concentrations of arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), adrenic acid (AdRA, 22:4n-6), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) were determined in 233 participants of the longitudinal Maastricht Aging Study (MAAS). A validated food-frequency questionnaire was used to measure fish consumption. The mental and physical components of health-related quality of life were assessed by means of the Short Form-36 (SF-36) questionnaire.

Results: The physical and mental health components of quality of life were not associated with plasma phospholipid LCPUFA concentrations. Fish consumption was not related to the mental health component of quality of life. However, fish consumption did show a positive association with physical well-being, which remained significant after correction for LCPUFA concentrations, suggesting that the relationship between fish consumption and the physical health component of quality of life is independent of the LCPUFA content of fish.

Conclusion: Quality of life does not appear to be related to plasma phospholipid LCPUFA concentrations. The physical health component of quality of life, however, shows a positive association with fish consumption, which is independent of LCPUFA concentrations. These findings suggest that fish consumption may serve as a proxy for a healthy lifestyle or a favorable nutritional status, which is reflected in better quality of life.

14.45 - 15.00

Plasma and erythrocyte fatty acid patterns in patients with recurrent depression: a matched case-control study

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Background: The polyunsaturated fatty acid (PUFA) composition of (nerve) cell membranes may be involved in the pathophysiology of depression. Studies so far, focused mainly on omega-3 and omega-6 PUFAs.

Objective: In the present study, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and PUFAs of the

omega-3, -6 and -9 series in plasma and erythrocytes of patients with recurrent major depressive disorder (MDD-R) were compared with controls.

Procedure: We carried out a case-control study. The sample consisted of 137 patients with MDD-R and 65 matched non-depressed controls.

Results: In plasma and erythrocytes of patients with MDD-R the concentrations of SFAs and MUFAs, and additionally erythrocyte PUFAs, all with a chain length >20 carbon (C) atoms, were significantly lower than in the controls. In contrast, the concentrations of most of the shorter chain members ($\leq 18C$) of the SFAs and MUFAs were significantly higher in the patients. Estimated activities of several elongases in plasma of patients were significantly altered, whereas delta-9 desaturase activity for C14:0 and C18:0 was significantly higher.

Conclusion: The fatty acid status of patients with MDD-R not only differs with regard to omega-3 and omega-6 PUFAs, but also concerns other fatty acids. These alterations may be due to: differences in diet, changes in synthesizing enzyme activities, higher levels of chronic (oxidative) stress but may also result from adaptive strategies by providing protection against enhanced oxidative stress and production of free radicals.

15.00 - 15.15

Fatty acid intake and depression in adolescents

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Background: Randomised controlled trials have demonstrated that omega-3 polyunsaturated fatty acids (n-3 PUFA) are beneficial in reducing symptoms of depression. However there is limited evidence regarding the influence of dietary n-3 PUFA intake on mood in adolescents from population studies.

Objective: In the present investigation, we aimed to address the question of the relationship between dietary and n-3 PUFA intake on depression symptomatology in a large prospective pregnancy cohort.

Procedure: Adolescents enrolled in the Western Australian Pregnancy Cohort (Raine) Study completed a Food Frequency Questionnaire (FFQ) to assess dietary fatty acid intake, as well as other dietary factors at 14 years (y). A fasting blood sample was collected for biochemical analyses to validate dietary intake. Participants also completed the Beck Depression Inventory for Youth (BDI-Y) at 14y (N=1,407) and 17y (N=995). Cross-sectional and longitudinal analyses were conducted using Spearman's correlations and linear regression in models unadjusted and adjusted for other dietary and metabolic factors.

Results: The FFQ and BDI-Y were completed by 1,407 adolescents at 14 years (y). In addition, 995 participants completed the FFQ at 14y and the BDI-Y at 17y. FFQ and erythrocyte values were significantly correlated for EPA, DHA, n-3 PUFA and total n-3 PUFA. Significant inverse relationships were observed between both n-3 and n-6 fatty acid intake at 14y and BDI-Y scores at both 14y and 17y. These associations were considerably attenuated however, following adjustment for other dietary and metabolic confounders.

Conclusion: This study highlights the potential short- and long-term benefits of essential fatty acid intake particularly n-3 PUFA in reducing depressive symptoms in adolescents. Much of this relationship may be accounted for by other dietary factors, including overall intakes of energy, fat and cholesterol, and metabolic factors.

This project was funded by a nationally competitive Heart Foundation/ Beyond Blue Strategic Research Initiative grant.

15.15 - 15.30

Dietary omega-3 and omega-6 intake and the risk of clinical depression: results from the nurses' health study

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Objective: To examine the relationship between different types of n-3 and n-6 fatty acids, and the risk of clinical depression.

Procedure: We prospectively studied 73,449 women from the Nurses' Health Study who were 46 to 79 years of age and free from depressive symptoms at baseline (1996). Information on diet was obtained from validated food frequency questionnaires completed four times before baseline (1984, 1986, 1990, 1994). Clinical depression was defined as reporting both physician-diagnosed depression and regular antidepressant medication use. During 10 years of follow-up (1996-2006), 3,406 incident cases of clinical depression were documented. Cox-proportional hazard models, adjusted for age and other

possible risk factors, were used to calculate relative risks (RR) of depression.

Results: Intake of n-3 from seafood was not associated with risk of depression (multivariate RR for 0.3 g/day increment = 0.96 [0.87 to 1.06]; P=0.411). Intake of alpha-linolenic acid was not associated with risk of depression, except in regression models adjusted for n-6 fatty acids (multivariate RR for 0.5 g/day increment = 0.78 [0.69 to 0.89]; P=0.0001). Intake of linoleic acid was associated with a higher risk of depression (multivariate RR for 5 g/day increment = 1.29 [1.11 to 1.49]; P=0.001), whereas arachidonic acid intake was not associated with risk of depression (multivariate RR for 0.1 g/day increment = 1.03 [0.90 to 1.17]; P=0.677).

Conclusions: The results of this large longitudinal study do not support a protective effect of marine n-3 fatty acids or fish intake on risk of depression. However, this study provides support for the hypothesis that a higher alpha-linolenic acid intake and a lower intake linoleic acid might reduce the risk of clinical depression, but this relation deserves further investigation.

Concurrent Session - 5

Biochemistry and Lipids : Lipid, genes and (epi)genetics

14.00 - 14.30

Invited Contribution: GROW Lecture

Developmental programming in maternal diabetes and in experimental maternal overfeeding and underfeeding

FA van Assche

KULeuven, Leuven, Belgium

Epidemiologic and experimental studies have provided evidence that an abnormal intra-uterine environment increases the risk of developing diseases in the offspring. For the diabetic intra-uterine environment it was suggested that an epigenetic effect of hyperglycemia in pregnancy was acting in addition to genetic factors to induce diabetes in the next generations and could be integrated in the framework of developmental programming. Developmental programming is a process of an insult in utero and or in early postnatal life, inducing a permanent response in the fetus and the newborn, leading to enhanced susceptibility to later diseases.

In maternal diabetes in the human it is well known that increased trans placental transfer of glucose and other nutrients induces pancreatic B cell hyperplasia, increased insulin secretion and macrosomia in the fetus. The hyperactivity of the fetal B cells may result in reduced capacity of insulin secretion in later life. At the contrary in intra-uterine growth restriction hypoplasia of the B cells and hypoinsulinism is present in the fetus, inducing reduced insulin receptor activity in the fetus and the newborn. It is clear that long term effects are related to abnormal fetal growth.

Animal models may discover the specific effects of an abnormal intra-uterine environment, independent of inherited traits. First evidence of developmental programming came from animal research in 1979, when it was demonstrated that mild diabetes in the pregnant rat induced gestational diabetes in the second generation and, as a consequence, macrosomia, increased insulin secretion and B cell hyperplasia in the fetus. Transgenerational effects were confirmed in subsequent studies. Furthermore in the offspring a reduced capacity of insulin secretion was observed.

Intra-uterine growth restriction was induced by maternal starvation or by reduction in the utero placental circulation. The fetuses showed low insulin and B cell hypoplasia; the adult offspring had insulin resistance and vascular lesions. Diet induced obesity in the rat during pregnancy resulted in fetal hyperinsulinism and permanent overweight and also insulin resistance in the offspring.

Fetal hyperinsulinism is present in maternal diabetes as well as in maternal obesity. It is crucial to avoid fetal hyperinsulinism by optimal maternal care, not only for the prevention of metabolic and vascular complication, but also for the prevention of cancer. Indeed it is known that insulin has mitogenic effects.

The transgenerational effects in relation to developmental programming opens new fields for the exploration of adaptive and epigenetic mechanisms.

14.30 - 15.00

Invited contribution

The impact of eicosapentaenoic acid (EPA) on regulation of fatty acid metabolism, metabolic flexibility and gene expression in human skeletal muscle cells

A Rustan

Department of Pharmaceutical Biosciences, University of Oslo, Oslo, Norway

Our recent studies with differentiated human skeletal muscle cells (myotubes) suggests a positive role for eicosapentaenoic acid (EPA) compared to the other fatty acids (FAs) in improving overall energy metabolism and metabolic switching in skeletal muscle, which may contribute to the beneficial effects of dietary intake of very long-chain n-3 fatty acids.

In a study using myotubes established from lean donors we showed that EPA increased suppressibility, the ability of acute glucose to suppress FA oxidation. Substrate-regulated flexibility, the ability to increase FA oxidation when changing from a high glucose, low fatty acid condition (“fed”) to a high fatty acid, low glucose (“fasted”) condition, was increased by EPA. Adaptability, the capacity to increase FA oxidation with increasing FA availability, was enhanced after pretreatment with EPA, linoleic acid (LA) and palmitic acid (PA). EPA *per se* accumulated less in the cells, however, EPA, LA and oleic acid (OA) treatment increased the number of lipid droplets (LDs) in myotubes. LD volume and intensity, as well as mitochondrial mass were independent of FA pretreatment.

Microarray and PCR analysis showed that EPA regulated more genes than the other FAs and specific pathways involved in carbohydrate metabolism were induced only by EPA. The present study suggest a favorable effect of EPA on skeletal muscle metabolic switching and glucose utilization. Additional experiments with ALA and DHA indicated that the metabolic effects could be due to a general quality of n-3 FAs. Based on finding from this study we also suggest the use of three parameters called suppressibility, adaptability and substrate-regulated flexibility in functional studies of fuel selection and energy metabolism in cell cultures.

In another study we wanted to identify the potential effects of EPA and a synthetic fatty acid derivative (TTA) on energy metabolism, insulin resistance and gene expression (PCR). Here we compared the effects of EPA, TTA, and OA in myotubes established from obese individuals with type 2 diabetes and obese healthy control subjects. Our results suggest that 1) mitochondrial dysfunction in diabetic myotubes was caused by disturbances downstream of fatty acid β -oxidation; 2) EPA promoted accumulation of triacylglycerol (TAG), enhanced β -oxidation, and increased glucose oxidation; and 3) TTA improved complete palmitic acid oxidation in diabetic myotubes, opposed increased lipid accumulation, and increased glucose oxidation.

15.00 - 15.15

Impact of different dietary fat quantity and quality on skeletal muscle fatty acid handling in subjects with the metabolic syndrome

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Objectives: Insulin resistance is characterized by disturbances in lipid metabolism and increased fat storage in ‘non-adipose tissues’ like skeletal muscle (SM). The aim of this study was to investigate whether SM gene expression and the fatty acid (FA) profile of the SM is affected by diets with different fat quantity and quality in subjects with the metabolic syndrome (MetS, NCEP criteria).

Procedure: 84 subjects (age 57.3±0.9y, BMI 30.9±0.4kg/m², 42men/42women) were randomly assigned to one of four isoenergetic diets: high-SFA (HSFA); high-MUFA (HMUFA) and two low-fat, high-complex carbohydrate (LFHCC) diets, supplemented with 1.24g/day of long chain n-3 PUFA (LCn-3) or placebo for 12 weeks each. Insulin sensitivity (SI) was determined by a frequently sampled insulin modified intravenous glucose tolerance test. SM biopsies were taken to determine mRNA expression of genes involved in lipid metabolism. In a subgroup (n=25) muscle TAG, DAG, free FA, and phospholipid content, their fractional synthetic rate (FSR) as well as degree of saturation were determined.

Results: Genes involved in lipogenesis (SREBP1c, SREBP2, ChREBP and ACC2) were downregulated after 12-weeks HMUFA

(mean fold change (FC) of -1.3) and after LFHCC LCn-3 (mean FC -1.7) in insulin resistant (IR) subjects (below the median of SI) whereas insulin sensitive (IS) subjects showed the opposite effect (mean FC +1,6 at both diets). HMUFA diet decreased the DAG content (paired t-test $p=0,027$) and tended to decrease the FSR in TAG ($p=0,055$) and DAG ($p=0,066$). LFHCC LCn-3 diet reduced the muscle TAG content ($p=0,032$) and tended to increase percentage saturation of DAG ($p=0,064$).

Conclusions: Both HMUFA and LFHCC LCn-3 resulted in a downregulation of lipogenic genes in IR subjects with the MetS. In a subgroup HMUFA and LFHCC LCn-3 reduced DAG or TAG content, respectively, suggesting that these diets may reduce muscle fat accumulation by affecting the balance between fat storage and oxidation.

15.15 - 15.30

Omega 3 long chain fatty acid synthesis in rats is regulated more by substrate levels than gene expression

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³Early Origins of Adult Health Research Group, Sansom Institute, Adelaide, Australia

Background: The conversion of omega 6 (n-6) linoleic acid (LA) and n-3 alpha-linolenic acid (ALA) to long chain polyunsaturated fatty acids (LCPUFA) involves desaturation and elongation. Although there is evidence that genes for these steps can be regulated by extremes of dietary PUFA, the degree to which there is meaningful regulation of LCPUFA levels in tissues by diet as a result of changes in expression of desaturase and elongase genes is unclear.

Objective: To determine whether, within the range of PUFA in normal human diets, there is any meaningful regulation of desaturases, elongases and transcription factors known to be involved in the regulation of hepatic lipid metabolism.

Procedure: Weaned male Hooded-Wistar rats were fed experimental diets which were composed of ALA level ranging from 0.2-2.9%en while keeping LA constant at 1%. A high PUFA diet (5.5%en as PUFA) and a low PUFA diet (0.4%en as PUFA) were used as two reference diets. Rats were fed the diets for 3 weeks. Plasma and livers were collected for fatty acid analysis. Hepatic gene expression level of delta 6 desaturase, elongase and relative transcription factors were determined by quantitative real-time PCR.

Results: While high n-3 PUFA diets consistently produced higher levels of EPA, DPA and DHA in rat tissues than low n-3 PUFA diets, expression of the delta 6 desaturase gene was increased only in animals fed the very low PUFA reference diet; There was no obvious relationship between the mRNA expression of desaturases, elongases or transcription factors and the levels of EPA, DPA or DHA in rat liver tissue as a result of feeding increasing ALA levels in this study.

Conclusion: Over the range of polyunsaturates encountered in human diets, it is probable that n-3 LCPUFA levels are regulated by substrate supply only, and not alteration in LCPUFA synthetic gene expression.

Concurrent Session - 6

Lipids and Nutrition : Maternal and infant nutrition I - Body composition

14.00 - 14.15

Effects of exposure to high fat in utero on phospholipid profiles in a mouse model of the metabolic syndrome

CR Smith, V Ledger, C Torrens, KD Bruce, FR Cagampang, CD Byrne, GF Clough, AD Postle
University of Southampton, Southampton, United Kingdom

Background: In utero exposure to high fat (HF) primes for the development of metabolic syndrome which is exacerbated by exposure to a HF diet in adult life (Bruce et al *Hepatology* 50;1796;2009).

Objective: To determine whether exposure to HF in utero and during weaning alters phospholipid profiles in liver and aorta and how age and dietary mismatch influence this in a murine model of the metabolic syndrome.

Procedure: Female C57 Black mice were fed a HF (45% kcal fat) or chow (C) diet (20% kcal fat) before and during pregnancy and lactation. Post-weaning the offspring were maintained on the same diet as their dams (C/C, HF/HF) or transferred to the opposite diet (C/HF, HF/C). Lipids were extracted from the aorta and liver of the male offspring at 15

and 30 weeks. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) molecular species were analysed by mass spectrometry.

Results: Age and diet both significantly altered liver PC composition. For example, PC16:0/18:2 was 28.0% of liver PC in the C/C group at 15 weeks but 23.7% at 30 weeks. Corresponding values for the HF/HF groups were 13.4% and 11.8%, and for the HF/C groups were 21.8% and 15.1%. Age-related changes to aorta PC were similar for all dietary groups. Diet had no effect on aortic PC or liver PE.

Conclusion: Exposure to HF in utero results in changes to phospholipid profiles in liver and aorta that were modified during the life course. HF exposure in utero primed subsequent changes to liver PC composition; HF mice weaned to chow diet exhibited a similar composition to control mice at 15 weeks, but were unable to maintain this composition at 30 weeks. The absence of corresponding diet-related changes to the aortic PC compositions illustrates the robust nature of homeostatic mechanisms controlling lipid profiles.

14.15 - 14.30

Late gestation undernutrition and post-natal diet program hepatic lipid composition and fatty acid profiles in adult sheep

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²Copenhagen University, Copenhagen, Denmark

Objective: To assess whether late-gestational undernutrition (LG-UN) programs hepatic lipid content and composition in the adult animals and whether the postnatal diet affects such foetal programming effects.

Methods: Twenty twin-pregnant ewes were fed either a NORM (- requirements) or LOW (50% of requirements) diet the last 6 wks of gestation (term=147d). From 3d to 6mo post-partum (around puberty), twin lambs were assigned to each their feeding: CONV (hay) or HCHF (High-Fat-High-Carbohydrate) supplemented with milk replacer from 0-8 wks. Lambs were slaughtered at 6mo and at 2yrs (young adulthood). Liver triacylglycerol (TAG), ceramide and phospholipids were analyzed.

Results: After 6 mo of differential postnatal feeding treatment, HCHF lambs became obese (BMI-28-32), but LG-UN did not impact fat deposition. HCHF feeding resulted in massive accumulation of TAG ($p < 0.0001$) and ceramide ($p < 0.01$) in the liver in 6 mo lambs, while the prenatal nutrition had no effect. At 2 yrs of age, TAG concentration remained higher ($p < 0.05$) in sheep exposed to LG-UN, and at this age, both pre- and postnatal nutrition caused striking differences in fatty acid composition in liver lipids. LG-UN decreased C16:1 n-7 and the ratio of n-6:n-3 PUFA in phospholipids and, noteworthy, altered concentrations of fatty acids derived from rumen microbiotic activity in TAG and PL.

Conclusion: LG-UN programmed for increased baseline levels of liver TAG in adult sheep as well as fatty acid composition in depot and structural lipids. Our findings interestingly suggest that gut microbiotic activity may be subjected to prenatal programming, which persists into adulthood.

Postnatal HCHF feeding caused severe liver steatosis in lambs, manifested both as increased TAG and ceramide. Long-term consequences of HCHF feeding involved repositioning of lipid deposition towards less renal and more abdominal fat in adult sheep.

14.30 - 14.45

Maternal omega-3 supplementation alters fat distribution in the offspring

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²University of South Australia, Adelaide, Australia

Background: Fat cell formation (adipogenesis) and lipid accumulation (lipogenesis) are highly sensitive to the nutritional environment during the period of fat cell development. Omega-3 long chain polyunsaturated fatty acids (LCPUFA) inhibit adipogenesis and lipogenesis in adult rats, however it is not known whether supplementing the maternal diet with omega-3 LCPUFA results in reduced fat deposition in the offspring.

Objective: This study aimed to investigate the hypothesis that maternal omega-3 supplementation during pregnancy and lactation would reduce body fat mass in the adult offspring.

Procedure: Female Wistar rats were fed either a standard chow (Control, n=8) or chow supplemented with 25mg/kg/day of the omega-3 LCPUFA Docosahexaenoic acid (DHA) during pregnancy and lactation (Omega-3, n=8). Tissues were collected from pups at 6wk of age and weights of visceral and subcutaneous fat depots recorded.

Results: Omega-3 fatty acid status (as a % of total fatty acids) was higher in offspring of omega-3 supplemented dams at 6 wks of age ($6.7 \pm 0.2\%$ vs $5.6 \pm 0.2\%$, $P < 0.001$). Birth weight and 6wk body weight were not different between groups. The relative mass of subcutaneous fat was higher in male (0.019 ± 0.001 g/g vs 0.014 ± 0.002 g/g, $P < 0.04$) and female (0.018 ± 0.001 g/g vs 0.013 ± 0.002 g/g, $P < 0.04$) omega-3 offspring compared to controls. Whilst there was no difference in the relative mass of visceral fat, the ratio of visceral to subcutaneous fat mass was lower in omega-3 offspring compared to controls in both males and females.

Conclusion: These data suggest that while maternal omega-3 supplementation does not result in an overall reduction in body fat mass in the offspring, it may be associated with a change in body fat distribution which could result in a more favourable metabolic phenotype.

14.45 - 15.00

Maternal n-6 and n-3 LC-PUFA status is related to body composition and glycaemic control in the offspring: the ABCD cohort

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Objective: To explore the association of maternal γ -linolenic acid (GLA, 18:3n-6), di-homo- γ -linolenic acid (DGLA 20:3n-6), AA (20:4n-6), EPA (20:5n-3) and DHA (22:6n-3) concentrations with offspring body composition, glucose levels and insulin sensitivity at age 5.

Procedure: Preliminary analysis of 1010 mothers and children of the ABCD cohort, who completed the 5th-year follow-up (data collection ongoing). Maternal fatty acid concentrations were determined in plasma phospholipids by gas-liquid chromatography around the 14th week of pregnancy. At follow-up, children's BMI (weight/height²), fat mass and fat free mass (bioelectrical impedance analysis) were measured. Capillary fasting glucose and C-peptide (subsample n=633) were used to calculate insulin sensitivity (homeostatic model assessment index). Linear regression analyses included standardized LCPUFA concentrations (z-scores, to allow comparison) and were controlled for pregnancy duration at maternal blood sampling and the child's age and gender.

Results: BMI (mean \pm SD) at follow-up was 15.33 ± 1.25 kg/m², fat mass 4.73 ± 1.46 kg and fat free mass 15.46 ± 2.19 kg. High BMI followed high concentrations of DGLA and AA but low concentrations of DHA (β_{DGLA} 0.089, β_{AA} 0.079, β_{DHA} -0.097; $P < 0.05$). Results were similar for fat mass (β_{DGLA} 0.085, β_{AA} 0.139, β_{DHA} -0.093), but the association was non-significant for DGLA ($P = 0.06$). No significant associations were observed for fat free mass. Mean glucose, C-peptide and insulin sensitivity values were 4.55 ± 1.67 mmol/L, 0.39 ± 0.17 nmol/L and 0.84 ± 0.37 , respectively. Glucose values were positively associated with AA but negatively with EPA (β_{AA} 0.043, β_{EPA} -0.048; $P < 0.02$). Insulin sensitivity (logarithmic transformation) was associated with AA, EPA, as well as DHA (β_{AA} 0.020, β_{EPA} -0.024, β_{DHA} -0.022; $P < 0.05$).

Conclusion: This preliminary analysis of the ABCD cohort suggests that maternal n-6 and n-3 LCPUFAs are related to the offspring's body composition and glycaemic control at age 5. More detailed analysis of a larger group of children (expected N 2500), including relevant covariables, are planned.

15.00 - 15.15

Determinants of infant n-3 LC-PUFA status and its associations with blood pressure and lipid profile

LB Schram, AD Andersen, KF Michaelsen, L Lauritzen

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Objective: Infant n-3 long-chain polyunsaturated fatty acid (LCPUFA)-status in erythrocytes (RBC) is influenced by intake of n-3LCPUFA from complementary food, breast milk and intrauterine nourishment. However, few studies have investigated the degree of influence conferred by determinants of n-3LCPUFA-status or effects of n-3LCPUFA-status on blood pressure (BP) and lipid profile in a cross-sectional design.

Procedure: We performed a cross-sectional study with 466 healthy Danish 9 month-old infants to investigate dietary determinants of n-3LCPUFA-status and its associations with BP and lipid profile. We assessed if they were still breastfed (BF), if fish had been introduced, RBC fatty acid composition, BP and plasma concentrations of LDL-C, HDL-C and triacylglycerol.

Results: The RBC content of n-3LCPUFA was 9.6 ± 2.3 FA% (mean \pm SD) (range: 3.3-17.3 FA%). 23% of the variation in n-

3LCPUFA-status was explained by infant age ($p=0.003$), maternal smoking during pregnancy ($p=0.006$), BF ($p<0.001$) and fish introduction ($p=0.033$). Infant age and maternal smoking were positively and negatively associated with n-3LCPUFA status, respectively. BF and fish introduction conferred a 19% and 16% higher status, respectively. They did however tend to interact ($p=0.073$) and a significant association between fish introduction and n-3LCPUFA-status was found only among non-BF infants ($p=0.003$). Inclusion of maternal fish intake ($p=0.001$) in a sub-analysis (28% of the infants) further increased the explained variation to 36%. n-3LCPUFA-status was not associated with BP or lipid profile.

Conclusion: Breastfeeding and introduction of fish are equally important determinants of n-3LCPUFA-status in 9 month-old healthy Danish infants. Associations between n-3LCPUFA-status and BP or lipid profile were not found.

15.15 - 15.30

Fish oil-supplementation has little effect on growth and body composition in late infancy

L Lauritzen, AD Andersen, KF Michaelsen
University of Copenhagen, Frederiksberg C, Denmark

Objective: Dietary marine n-3 long-chain polyunsaturated fatty acids (LCPUFA) have in animals been shown to affect development of adipose tissues. In humans, n-3 LCPUFA-enriched infant formulas or fish oil-supplementation of lactating mothers have generally been shown not to influence infant growth. However, none of the studies have aimed at investigating the impact on adipose tissue growth.

Procedure: We performed a double-blinded intervention study in which 154 healthy Danish infants were randomly allocated to supplementation with (5ml/d) fish oil (FO) or sunflower oil (SO) from 9 to 18 months of age. The primary endpoints were z-scores of anthropometric assessments of body size and composition. The relative content of fatty acids (FA%) in erythrocytes (RBC) was determined as a biomarker of compliance.

Results: 133 infants completed the study. The estimated median oil intake was 3.8 (25-75 percentile=3.2-4.2) g/d and did not differ between groups. Infants in the FO-group had larger increases in RBC n-3 PUFA than the SO-group (12.2 ± 0.7 (mean \pm SE) vs. 2.0 ± 0.4 FA%, $p<0.001$) and larger decreases in n-6 PUFA (-8.9 ± 0.7 vs. -0.9 ± 0.6 FA%, $p<0.001$). Adjusted ANCOVA-models showed no effects of n-3 LCPUFA-supplementation on z-scores for height, weight, BMI or skin fold (SF) thicknesses. However, at 18 months infants in the FO-group had a lower SF-index (triceps/sub-scapular) than the SO-group ($p=0.02$). Furthermore, the consumed amount of oil, irrespective of type, was found to affect both triceps ($p=0.02$) and sub-scapular ($p=0.04$) SF thicknesses.

Conclusion: This study shows no effect of n-3 LCPUFA intake on adipose tissue mass, but indicates an effect on fat distribution with more trunk subcutaneous fat. The adipogenic effect of oil intake already in infancy could be of public health concern.

Plenary: Biochemistry of Lipids 1: Lipids, genes and metabolomics

16.00 - 16.45

New insights in the role of PPARs

W Wahli
Université de Lausanne, Lausanne, Switzerland

Peroxisome proliferator-activated receptors form a small family of three nuclear receptors (PPAR α , PPAR β , also called PPAR δ and PPAR γ), which act as lipid sensors to modulate gene expression. Initially, roles were identified for PPAR α in fatty acid catabolism. However, PPAR α is also involved in sexual dimorphism especially by down-regulating gene expression. Using the steroid hydroxylase Cyp7b1 gene as a model, the molecular mechanism of this PPAR α -dependent repression was elucidated. Physiologically, this repression confers protection against estrogen-induced intrahepatic cholestasis.

The activation of PPAR β induces a protection against diabetes and obesity. Ablation of *PparB* in the pancreas leads to hyperinsulinemia due to an increase of pancreatic β -cell mass and insulin secretion. The results obtained provide evidence for a repressive role for PPAR β in β -cell mass and insulin exocytosis and shed new light on its metabolic action.

Healing of cutaneous wounds proceeds via a well-tuned pattern of events, which are regulated spatio-temporally by a variety of growth factors and cytokines. Activation of PPAR β promotes keratinocyte survival, directional sensing and migration. In addition, IL-1 produced by the keratinocytes activates PPAR β expression in the underlying fibroblasts, which

in turn inhibits the mitotic activity of keratinocytes via inhibition of the IL-1 signaling pathway. Furthermore, PPAR β is involved in skin tumor development after UV irradiation. In conclusion, PPARs are implicated in important processes controlling cellular fate as well as in major metabolic and inflammatory regulations with obvious medical implications, especially related to metabolic diseases and tissue repair.

16.45 - 17.30

NUTRIM lecture

Metabolomics for understanding of metabolic derangements in obesity and Type 2 diabetes

C Newgard

*Sarah W. Stedman Nutrition and Metabolism Center & Department of Pharmacology & Cancer Biology
Duke University Medical Center, Durham, United States of America*

We seek to apply comprehensive metabolic analysis tools (sometimes called “metabolomics”) for understanding of mechanisms underlying chronic human diseases such as diabetes, obesity, and cardiovascular disease. Current approaches include analysis of metabolic flux by ^{13}C NMR-based mass isotopomer analysis (in collaboration with Drs. Shawn Burgess and A. Dean Sherry and associates, Dallas, TX) and metabolic profiling of important groups of metabolic intermediates by both “targeted” and “unbiased” mass spectrometry (in collaboration with Drs. James Bain, Robert Stevens, Olga Ilkayeva, Brett Wenner, Michael Muehlbauer, Mark Butler, and David Millington at Duke). These tools have been used to investigate the metabolic mechanisms underlying development of peripheral insulin resistance in animals and humans. Studies led by Drs. Debbie Muoio and Tim Koves at the Stedman Center have demonstrated a “disconnect” between mitochondrial b-oxidation and TCA cycle activity that leads to accumulation of incompletely oxidized lipids in the mitochondria and impairment of insulin actions. We have also recently identified perturbations of branched chain amino acid (BCAA) catabolism in multiple cohorts of insulin resistant humans compared to normally insulin sensitive controls and have translated these findings to rodent models to demonstrate a contribution of BCAA to development of insulin resistance that is independent of body weight. Finally, in collaboration with Dr. Alan Attie at the University of Wisconsin, we have integrated transcriptomic and metabolomic analysis to identify new pathways that control hepatic gluconeogenesis and PEPCK expression. These examples will serve to illustrate the potential of comprehensive metabolic profiling methods for providing insights into diabetes and obesity mechanisms.

Posters - Sunday May 30

Lipids and Health

P001

The effects of arachidonic acid and docosahexaenoic acid on proliferation of and osteoclast formation from raw 264.7 murine monocytes

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Background: Several human and animal studies have shown that n-3 PUFAs may have a positive effect on bone. Recent findings indicate that docosahexaenoic acid (DHA) may inhibit osteoclast-mediated bone resorption while the effect of arachidonic acid (AA) is unknown. In addition, the exact cellular mechanisms and the extent of these effects are unclear.

Objectives: To compare the effects of AA and DHA on proliferation of RAW 264.7 monocytes and osteoclastogenesis from these cells.

Procedures: For proliferation studies, RAW 264.7 cells were exposed to ethanol (vehicle control), AA and DHA at concentrations of 5µg/ml to 20µg/ml. After 72 hours exposure, cell proliferation was determined using a standardised crystal violet staining procedure and results graphed as the percentage of control. A triple stain fluorescent procedure was performed to investigate possible mechanisms by which AA or DHA affected proliferation. Osteoclasts were generated by exposing the RAW 264.7 cells to RANKL for 5 days in the absence or presence of AA or DHA at 5µg/ml to 20µg/ml. Tartrate-resistant acid phosphatase (TRAP) activity in the conditioned media and presence of osteoclasts were determined by means of a standardised colorimetric method and TRAP staining respectively.

Results: Cell proliferation was inhibited by AA at concentrations above 10µg/ml with DHA having a significant inhibitory effect across all concentrations. The triple stain procedure revealed an increase in acid content of cells exposed to 20µg/ml DHA compared to controls. AA had a slight inhibitory effect on osteoclastogenesis in contrast to DHA that dose-dependently reduced osteoclast formation.

Conclusion: Both AA and DHA affected proliferation; the triple stain suggested an increased number of lysosomes or the formation of autophagic vacuoles in the presence of DHA, an indication of a form of cell death. Both AA and DHA inhibited formation of osteoclasts, with DHA having a more pronounced effect.

P002

An n-3 PUFA diet lowers liver triglycerides but increases weight gain and triggers some pro-inflammatory responses

SR Shaikh, B Rockett, K Carraway, K Morrison
Brody School of Medicine, East Carolina University,
Greenville, United States of America

Background: N-3 PUFAs have great potential for the treatment of metabolic and inflammatory diseases. A major limitation of enhancing the translation of these fatty acids into the clinic has been an incomplete understanding of their targets and mechanisms.

Objective: In the present study, we addressed if a high fat (HF) n-3 PUFA diet made of fish/flaxseed oil could have a therapeutic niche for improving the metabolic and inflammatory profile compared to other diets.

Procedure: C57BL/6 mice were fed the HF n-3 PUFA diet for 14 weeks and compared to a low fat normal diet (ND), and HF diets enriched in saturated (SFA), hydrogenated (HFA), and monounsaturated (MUFA) fatty acids.

Results: The HF n-3 PUFA diet lowered liver triglycerides relative to the other diets, consistent with the known effects of n-3 PUFAs. The n-3 PUFA diet also promoted the greatest weight gain as a function of time, relative to the other diets, due to accumulation of white and brown adipose tissue. The adipose tissue of mice fed the HF n-3 PUFA diet accumulated macrophages, albeit unknown if they are M1 pro- or M2 anti-inflammatory. The mice displayed splenomegaly and splenic B cells from the HF n-3 PUFA fed mice upon activation with LPS *ex vivo* secreted higher levels of IL-6 and IFNγ relative to controls.

Conclusions: Overall, our data suggest that high doses of n-3 PUFAs have potential clinical utility for lowering triglycerides but are also associated with some pro-inflammatory side effects. Additional studies, which are in progress, are required to address if long and short term consumption of HF n-3 PUFAs, compared to other diets, improve insulin sensitivity and the systemic inflammatory profile at the genetic level.

P003**Fucoxanthin increases docosahexaenoic acid through the up-regulation of delta6-desaturase in the liver of mice**

T Tsukui, M Hosokawa, K Miyashita
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Background: Fucoxanthin (Fx) is a major marine carotenoid which is found in edible brown seaweeds such as *Undaria pinnatifida* and *Sargassum fulvellum*. It has a unique structure, with allenic bond and 5,6-monoepoxide, which is different from common carotenoids such as β -carotene and lycopene. In our recent study, we have observed enhancement of docosahexaenoic acid (DHA) levels in the liver of diabetic/obese KK-A^y mice fed Fx diet.

Objective: We investigated the effect of dietary xanthophylls (Fx and astaxanthin) and phytol on DHA levels in the liver of normal C57BL/6J mice. Furthermore, the expression level of delta6-desaturase (D6D), which is the rate-limiting enzyme in biosynthesis of n-3 and n-6 polyunsaturated fatty acids (PUFA) such as DHA and arachidonic acid was examined.

Procedure: The mice (4 weeks old) were fed the experimental diets containing xanthophylls or phytol for 4 weeks. AIN-93G containing 7.0% soybean oil was used as the basal diet in this study. Fx (0.2%), astaxanthin (0.05 and 0.2%) and phytol (0.2 and 0.5%) were added in the basal diet, respectively. We analyzed fatty acid composition of total lipids in the liver of Fx-fed mice by GC. D6D expression level in the liver was analyzed by Western blotting.

Results: After 4 weeks feeding, the body weights and liver lipid contents of all the groups of mice were not significantly different. The mice fed Fx significantly increased the amount of DHA in the liver. In addition, the expression level of D6D in the liver was increased compared to control. On contrary, the amount of DHA in the liver of mice fed astaxanthin and phytol was not significantly different compared to control and D6D expression level was significantly decreased.

Conclusion: Dietary Fx enhances hepatic DHA level through the up-regulation of D6D in the liver of mice.

P004**Salmon consumption by pregnant women reduces ex vivo umbilical cord endothelial cell activation**

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Background: Inhibition of cell adhesion molecule (CAM) expression on endothelial cells (EC) is likely to ameliorate inflammatory and cardiovascular disease (CVD). In vitro exposure of EC to long chain (LC) n-3 polyunsaturated fatty acids (PUFA) has been shown to reduce CAM expression and leukocytic cell adhesion. Animal studies have shown similar effects.

Objective: The aim of the current study was to assess whether salmon (rich in LC n-3 PUFA) consumption twice a week during pregnancy affects umbilical vein endothelial cell (HUVEC) activation.

Procedure: HUVEC were isolated and cultured from a subset of participants (n=10) of the UK salmon in pregnancy study (SIPS). Cell surface expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) was assessed by flow cytometry in the presence or absence of 24h LPS stimulation. In addition mediator secretion was measured by multiplex assay.

Results: The level of LPS induced ICAM-1 (p=0.006) and VCAM-1 (p=0.034) expression was significantly lower in the salmon diet group as compared to controls.

Moreover, IL-6 secretion increased significantly upon LPS stimulation in the control group (p=0.003) but not in the salmon group, whereas TNF- α release was not affected by the salmon intervention. Furthermore, growth factor G-CSF, known to suppress ICAM-1 expression, was significantly enhanced by the salmon diet (p=0.009).

Conclusion: Increased dietary salmon intake was found to dampen EC activation, implicating a role for LC n-3 PUFA in suppression of inflammation and prevention of CVD in humans.

P005**90-Day feeding and genotoxicity studies on a refined arachidonic acid-rich oil (RAO)**

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Background: Long-chain polyunsaturated fatty acids, including arachidonic acid, are associated with normal growth and maturation of numerous organ systems and are often added to infant formula.

Objective: To evaluate safety of a refined arachidonic acid-rich oil (RAO).

Procedure: Safety was evaluated for reverse mutation in *Salmonella typhimurium* and *Escherichia coli*, chromosome aberration in CHO cells and gene mutation TK locus mouse lymphoma cells, and in a 90-day Wistar rat feeding study with in utero exposure. The in utero phase involved dietary exposure to 0.5%, 1.5% and 5% RAO and 2 controls diets-a standard low-fat diet and a high-fat

diet with 5% corn oil. This exposure covered 4 wks prior to mating, through mating, gestation and lactation until off spring (F1) weaning. A subsequent 90-day feeding study in F1 rats evaluated the same test and control diets.

Results: Genotoxicity assay results were all negative. Statistically significant effects were seen for selected histopathology, clinical chemistry and organ weight endpoints; however, other than increased absolute and relative monocytes seen in both sexes of high-dose rats, the observations were not attributed to treatment for one or more reasons. Based on these findings, no adverse treatment-related effects for RAO were seen at up to 5% in the diet, equivalent to an overall average RAO intake of 3,170 mg/kg bw/d.

Conclusion: These and similar findings for other refined ARA-rich oils establish a strong body of evidence for the safety of this RAO.

P006

A novel application for DNA adducts in the investigation of neurodegenerative disease with phospholipid emulsion

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Objective: The lipid soluble nature of toxins has led us to address the complexity of neurological disease oriented to cell membrane architecture. Identification of nuclear DNA adducts as chemicals, pesticides, and metals at Acumen Laboratory in Devon, England and red cell lipid analysis at Johns Hopkins, Peroxisomal Diseases Laboratory were obtained on subjects with Multiple Sclerosis, Autism, Post Stroke, Alzheimer's, Motor Neuron, and Parkinson's Disease. The information from the DNA adducts has added to our previous findings in linking toxic environmental exposure and characteristic accumulation of very long chain fatty acids (VLCFAs) in the form of lipid rafts or ceramides, and the development of cell membrane derangement and dysfunction. Membrane phospholipid abnormalities with elevation of VLCFAs may be indicative of exposure to fat-soluble neurotoxins resulting in suppressed peroxisomal beta oxidation of VLCFAs.

Procedure: Our treatment protocol includes oral and intravenous infusion of phenylbutyrate, phosphatidylcholine, glutathione and methylation factors (folinic, methylcobalamin, tetrahydrobiopterin, riboflavin) to clear bioaccumulation of toxins and stabilization of cellular structures.

Results: We have previously shown that the use of oral and IV lipids facilitated stabilization of phospholipids in cellular membranes in 300 subjects. This study shows for the first time the clearance of toxins from the DNA of patients with neurological disease. This clearance corresponds to marked clinical improvement in our subjects within the first few weeks of lipid therapy.

Conclusion: We have documented significant neurological improvement in our subjects following six months of an oral and intravenous lipid regime. The administration of our lipid protocol may offer a new therapeutic strategy for neurological disorders.

P007

Enhanced EPA/DHA incorporation in erythrocyte membranes in response to long-term omega-3 fatty acid supplementation from triacylglycerides vs. ethyl esters

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Background: An increased consumption of omega-3 fatty acids (n-3 FAs) is associated with a number of health benefits and is recommended by various scientific associations. Currently, there is a debate about whether different chemical forms of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are absorbed quantitatively identically.

Objective: The objective of the study was to investigate the response of the EPA and DHA content in erythrocyte membranes (omega-3 index) to supplementation with two different n-3 FA formulations in humans.

Procedure: In the double-blind, randomised placebo-controlled trial, 150 volunteers were allocated to one of three groups: 1) fish oil concentrate with EPA+DHA (1.00g + 0.67g) given as triacylglycerides (n-3 FA TAG group); or 2) corn oil (placebo-group); or 3) fish oil concentrate with EPA+DHA (1.00g + 0.67g) given as ethyl-esters (n-3 FA EE group). Proband daily consumed 4 gelatine-coated soft capsules over a period of 6 months, resulting in a total intake of 2016mg n-3 FAs (1008mg EPA, 672mg DHA). The omega-3 index was measured at baseline (t₀), after 3 months (t₃), and at the end of the intervention period (t₆).

Results: The omega-3 index increased significantly in both n-3 FA-treated groups from baseline to t₃ and t₆ (p<0.001), whereas the boost was significantly higher in the n-3 FA TAG group than in the n-3 FA EE group (t₃: 186% vs. 161% [p < 0.001]; t₆: 197% vs. 171% [p < 0.01]). No changes were observed in the placebo group.

Conclusion: A 6 month lasting supplementation of EPA+DHA as TAGs led to a faster and higher increase in

omega-3 index compared to identical doses of EPA+DHA as EEs. These obvious differences imply a higher bioavailability of n-3 FA TAGs for incorporation in human cells compared to n-3 FA EEs and should be considered in the n-3 FA intake recommendations.

P008

Evidence of beneficial effects of enteral docosahexaenoic acid on cytokine production and clinical outcomes in surgical neonates

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Background: Surgical patients are on risk to build-up uncontrolled inflammatory response that predispose them to sepsis and multiorgan dysfunction. Since neonates have an immature immune system, they are in higher risk to develop adverse clinical outcomes (ACO).

Objective: To evaluate the effect of the acute, enteral administration of docosahexaenoic acid (DHA) to surgical neonates, on the cytokine production and ACO.

Procedure: In a randomized double-blind design, 20 neonates received 75 mg/kg/day of DHA (G-DHA) (Neuromins for Kids, Martek Inc®), or sunflower oil (G-SO) by enteral feeding, two days before and throughout six days after cardiovascular surgery. Percentage of leucocytes producing intracytoplasmic cytokines IL-1beta, IL-6, TNF-alpha, IL-10 and IL-1ra in whole blood was determined by flow-cytometry at baseline, 24h and day seven after surgery. ACO such as: presence of severe sepsis, respiratory and cardiovascular dysfunction, and length of hospitalization at neonatal intensive care unit were assessed.

Results: Ten neonates received DHA and ten SO. G-DHA showed lower percentage of TNF-alpha+ cells (2.51% ± 1.45% vs. 8.36% ± 1.38%; P<0.05) and IL-10+ (0.51% ± 1.45% vs. 3.35% ± 1.14%; P<0.05) than G-SO at 7-d after surgery. Repeated measures ANOVA, adjusting by confounders, demonstrated that the percentage of leucocytes producing cytokine was lower in G-DHA than in G-SO: IL-1beta+ (-1.1%), IL-6+ (-9.77%), IL-1ra+ (-3.67%) and IL-10+ (-9.34%), (P<0.05). Likewise, G-DHA presented fewer ACO than G-SO: two vs. seven organ dysfunctions, respectively P=0.035; by considering together severe sepsis and organ dysfunctions, G-DHA presented 3 events compared to 11 in the G-SO (P=0.012). Length of hospitalization was borderline shorter in G-DHA than in G-SO (9.3 ± 1.9 d vs. 16.7 ± 4.4 d, P=0.074).

Conclusion: Preliminary results suggest that enteral DHA administration reduces the inflammatory response and improve clinical outcomes in neonates suffering surgical procedures.

P009

Algal-docosahexaenoic acid ethyl ester reduces triglyceride and cholesterol levels and fatty liver in wistar rats fed a hyperlipidemic diet

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Background: Preclinical and clinical studies have demonstrated that the omega-3 long-chain polyunsaturated fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) as a triglyceride (TG) oil or an ethyl ester provide cardiovascular benefits. Martek Biosciences Corporation has developed a concentrated ethyl ester of DHA (900 mg/g) from microalgae (MATK-90).

Objectives: This study evaluated the effects that different doses of MATK-90 had on TG and cholesterol (CHOL) levels and histopathology in Wistar rats fed a high fructose diet used to induce hypertriglyceridemia. Effects of MATK-90 were compared with those produced by a drug (Lovaza™, P-OM3; 465 mg EPA+ 375 mg DHA/g), a TG oil used in food (DHASCO®, algal-DHA, 40% DHA by weight), and a control (corn oil).

Procedures: Doses of MATK-90 (0.6, 1.3, 2.5, 5.0 g/kg/day), algal-DHA (5g/kg/day=2g DHA), P-OM3 (5g/kg/day) and corn oil (5g/kg/day) were administered by oral gavage for 28 days. The fructose diet was provided for 4-5 weeks until the TG level was ≥ 300 mg/dL. Selected tissues were examined macro- and microscopically and were graded on a scale of 1-4 (minimal, mild, moderate, marked) according to the intensity and extent of change.

Results: After 28 days, a statistically significant dose-related decrease was observed in TG and CHOL levels in all but the low dose of MATK-90 treatment groups vs. control. In the high-dose group of MATK-90 and in the P-OM3 group reductions in TG were similar. At 28 days, the high dose group of MATK-90 but not P-OM3 group showed a significant reduction of CHOL vs. control. Compared with P-OM3, the highest dose of MATK-90 was more efficacious in decreasing the incidence and severity of hepatocellular cytoplasmic rarefaction and vacuolation.
Conclusion: The benefits using combined ethyl esters of DHA and EPA from fish oil are also attainable with an ethyl ester of algal-DHA alone.

P010

Incorporation of n-3 PUFA into human blood mononuclear cells according to intakes representing daily consumption of oily fish

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Background: N-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), influence immune cell function. The functional effects of these fatty acids appear to be closely linked with their appearance in cell membrane phospholipids. Studies performed to date investigating the incorporation of EPA and DHA into human immune cells have used marine oils consumed daily. Although much is known about the effect of very frequent intake of EPA and DHA, this situation does not mimic what occurs with less frequent intake of these fatty acids such as when oily fish are consumed just a few times per week.

Objective: To describe the effect of increased n-3 PUFA intake mimicking the consumption of one, two or four oily fish meals per week on the fatty acid composition of human peripheral blood mononuclear cells (PBMCs).

Procedure: Healthy male and female volunteers (n = 204) aged 20 to 80 y were recruited into a double blind, placebo controlled trial and were randomly assigned to one of five treatment groups representing different patterns of oily fish intake. Blood was collected at several time points up to 3 months and PBMCs prepared using standard techniques. PBMC fatty acid composition was determined by gas chromatography.

Results: EPA and DHA significantly increased in the "4 portions/wk" group when compared to the control group after 1 week of intervention. AA was significantly lower in the "4 portions/wk" dosage group at 2 weeks and 2 months when compared with the control group. There were few other differences in the EPA, DHA and AA contents of PBMCs among the groups over this time period.

Conclusions: From this preliminary data, it appears that providing EPA+DHA at the level of 11.2 g/wk spread over 4 d/wk can affect PBMC fatty acid composition and may influence PBMC function.

P011

Dietary fish oil supplementation in a porcine model of aortic vascular prosthetic graft infection - effects on three acute phase proteins

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Background: Aortic Vascular Prosthetic Graft Infection (AVPGI) is a feared postoperative complication, a frequently involved pathogen is *Staphylococcus aureus* (*S. aureus*). Due to lack of powerful randomized humane trials in this area, a porcine model has been developed.

Fish oil n-3 Polyunsaturated Fatty Acids (PUFA) is considered anti-inflammatory and may affect potency of AVPGI and the risk of developing sepsis in this porcine model.

Objective: The objective of the present study was to compare dietary fish oil (n-3 PUFA) supplementation to sunflower oil (n-6 PUFA) and saturated fat (animal fat) supplementation, when looking at the response of three acute phase proteins (C-reactive protein (CRP), haptoglobin and fibrinogen) in this porcine AVPGI model.

Procedure: In this study, 84 pigs (fourteen litters with six female littermates) were randomized to one of three diets containing 10% fat, based on fish oil (n=28), sunflower oil (n=28) or animal fat (n=28). The pigs were fed the diets for 35 days. On day 21 an aortic vascular prosthetic graft was inserted in the infra renal part of the abdominal aorta, and the grafts were artificially infected with *S. aureus* (strain ATCC29213, 10⁶ colony forming units (CFU)). Blood samples were drawn once prior to surgery (day 21) and 5 times in the 14-day follow-up period. Acute phase proteins were analyzed in an auto analyzer ADVIA 1650[®] (Siemens Medical Solutions). A commercial Porcine C-Reactive Protein assay and a Haptoglobin assay from Tridelta Developments Ltd were used for CRP and haptoglobin detection. Fibrinogen was detected with particle-bound polyclonal rabbit anti fibrinogen antibody (Q 0122, DakoCytomation, Denmark).

Results: Statistical analyses are in progress. Diet fixed effects on the acute phase protein response, will be estimated by a linear model. Litter design and correlation due to repeated measurements will be taken into account when fitting the model.

Conclusion: The conclusion awaits results.

P012

The effects of polymorphisms in phospholipase A2 and cyclo-oxygenase 2 genes on interferon-alpha-induced depression and polyunsaturated fatty acids levels

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Objective: The hypothesis of this study is that genetic variations in phospholipase A2 (PLA2) and cyclo-oxygenase 2 (COX2) would have effects on interferon-alpha-induced depression and somatic symptoms, as well as PUFA levels, in patients with hepatitis C viral (HCV) infection.

Methods: Patients with chronic HCV (n=132) were assessed to examine the effects of single nucleotide polymorphisms (SNPs) of COX2 and PLA2 genes on the development of depression and on the erythrocyte levels

of three main PUFAs, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid, during interferon-alpha therapy. A second "replication" group of patients (n=82) with major depression was used to replicate the effects of genotypes on specific clusters of depressive symptoms.

Results: 28% of participants developed interferon-alpha-induced depression. Participants with PLA2 BanI GG or COX2 rs4648308 AG genotypes had a higher risk of IFN-alpha-induced depression (odds ratio=3.1 and 3.5, respectively). The "at risk" PLA2 polymorphism was associated with lower EPA levels, and the "at risk" COX2 polymorphism was associated with lower DHA and EPA levels, during IFN-alpha therapy. The PLA2 BanI GG was also associated with more somatic symptoms, both in patients with interferon-alpha-induced depression and the replication sample of patients with major depression.

Conclusions: Genetic variations in the COX2 and PLA2 genes have effects on IFN-alpha-induced depression, possibly by affecting the levels of EPA and DHA. PLA2 genotypes is associated with somatic symptoms in depression, hence it may be a potential biomarker to understand clinical subtypes of depressive disorders.

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P013

Effect of n-3 polyunsaturated fatty acid supplementation on prostaglandin E and F isomer production by ovine uterine endometrial cells

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Background: Regulation of uterine prostaglandin (PG) production is essential for normal reproduction. n-3 PUFAs are the precursors of 3-series PGs, while n-6 PUFAs produce 1- and 2- series PGs. Both PUFA families utilise the same enzymes for PG production, so n-3 PUFA supplementation can alter 1- and -2 series PG production. Most previous studies utilized assays which could not distinguish these different PG isomers due to high cross-reactivity of the antisera.

Objectives: To examine the effect of n-3 PUFA supplementation on 1-, 2- and 3- series PGE and PGF production by ovine uterine endometrium.

Procedures: Stromal and epithelial cells isolated from cyclic ewes were separated and cultured to confluence in DMEM/F12 medium with 10% fetal calf serum. They were supplemented with 0 (CONT), 20 or 100 µM of α-linolenic

acid (ALA), stereodonic acid (SDA) or eicosapentaenoic acid (EPA) in serum free medium for 24 h. PG isomers in spent medium were isolated using HPLC and quantified by radioimmunoassay. COX isoforms in the treated cells were measured using qPCR. Each treatment was quadruplicated with cells from 10 ewes.

Results: In the epithelial cells, all supplemented n-3 PUFAs increased PGF_{1α} while their effect on PGF_{2α} was moderate. ALA and EPA stimulated PGF_{3α} up to 4-fold (CONT 2.4±0.38 vs EPA 10.7±12.19 ng/ml, p<0.01). n-3 PUFAs inhibited COX-1 expression but did not alter COX-2 expression significantly. In the stromal cells, EPA and SDA significantly suppressed PGE₁ production (CONT 4.9±1.68ng/ml vs EPA 1.4±0.29 ng/ml); PUFAs altered PGE₂ moderately (only SDA increased PGE₂ production, P<0.01); 100µM EPA increased PGE₃ by 2-fold (CONT 1.6±0.39 vs EPA 3.2±0.54 ng/ml, P<0.01) whereas other tested PUFAs did not change PGE₃ production significantly. n-3 PUFAs inhibited COX-1 expression, but stimulated COX-2 expression (P<0.05-0.01).

Conclusion: n-3 PUFA supplementation significantly altered PG isomer production and this may affect reproductive processes.

P014

Effects of an arachidonic acid/ docosahexaenoic acid mixture on the development of obesity and its related disorders

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Background: Arachidonic acid (ARA) and docosahexaenoic acid (DHA) are n-6 and n-3 long-chain polyunsaturated fatty acids, respectively, and have been proven to support brain development and vision. Their dietary supplementation is therefore considered to be health beneficial, especially during early life stages. Regarding body weight development, high intake of n-6 polyunsaturated fatty acids with declining consumption of n-3 fatty acids has been associated with an increased risk for obesity.

Objective: This study addresses whether prolonged ARA-DHA supplementation, at relatively low amounts, is effective in reducing the development of obesity and its related disorders. This was tested in ApoE3L-transgenic mice, a humanized animal model for hyperlipidemia with mild obesity and insulin resistance, which were fed a high-fat/high-carbohydrate (HFHC) diet.

Procedure: Male ApoE3L-mice were fed ad libitum with a HFHC-diet (28% fats, 42% carbohydrates) in the absence or presence of 0.129wt% ARA and 0.088wt% DHA. Food intake, body weight, plasma cholesterol, plasma triglycerides and both fasting glucose and insulin were

measured during the course of a 16-weeks feeding period. At the end of the experiment, plasma levels of HDL-, LDL-, VLDL-cholesterol and fat tissue and liver weights were determined.

Results: Throughout the feeding period, mice that were fed the ARA-DHA supplemented HFHC-diet gained less body weight in comparison to the control group with the HFHC-diet only. ARA-DHA supplementation also lowered plasma cholesterol and fasting glucose levels. At the end of the experiment, the ARA-DHA supplemented mice had a reduced relative liver weight and an increased HDL/LDL-ratio as compared to their controls. No effects of ARA-DHA supplementation were found on food intake, insulin levels, and fat tissue weights.

Conclusion: This study shows that in a HFHC-dietary context, supplementation of ARA and DHA in relatively low amounts has health benefits on body weight development, plasma cholesterol, plasma glucose levels and liver health.

P015

Altered n-6 and n-3 fatty acid metabolism in a cystic fibrosis cell line

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Background: Abnormalities of fatty acid (FA) turnover with low levels of linoleic (LA) and docosahexaenoic (DHA) acid are observed in cystic fibrosis (CF) patients possibly due to alterations in the basal lipid metabolism.

Objective: To study in CF and non CF cell lines the metabolism of n-6 and n-3 FA and to elucidate mechanisms possibly responsible of the altered FA status in CF, focusing on FA desaturase enzymes.

Procedure: 16HBE14o- cells (a human bronchial epithelial cell line) were used, with CF phenotype (16HBE14o-AS3) and with no CF phenotype (16HBE14o-S1). The two cell lines were incubated with EMEM without FBS for 24h, for the analysis of total FA profile by GC and of the expression of FA desaturases. In addition cells were incubated with [1-¹⁴C] linoleic or α -linolenic acid (ALA) (1 μ Ci/plate), respectively precursors of n-6 and n-3 FA, for the analysis of labelled FA from total lipids and of the incorporation of labelled LA or ALA into different lipid classes.

Results: The FA composition of cell total lipids is similar in the CF line with its isogenic control, with high levels of saturated and monounsaturated FA and low polyunsaturated FA (PUFA). Both [1-¹⁴C] LA and ALA total conversion to eicosapentaenoic acid is increased in AS3 cells versus S1 and this is due to an enhanced Δ 6 desaturase activity for LA and Δ 5 for ALA. The incorporation of [1-¹⁴C] LA and ALA in phospholipids in CF cells is increased while it is lower in triglycerides and

cholesterol esters than in control cells. There is no difference in the expression of both desaturase enzymes between CF and control cells.

Conclusion: The FA profile (analyzed by GC) of the two cell lines is similar, but the metabolism of [1-¹⁴C] LA and ALA is increased in CF cells versus controls.

P016

Blood n-3 PUFA vary in relation to ages and physiological conditions and are lowest in children. Studies in Italian subjects

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Background: Long Chain PolyUnsaturated Fatty Acids (LCP), especially the n-3 (EPA 20:5 and DHA 22:6), modulate key functional processes in biomembranes. Circulating levels of LCP of the n-3 and n-6 series are representative of their "status" in the organism, and their assessment is a prerequisite for requirements and recommendations, which may vary at different ages.

Objective: To compare the FA status in Italian subjects at different ages, assessed through the determination of FA profiles by gas chromatography in a drop of blood from a fingertip.

Methods: Data from published and ongoing studies in different age groups, with an equal gender distribution, are reported (n° in parenthesis): 1. Neonates 4 days old (53), 2. 18 months children (21), 3. 3-9 years children (261), 4. 3-15 years (30), 5. Adults < 40 years (100), 6. Adults > 55 years (650).

Results: LCP of both series (n-3 and n-6) are higher in neonates than at subsequent ages, reflecting the efficient transfer of these FA to the foetus. Then, changes of AA (20:4 n-6) and DHA follow different trends. AA rapidly declines in children, increases progressively up to adolescence, it falls in younger adults and rises again in older subjects. DHA, undergoes marked reduction in children, it increases in younger adults, and it declines in the older adults. The lowest levels of DHA and total n-3 PUFA are present in children, possibly due to high rates of utilization during rapid growth vs inadequate intakes of FA present in limited amounts in diets.

Conclusion: The assumption that children are substantially healthy and do not require special dietary recommendations may result in inadequate n-3 LCP intakes. The low levels of DHA in an age period coinciding with the growth and functional development of the major biological systems may result in inadequate protection vs subsequent chronic diseases.

P017**Non-invasive assessment of fasting-induced myocardial triglyceride accumulation in fatty acid oxidation deficient mice**

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Background: Patients with genetic defects in mitochondrial fatty acid β -oxidation (FAO) often present cardiac abnormalities, as fatty acids are the heart's main energy source. Besides chronic energy shortage, toxicity of accumulating metabolites may contribute to the pathogenesis of hypertrophic cardiomyopathy. As fasting will increase the heart's reliance on FAO, we hypothesized that it will elevate myocardial triglyceride levels and decrease cardiac function in FAO disorders.

Objective: To non-invasively assess the consequences of a FAO deficiency for the heart by applying in vivo magnetic resonance (MR) techniques in the long chain acyl-CoA dehydrogenase (LCAD^{-/-}) knock-out mouse at baseline and after fasting.

Procedure: Male LCAD^{-/-} ($n=4$) and C57BL/6 wild type mice ($n=4$) underwent baseline measurements in a 9.4 Tesla MR scanner at 13 weeks of age. Two weeks later, the protocol was repeated after 24 hrs of fasting. Left ventricular (LV) function and mass were determined from cinematographic MR image series. Localized proton MR spectroscopy (¹H-MRS) was performed in vivo to assess myocardial triglyceride content. Repeated measures ANOVA was used for statistical analysis.

Results: LV myocardial mass normalized to body weight was larger in LCAD^{-/-} mice than in controls ($P<0.01$). There was a trend for decreased ejection fraction (EF) in the LCAD^{-/-} mice after fasting ($P=0.098$), suggesting reduced systolic function. No significant effect of fasting on myocardial triglyceride content was observed in wild type animals (paired t -test: $P=0.143$). After fasting, myocardial triglyceride content was increased in LCAD^{-/-} mice (paired t -test: $P=0.015$) and was larger compared to controls (unpaired t -test: $P<0.001$).

Conclusion: FAO-impaired LCAD^{-/-} mice displayed LV hypertrophy and elevated myocardial triglyceride levels. Fasting may affect systolic function in LCAD^{-/-} mice, as a trend for a decreased EF was observed. The non-invasive MR protocol will be applied to further investigate consequences of FAO disorders during longitudinal dietary studies.

P018**Effect of cigarette smoking on the relationship between fatty acids and subclinical atherosclerosis**

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Background: Cigarette smoking (CS) is a determinant of carotid atherosclerosis. Among mechanisms potentially responsible of this effect, e.g activation of extensive peroxidation processes, changes of fatty acid (FA) levels in smokers, especially the reduction of polyunsaturated FA (PUFA) in plasma and tissues, may be involved.

Objective: To evaluate whether CS interacts with circulating FA in affecting the individual values of carotid artery intima media thickness (C-IMT).

Methods: Plasma FA composition of 32 Smokers and 32 Never-smokers, matched for age, sex and status of hypercholesterolemia was determined by GC analysis after FA transmethylation. C-IMT was measured by B-mode ultrasound. To reach the study objective, correlations between C-IMT and each FA were investigated in both smokers and never-smokers and interactions assessed.

Results: As expected, saturated (14:0, 16:0) and monounsaturated (16:1, 18:1, 24:1) FA were higher and PUFA (20:4 n-6 and 22:5, 22:6 n-3) were lower in Smokers than in Never-smokers. The effect of CS on FA was dose-dependent for both 20:1 and 18:3 n-3, where a negative correlation with pack-years, an index of global smoking exposure, was found ($r=-0.402$, $p=0.015$ and $r=-0.346$, $p=0.039$, respectively). Interactions were evaluated after adjustment for fibrate therapy (the unique variable for which the two groups differ) as well as for matching (an adjustment done to take into account that groups were matched). CS significantly interacts with 20:3 n-6 (Binteraction=-0.16), 20:5 n-3 (Binteraction=0.25), 22:5 n-3 (Binteraction=0.43), 22:6 n-3 (Binteraction=0.09) and n-3 (Binteraction=0.04) (all $p<0.05$).

Conclusion: CS interacts especially with some circulating PUFA in affecting the individual burden of subclinical atherosclerosis.

P019**Major depression, cardiovascular risk factors and the omega-3 index**

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Background and Objective: Cardiovascular disease (CVD) and major depressive disorders (MDD) are frequent

diseases worldwide with a high comorbidity rate. Omega 3 fatty acids have been suggested as disease modulators for both CVD and MDD. Therefore, we studied whether polyunsaturated fatty acids and the Omega-3 Index may represent markers for assessment of the cardiovascular risk in physically healthy patients suffering from MDD. **Procedure:** Case-control study in 166 adults (86 MDD patients without CVD, 80 matched healthy controls). Baseline examinations included depression ratings, conventional cardiovascular risk factors, fatty acid, and interleukin-6 determinations.

Results: Several conventional risk factors were more prevalent in MDD patients. The Omega-3 Index and individual omega-3 fatty acids were significantly lower in MDD patients. An Omega-3 Index <4% was associated with high concentrations of the proinflammatory cytokine IL-6. **Conclusion:** Conventional cardiovascular risk factors, the Omega-3 Index and IL-6 indicated an elevated cardiovascular risk profile in MDD patients currently free of CVD. Our results support the employment of strategies to reduce the cardiovascular risk in yet cardiovascularly healthy MDD patients by targeting conventional risk factors and the Omega-3 Index.

P020

Endothelial function during raised non-esterified fatty acid levels is enhanced by moderate substitution of SFA with n-3 PUFA

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Background: Type II diabetes mellitus and obesity are characterised by raised serum non-esterified fatty acids (NEFA). Increased NEFA levels impair endothelial function through the effects on insulin and subsequent impact on the activation of endothelial nitric oxide synthase (eNOS). However, it is unknown whether this effect is consistent for all fatty acid types.

Objective: To examine the impact of raised NEFA achieved by the oral consumption of SFA or SFA plus n-3 PUFA and an infusion of heparin on endothelial function.

Procedure: On separate study days, healthy volunteers (n = 55) were given drinks either rich in SFA (0.42g palm stearin per kg bodyweight) or SFA and n-3 PUFA (0.36g palm stearin + 0.06g DHA-rich fish oil per kg bodyweight). Approximately 33% of total fat was given at 0 h, with smaller amounts (≈11%) given every 30 min until the end of the 4 h study. In order to activate lipoprotein lipase and thus raise NEFA levels, an intravenous infusion of heparin was administered from 2-4 h. Endothelial function was measured using flow-mediated dilatation (FMD) at baseline and 3.5 h.

Results: Total NEFA levels throughout the study days were similar for both fat types. The FMD response decreased following consumption of SFA, and increased following SFA

and n-3 PUFA. The levels of circulating nitrate, a marker of bioavailable nitric oxide, also declined to a greater extent on the SFA study day.

Conclusion: The effect of raised NEFA on endothelial function appears to be dependent on the fatty acid composition of NEFA rather than simply the total NEFA concentration. Moderate substitution of SFA with n-3 PUFA may enhance endothelial function during high NEFA levels.

P021

Skeletal muscle UCP3: protecting mitochondrial oxidative capacity when facing fatty acids?

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Background: Uncoupling protein 3 (UCP3) is suggested to protect mitochondria against lipid-induced oxidative damage. One of the potential mechanisms involved is mitochondrial uncoupling.

Objective: Here, we investigated whether lack of UCP3 indeed leads to decreased mitochondrial uncoupling and exacerbates the decrease in oxidative capacity in the presence of fatty acids (FAs).

Procedure: To this purpose, isolated skeletal muscle mitochondria from male C57Bl/6 mice lacking UCP3 (UCP3^{-/-}) vs. wild type (WT) controls were studied (n=5). Mitochondrial uncoupling was analyzed using respirometry, in the presence of potential UCP3 activators (superoxide and FAs). High superoxide production was established by addition of succinate without rotenone, creating reverse electron flow. Maximal oxidative capacity was measured by respirometry upon titration of FCCP, both in presence or absence of palmitate. To assess lipid-induced mitochondrial damage, mitochondrial swelling was measured spectrophotometrically upon oleate incubation while lipid peroxidation was determined by western blotting of 4-HNE protein adducts after 1h incubation with linoleic acid and succinate (i.e. high levels of superoxide).

Results: Mitochondrial uncoupling was similar in UCP3^{-/-} and WT, both in the presence of high superoxide (P=0.34) and upon increasing levels of FAs (P=0.80). FAs decreased mitochondrial maximal oxidative capacity (P=0.02 in WT and P=0.01 in UCP3^{-/-} mice) but this decrease was similar in both genotypes (-86.8 ± 14.1 vs. -74.4 ± 6.7 nmol O₂/min/mg in UCP3^{-/-} vs. WT resp. P=0.46). Also mitochondrial swelling upon oleate was similar in UCP3^{-/-} vs. WT (P=0.80). However, 4-HNE protein adducts were 33% higher in UCP3^{-/-} (P=0.02).

Conclusion: In summary, we did not find support that UCP3 is involved in mitochondrial uncoupling and protection against lipid-induced damage in our acute

experiments. However, this might be due to a time effect since longer-term incubation (1h) of mitochondria lacking UCP3 with high levels of superoxide and FAs did result in higher levels of lipid peroxidation.

P022

Leptin receptor mutation in *db/db* mice does not protect against lipodystrophy induced by *trans*-10,*cis*-12 conjugated linoleic acid (CLA)

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Background: CLA has divergent effects on adiposity, insulin sensitivity and hepatic steatosis in *ob/ob* mice (mutation in leptin gene) compared to *fa/fa* Zucker rats (mutation in leptin receptor). These differences could be due to species or leptin genotype.

Objective: To determine the effects of CLA isomers on adiposity, insulin sensitivity and hepatic steatosis in *db/db* mice (mutation in leptin receptor).

Procedure: *db/db* mice (7 wk old) fed *cis*-9,*trans*-11 (*db9*-11) or *trans*-10, *cis*-12 (*db10*-12) CLA (0.4% w/wt) or control (*dbCTL*) diet were compared to C57BKS/J lean mice fed control diet for 6 weeks. Feed intake, body weight, adiposity, fasting serum glucose, insulin, leptin and total hepatic lipids were assessed. Activation of key signalling molecules in adipose tissue was determined by Western blotting.

Results: The *db10*-12 mice had reduced body weight and significant depletion of adipose mass, despite increased feed intake, compared to the *db* groups. Although serum leptin concentration was not different from *dbCTL*, the greater fat ablation in *db10*-12 mice was associated with 2-fold higher serum leptin when expressed per adipose mass and 9-fold higher leptin protein levels in epididymal adipose tissue. The *db10*-12 mice had higher liver weight (g/bwt) and greater hepatic steatosis compared to *dbCTL*. The CLA isomers did not worsen or improve assessments of insulin sensitivity (fasting serum glucose, HOMA) except *db9*-11 had greater fasting hyperinsulinemia than *db10*-12 mice. However, signalling through phosphatidylinositol 3-kinase (PI-3K) was altered in adipose tissue of *db10*-12 compared to *dbCTL* (elevated p-p85 PI-3K, reduced pPDK1, unchanged pAkt). The *db9*-11 mice were similar to the *dbCTL* group except for higher fasting serum leptin concentrations.

Conclusion: Dietary *trans*-10,*cis*-12 CLA produces lipodystrophy in *db/db* mice similar to previous studies in *ob/ob* mice, which indicates a species-dependent response to CLA in mice and rats rather than a leptin genotype effect.

P023

The effects of stearidonic acid-enriched soybean oil on the omega-3 index and other heart health markers

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Background: Stearidonic acid (SDA) is the product of the rate-limiting enzyme (delta-6 desaturase) in the conversion of ALA to EPA, and through biotechnology, can be produced at levels of 20%-30% in soybean oil (SDA SBO).

Objective: To compare the effects of SDA SBO to EPA and to regular SBO on red blood cell (RBC) EPA+DHA levels (the omega-3 index) and other markers of cardiovascular health.

Procedure: Healthy volunteers were randomized to treatment with SDA SBO (15 g/day providing 4.1 g SDA) plus 1 g/day SBO oil in gelcaps (SDA group) or to 15 g/day regular SBO with either EPA ethyl esters (1 g/d; EPA group) or 1 g/day SBO oil in gelcaps (control group). The omega-3 index, serum lipids, blood pressure, heart rate, adiponectin, C-reactive protein and safety laboratory measures were obtained at baseline and after 12 weeks.

Results: A per-protocol analysis was conducted on 157 subjects. The overall mean (\pm SD) omega-3 index at baseline was $4.3 \pm 1.1\%$. The omega-3 index was unchanged at 12 weeks in the control group but increased by 17.7% in the SDA group and by 19.7% in the EPA group (ANCOVA, both $p < 0.001$) vs. control. Variability in individual response was observed. Relative to EPA, SDA increased RBC EPA with about 18% efficiency on average. None of the other endpoints were affected by SDA or EPA treatment. Fasting triglycerides were reduced 26-30% by SDA or EPA treatment vs. control ($p = 0.029$) for a subset population with baseline triglyceride levels > 150 mg/dL, when assessed by a post hoc repeated measures analysis. There were no adverse treatment-related effects.

Conclusion: SDA SBO significantly raised the omega-3 index, an emerging independent marker of cardiovascular risk. SDA SBO may be a viable plant-based approach to providing meaningful intakes of cardioprotective omega-3 FAs.

Sponsorship: Monsanto Co, Solae LLC

P024

Fatty acid synthesis and cellular regulation - an intimate affair

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Background: Acetyl-CoA carboxylase (Acc1p) catalyzes the rate limiting step in fatty acid de novo biosynthesis by carboxylation of acetyl-CoA to malonyl-CoA. Acc1p is deactivated by phosphorylation by Snf1p kinase (yeast ortholog of mammalian AMP-activated protein kinase AMPK). Because Snf1p has pleiotropic functions in the cell, mutants lacking Snf1p display multiple cellular malfunctions in addition to deregulated lipid metabolism.

Objective: A yeast strain was constructed, which has the serine residue (phosphorylation site of Acc1p) replaced by an alanine (*acc1S/A*), thus locking Acc1p in a constitutively hyperactivated state. This point mutant was used as a tool to investigate the impact of deregulated fatty acid synthesis on lipid metabolism and cellular physiology.

Procedure: The point mutant was characterized including phenotypical (growth) analysis, fluorescence microscopy, HPLC-ESI/MS² and RT-PCR.

Results: The *acc1S/A* mutant strain showed a severe growth defect on media lacking inositol, which was due to the repression of the *INO1* gene by its repressor Opi1p. In wild type, Opi1p is bound to phosphatidic acid (PA) on the ER. In the absence of phosphorylation in the *acc1S/A* mutant, fatty acid and triacylglycerol synthesis were increased and PA levels declined. This resulted in the translocation of Opi1p into the nucleus leading to a repression of *INO1*.

Conclusion: Here we show that Acc1p hyperactivity not only affects fatty acid and triacylglycerol biosynthesis but also plays a regulatory role in lipid metabolism through regulating PA levels.

P025

Maternal supplementation of long chain polyunsaturated fatty acids alters the 'ex vivo' response of splenocytes to concanavalin A and ovalbumin

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Background: Feeding infant's formula with added long chain polyunsaturated fatty acids (LCP), docosahexaenoic acid (DHA) and arachidonic acid (ARA) alters immune development. However, it is not known if providing LCP in the maternal diet influences immunity in the suckled infant.

Objective: This study was designed to determine the effect of adding LCP (4.6% DHA, 0.8% EPA, 0.5% AA w/w of fat) into the diet of lactating dams on immune cells from their offspring's ex vivo response to polyclonal mitogens Concanavalin A (ConA), lipopolysaccharide (LPS) or the food protein, ovalbumin (OVA).

Procedure: At birth, pups (n=60) were randomized to dams (n=6/diet) fed isocaloric adequate diets (15% w/w fat, P/S ratio=0.5) with (+LCP) or without (-LCP) LCP and suckled for 3 weeks. At 21d of age pups (n=2/dam) were killed and splenocytes isolated to determine the effect on cell phenotypes (by flow cytometry), ability to proliferate (stimulation index) and produce cytokines (via ELISA).

Results: At 21d of age, there was no effect of maternal diet on the body and spleen weight of the pups. Diet had only small effects on the phenotype distribution in spleen and did not alter splenocyte proliferative response to mitogens nor LPS-stimulated cytokine production.

However, after ex vivo Con A stimulation isolated splenocytes from (55%) than-LCP pups the +LCP pups produced more IL-10 (220%) and less IFN- (P<0.05). With ex vivo OVA stimulation, splenocytes from +LCP pups produced (170%) than -LCP pups (P<0.05).

Conclusions: The cytokine response to OVA and the IL-10 response to Con A is similar to our previous findings in artificially reared formula-fed pups and infants supplemented with LCP. This research demonstrates that early supplementation with LCP, whether via formula or through human milk, alters the cytokine response to mitogens and food proteins in healthy infants. Funded by NSERC.

P026

Phospholipid profile in the postmortem hippocampus of patients with schizophrenia and bipolar disorder: no changes in docosahexaenoic acid species

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Background: Previous studies with postmortem brain tissues showed abnormalities not only in n-3 long-chain polyunsaturated fatty acids (PUFA) but also in phospholipid metabolism in the cortex of individuals with schizophrenia and mood disorder.

Objective: In this study we investigated whether there is similar abnormality in n-3 long-chain PUFAs and/or in phospholipid profile in the hippocampus of schizophrenia and bipolar disorder patients compared to unaffected controls.

Procedure: Using high-performance liquid chromatography/electrospray ionization-mass spectrometry (LC/MS), the phospholipid contents in the postmortem hippocampus from 35 individuals with schizophrenia, 34 individuals with bipolar disorder and 35 controls were evaluated.

Results: Unlike the previous findings from orbitofrontal cortex, we found no significant differences in either n-3 long-chain PUFA or total phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylcholine

(PC). However, docosapentaenoic acid (n-6, 22:5n-6)-PS and 22:5n-6-PC were significantly lower in individuals with schizophrenia or bipolar disorder than the controls. When fatty acid contents were estimated from PS, PE and PC, 22:5n-6 was significantly lower in both patient groups compared to the controls.

Conclusion: From these results we concluded that DHA loss associated with these psychiatric disorders may be specific to certain regions of the brain. The selective decrease in 22:5n-6 without affecting DHA contents suggests altered lipid metabolism, particularly n-6 PUFA rather than n-3 PUFA, in the hippocampus of individuals with schizophrenia or bipolar disorder.

P027

DHA-rich fish oil modulates cerebral haemodynamics in a dose response manner in healthy young adults

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Background: The impact of dietary supplementation with n-3 PUFAs on behavioural outcomes has been well researched but very little attention has focussed on their impact on brain function in healthy adults.

Objective: To evaluate the cognitive and cerebral haemodynamic effects of 1 g and 2 g of DHA-rich fish oil in healthy adults.

Procedure: Sixty-four healthy, non-smoking, non consumers of oily fish aged 18-29 years took part. Relative changes in the concentration of oxyhaemoglobin (O2Hb), deoxyhaemoglobin (HHb) and total haemoglobin (THb) were assessed using Near Infrared Spectroscopy (NIRS); cognitive performance was assessed using a computerised battery of working memory, attention and executive function tasks. Participants completed the tasks with concurrent NIRS recording on a single occasion following 12 weeks daily dietary supplementation with either 1 g DHA-rich fish oil (450 mg DHA + 90 mg EPA), 2 g DHA-rich fish oil (900 mg DHA + 180 mg EPA) or placebo (olive oil).

Results: Supplementation with DHA-rich fish oil, in comparison with placebo, resulted in a significant dose-response increase in THb during all of the cognitive tasks; a similar pattern was observed for O2Hb. No treatment-related effects were observed for HHb. A beneficial effect of treatment was revealed on two of the tasks; participants in both treatment groups (1 g, 2 g) were faster than placebo on a choice reaction time task and those in the 2 g group were faster on a rapid visual information processing task.

Conclusion: Supplementation with DHA-rich fish oil modulated task-related cerebral blood flow response in a dose-response manner and improved performance on

tasks requiring sustained attention and response inhibition. Combining imaging techniques in the future could further elucidate the relationship between brain function and behaviour with a view to inform healthy ageing and the prevention and treatment of conditions associated with reduced cerebral blood flow.

P028

The cognitive and mood effects of DHA-rich and EPA-rich fish oil following 12 weeks dietary supplementation in healthy young adults

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Background: The n-3 PUFAs DHA and EPA must be acquired via dietary sources but are consumed in small quantities in the typical 'Western' diet. Findings from previous studies that have demonstrated improved cognitive performance following dietary supplementation with n-3 PUFAs in healthy young adults are equivocal.

Objective: The current study aimed to investigate the cognitive and mood effects of 2 types of fish oil supplement containing different ratios of DHA and EPA in healthy young adults.

Procedure: One hundred and forty males and females aged 18-35 were recruited. Participants were randomly allocated to one of three treatment groups: 1 g DHA-rich fish oil (FO; DHA 5:1 EPA), 1 g EPA-rich FO (EPA 3:2 DHA) or 1 g olive oil (placebo). Cognitive performance was assessed before and after 12 weeks daily dietary supplementation using a battery of computerised tasks designed to assess various aspects of attention and memory. Mood was also assessed as a secondary outcome. A blood sample was obtained before and after the intervention period and later analysed for serum fatty acid content to ensure treatment compliance.

Results: Improvements in the DHA-rich FO treatment group were observed on the Stroop task. Those in the EPA-rich FO group reported less mental fatigue during 30 minutes of cognitively demanding tasks. Both treatment groups performed worse than placebo on a Name-to-Faces recognition task. There was no evidence to suggest a treatment effect on any of the mood outcomes.

Conclusion: Twelve weeks administration of either DHA- or EPA-rich FO resulted in minimal modulation of cognitive function in healthy volunteers, but a consistent pattern emerged regarding reduced mental fatigue in response to cognitive demand in the EPA-rich FO group. Treatments lead to a differential pattern of behavioural change that may be explained by the different biological mechanisms of action associated with each fatty acid.

P029**Dietary supplement mix containing anti-oxidants and fish oil reduces inflammation, metabolic and oxidative stress in overweight men**

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Background: Low-grade chronic inflammation in overweight subjects is thought to play an important role in disease development.

Objective: It was hypothesized that dietary components are able to reduce this low grade inflammation, as well as metabolic stress and oxidative stress.

Procedure: Therefore, food components with evidence based anti-inflammatory properties were combined and supplemented to 36 healthy overweight men with mildly elevated C-reactive protein levels. It was a double-blind, placebo controlled, cross-over study with treatment periods of 5 weeks. Analyses comprised established blood and urine parameters such as inflammatory and anti-oxidant markers. Furthermore, 120 plasma proteins, >250 plasma metabolites (lipids, free fatty acids and polar compounds) and transcriptome of peripheral blood mononuclear cells and adipose tissue were included.

Results: Integrated analyses of data indicated reduced inflammation of adipose tissue, increased liver fatty acid oxidation, enhanced endothelial function, improved glycemic control and lowering of oxidative stress.

Conclusion: In this study we were able to show that an intelligent designed food mix was able to influence processes of metabolic stress, inflammation and oxidative stress in human without changing their habitual lifestyle. It was only possible to accurately follow nutrition-health relationships by using comprehensive techniques like metabolic, protein and gene profiling.

P030**Systematic evaluations of molecular species in ethanolamine phospholipids from postmortem anterior prefrontal cortex of patients with schizophrenia**

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Background: A multiplicity of theories have been proposed over the years that aim to conceptualize the pathological processes inherent to schizophrenia (SZ). We and others have observed evidence suggesting that membrane phospholipid defects could potentially explain many seemingly unrelated neurochemical observations in schizophrenia.

Objective: To further explore the role of phosphatidylethanolamine (PE) in the schizophrenia pathology, we systematically evaluated molecular species of plasmalogen (PlsEtn) and phosphatidylethanolamine (PtdEtn) in cellular membranes of predominantly gray matter from human brain.

Procedure: Brodmann area 10 (BA10) of postmortem anterior frontal cortex (AFC) samples were analyzed by electrospray ionization mass spectrometry (ESI/MS).

Results: There are distinct PlsEtn and PtdEtn molecular species in normal human AFC gray matter. Approximately, 45% of PE are PlsEtn. The ion peak corresponding to PlsEtn molecular specie at m/z 723 (16:0 - 20:4) was significantly lower in SZ patients (1.14±0.19 nmol/mg protein, n=7), not in patients with bipolar disorder and major depression (BD/MD, 1.28±0.21 nmol/mg protein, n=10), than in control subjects without psychiatric illness (1.53±0.41 nmol/mg protein, n=7). No other significant differences in either PlsEtn or PtdEtn molecular species were demonstrated. On the other hand, there exist tight correlations between specific pair of molecular species of either PlsEtn or PtdEtn in control subjects, although some of these correlations persist across disease, others appear to be lost among SZ and BP/MD patients.

Conclusion: Taken together, above findings provide further support of a role of membrane phospholipids turnover and arachidonic acid signaling in schizophrenia pathology.

P031**Safety assessment of a highly purified ethyl ester of algal-docosahexaenoic acid (DHA) in sprague-dawley rats**

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Background: Preclinical studies have shown that DHA derived from microalgae (DHASCO®), is neither mutagenic nor toxic in acute, subchronic or developmental tests. DHASCO®, a triglyceride oil from the fermentation of *Cryptocodinium cohnii* that contains 40-50% DHA by weight, is generally recognized as safe for use in foods. Martek Biosciences Corporation has developed a concentrated ethyl ester of DHA (900 mg/g) from *C. cohnii* (MATK-90).

Objectives: This study evaluated the safety of MATK-90 when administered to Sprague-Dawley rats for 90 days

and determined the persistence, reversibility, or delayed occurrence of any finding after a 1-month recovery. Effects of MATK-90 were compared with those produced from DHASCO® and a control (corn oil). Procedures: Doses of MATK-90 (1.3, 2.5, 5.0 g/kg/day) and DHASCO® (5g/kg/day=2g DHA) were administered once-daily by oral gavage at a volume of 10 mL/kg. The corn oil was also administered by oral gavage (10 mL/kg). Each treatment group consisted of 10 animals. There were 5 recovery animals in the corn oil, DHASCO®, and highest dose treatment group of MATK-90. Safety was evaluated based on clinical observations, ophthalmic examination, and clinical pathology following necropsy and tissue collection from all animals on day 92 and from the recovery animals on day 120.

Results: Mean body weights, ophthalmic results, or urinalysis were unaffected by treatments after 90 days. At day 92 in the highest dose treatment group of MATK-90 marked lesions in mesenteric lymph nodes were observed (n=3) but were minimal to moderate by day 120.

Cytoplasmic vacuolation in adrenal glands and macrophage infiltration in the spleen were present at all dose levels but were also present in the control group. No adverse effects were associated with the administration of MATK-90 at doses of ≤ 2.5 g/day or in the DHASCO® treatment group (5g/kg/day).

Conclusion: The no-observable-adverse-effect level (NOAEL) for MATK-90 was 2.5g/kg/day.

P032

Reduced docosahexaenoic acid content in plasma phospholipids is associated with higher stearoyl-CoA desaturase activity in obese children

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Background: Stearoyl-CoA desaturase (SCD)-1 activity plays a crucial role in the development of obesity, and palmitoleic acid content is a biomarker of human adiposity, while docosahexaenoic acid (DHA) has an anti-obesity effect. The aim of this study was to investigate the relationship between DHA and SCD activity in obese children.

Subjects and Methods: Thirty-two obese children aged 12.0±2.6 years (mean±SD) with relative body weight greater than 120% were included. For each subject, the fatty acid composition of the plasma phospholipids was analyzed by gas chromatography, and the activity of SCD was estimated using the palmitoleic acid/palmitic acid ratio.

Results: Body mass index (BMI) correlated positively with SCD activity ($r=0.439$, $p=0.0120$), and palmitoleic acid content ($r=0.456$, $p=0.0088$). In addition, DHA content correlated negatively with SCD activity ($r=-0.373$, $p=0.0357$) and palmitoleic acid content ($r=-0.411$, $p=0.0196$), and tended to have an inverse relationship with BMI ($r=-0.377$, $p=0.0592$) and triglyceride concentrations ($r=-0.304$, $p=0.0967$). Thus, obese children with reduced DHA content had higher SCD activity.

Conclusions: DHA may suppress SCD activity, contributing to an anti-obesity effect.

P033

Diets rich in saturated fat do not stimulate hepatic lipogenic gene expression in the hamster

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Background: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disorder in Western populations. Recent evidence has shown that, in mice, high fat diets, rich in saturated fatty acids, induce expression of genes for hepatic lipogenic enzymes and promote the accumulation of hepatic triacylglycerol (TAG)¹. In particular, such diets promote oleic acid (C18:1) synthesis through stimulating elongation and desaturation of palmitic acid (C16:0)¹.

Objective: To investigate whether diets rich in saturated fatty acids stimulate hepatic lipogenesis and thus promote accumulation of hepatic TAG in the Hamster.

Procedure: Groups of 8 male Golden Syrian hamsters were fed; 1) a high starch/low fat chow, or such chow enriched in 2) sucrose, 3) dairy fat or 4) fat formulated to mimic the fatty acid profile of a typical 'Western' diet. Diets were fed for 4 weeks before animals were sacrificed. Liver TAG (mg/g liver) was measured enzymatically and fatty acid composition determined by gas chromatography. The ratio of C16:1/(C16:0 +C16:1) was used as a measure of SCD activity. Hepatic mRNA concentrations for Stearoyl CoA desaturase 1 (SCD1) and fatty acid synthase (FAS) were determined by Q-PCR.

Results:

	Diet 1	Diet 2	Diet 3	Diet 4
Hepatic TAG	4.06±1.4	3.79±0.8	3.33±0.7	4.12±0.8
C16 ratio	3.50±1.4	4.68±0.8 ^a	4.27±1.1 ^b	2.26±0.3 ^{a,b}
SCD1mRNA	0.97±0.6	1.32±0.6 ^a	0.82±0.4	0.41±0.2 ^a
FAS mRNA	1.56±0.8	1.93±0.6 ^{a,b}	0.91±0.4 ^a	0.98±0.4 ^b

Values within rows sharing common superscript are significantly different, $p<0.05$

Conclusion: Saturated fat-rich diets do not induce hepatic steatosis in the hamster. Contrary to previous findings in the mouse, such diets tend to reduce hepatic

lipogenic gene expression and Western fat specifically reduces hepatic SCD1 expression and activity.

¹ Oosterveer, MH et al PLoS ONE 4(6), e6006

P034

Effects of α -linolenic acid enriched diet on metabolic syndrome: improvement of liver insulin resistance by modulating insulin signaling pathway

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Background: The metabolic syndrome (MS) is a complex disease defined as the coexistence of three or more of the following components : abdominal obesity, hypertriglyceridemia and high fasting glucose... Insulin resistance is the pathogenic denominator that links all components of the MS. The mechanisms responsible for the onset of the MS involve a combination of genetic and environmental factors. Among them, dietary lipids play an important role. However, not all types of fatty acids promote MS, and omega-3 poly-unsaturated fatty acids (PUFA) even appear protective. However, the mechanisms sustaining these protective effects remain unclear.

Objective: Because hepatic insulin resistance, characterized by a defect of insulin signaling in hepatocytes, is a key component of the pathogenesis of fasting hyperglycemia, we investigated the effects of n-3 PUFA enriched diet on hepatic insulin signaling pathway (more specifically phosphoinositol-3-kinase pathway [PI3K]) during the establishment of MS.

Procedure: For that, fatty Zucker rats were used as model of MS in comparison with their lean littermates, and were fed a control or a linseed oil diet from gestation until sacrifice at three-months-old.

Results: Our results showed that an α -linolenic acid rich diet significantly decreased glucose intolerance and tended to reduce hyperinsulinemia observed in fatty Zucker rats fed the control diet. In the liver, the early steps of PI3K insulin-receptor signaling (insulin receptors substrates 1 and 2) were disturbed in control fatty rats whereas no difference was observed in the late steps (total and phosphorylated Akt/protein kinase B). The n-3 PUFA rich diet tended to restore liver PI3K insulin signaling pathway in fatty Zucker rats.

Conclusion: This study evidences that an α -linolenic rich diet is able to modulate liver insulin sensitivity during the establishment of MS. These results suggest new nutritional approaches for early insulin resistance improvement.

P035

Maternal LCPUFA status does not explain the association between short interpregnancy interval and birth weight in the abcd cohort

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Objective: To evaluate the role of maternal EPA (20:5n-3), DHA (22:6n-3), DGLA (dihomo- γ -linolenic acid, 20:3n-6) and AA (20:4n-6) blood concentrations during early pregnancy in the relation of short interpregnancy interval (IPI) with birth weight and smallness for gestational age (SGA).

Procedure: Subsample of the ABCD cohort (inclusion Jan 2003-March 2004): n=1659 multiparous women who gave birth to a liveborn singleton. Fatty acid concentrations were determined in plasma phospholipids by gas-liquid chromatography around the 14th week of pregnancy. Data on interpregnancy interval and potential confounders (physiological, lifestyle-related and socio-demographic factors) were collected by a pregnancy questionnaire. Multivariate regression analyses were performed to assess the relation between the selected fatty acid concentrations in quintiles, interpregnancy interval, and birth weight and SGA, respectively.

Results: High concentrations of EPA, DHA and DGLA, and low concentrations of AA during early pregnancy were associated with a reduced risk of low birth weight and/or SGA (effect estimates birth weight: -118.2 to -182.5 g; OR for SGA: 1.72 to 2.09; P<0.05). No significant associations were observed between short IPIs (<6 months, reference category 18-23 months) and the maternal fatty acid concentrations. Short IPIs were associated with a mean decrease of 207.6 g (SE: 73.1) in birth weight (P=0.005) and a twofold increased risk of SGA (OR: 2.05; CI: 0.93-4.51, P=0.074). Adjustment for maternal fatty acid concentrations did not affect these results to any meaningful extent.

Conclusion: This study does not support an important role for maternal EPA, DHA, DGLA or AA concentrations in the association between short interpregnancy interval and adverse pregnancy outcome as reflected by low birth weight or SGA.

P036

Targeting the brain with a structured DHA-containing phospholipid. Evaluation of the neuroprotective effect after stroke

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Introduction and purpose: Docosahexaenoic acid (DHA) is crucial for brain development and learning activity. Previous investigations showed us that the brain accretion of DHA is more efficient when it circulates in lysophosphatidylcholine (lysoPC-DHA) than in its non-esterified form (DHA), both being carried by serumalbumin. Our aim was to assess the neuroprotective potential of a biomimetic stable form of lysoPC-DHA in comparison with DHA using magnetic resonance imaging (MRI) in an animal model of acute stroke.

Procedure: We have then synthesized 1-acetyl,2-docosahexaenoyl-glycerophosphocholine (AceDoPC), by a one-step conversion of PC-DHA (from microalgae) in using an immobilized triacylglycerol lipase. For stroke, Sprague-Dawley rats underwent a sixty-minute proximal middle cerebral artery occlusion with the intraluminal thread model, confirmed by magnetic resonance angiography. Immediately following reperfusion, animals were randomly and blindly treated by intravenous injection of i) saline, or ii) plasma from donor rats, or iii) DHA or iv) AceDoPC, both solubilised in plasma. Twenty-four hours after reperfusion, animals were submitted to behavioural tests before being sacrificed. Multi-contrast MRI was performed at H0 during occlusion and at H24 before sacrifice.

Results: Median neuroscore was significantly decreased in the AceDoPC group (3.5) compared to saline (8), reflecting fewer deficits in animals receiving AceDoPC. Plasma and DHA groups exhibited a non significant trend to decrease (6 and 5, respectively) compared to controls. Between H0 and H24, lesion size increased in the saline group (+18%±20%), was stable in the plasma group (-8%±38%), decreased in the DHA group (-18%±18%), and further decreased in the AceDoPC group (-36%±33%, p<0.05 compared to saline).

Discussion and Conclusion: The results provide an in vivo evidence of neuroprotection in acute stroke by AceDoPC. Brain accumulation of DHA and/or DHA metabolites has to be confirmed by post-mortem tissue measurements.

P037

High pancreatic ω 3 fatty acid level prevents chemically-induced type 1 diabetes in fat-1 transgenic mice

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Objective: Central to type-1 diabetes mellitus, β -cell destruction is partially mediated by pro-inflammatory cytokines and triggered by cytokine-induced activation of the transcription factor NF- κ B. Due to differences in diet and ω 3 sources, the effects of ω 3 polyunsaturated fatty acids (PUFA) on diabetes remain not totally elucidated. We evaluated whether high levels of pancreatic ω 3 PUFA could prevent chemically induced Type-1 diabetes, using fat-1 transgenic mice able to convert ω 6 into ω 3 PUFA. **Research Design and Methods:** Autoimmune diabetes was induced by multiple low-doses streptozotocin (MLD-STZ) to produce β -cell injury. When blood glucose levels exceeded 300 mg/dl, blood glucose and plasma insulin levels, cytokines, chemokine, GLUT-2 mRNA expression and NF- κ B p65 protein expression were determined. Pancreatic islets were immunostained for insulin and cleaved caspase-3 assessment and pancreas lipidomic analysis were performed.

Results: STZ-treated fat-1 mice, presenting pancreatic ω 3 fatty acid enrichment and a balanced ω 6/ ω 3 ratio, did not develop hyperglycemia compared to wild-type mice; STZ-induced β -cell injury was prevented as evidenced by no histological pancreatic damage or reduced insulin secretion. β -cell injury prevention was associated with pro-inflammatory cytokine inhibition (TNF- α , IL-1 β , iNOS) and decreased NF- κ B expression. In the fat-1-STZ-treated mice, pro-inflammatory AA-derived mediators as PGE2 and 12-HETE were decreased and the anti-inflammatory LXA4 was detected. Moreover, ω 3 precursor of the anti-inflammatory RvE1, was highly increased. the 18-HEPE,

Conclusions: Collectively, these findings indicate that the inflammatory process occurred in type-1 diabetes is decreased with high pancreatic ω 3 PUFA, suggesting that a low ω 6/ ω 3 PUFA ratio may play a crucial role in type-1 diabetes prevention and treatment.

P038**Role of arachidonic acid in diabetes: a review of the literature**MJ Bruins*DSM, Delft, The Netherlands*

Background: Long-chain polyunsaturated fatty acids (LCPUFA) of the n-6 series (≥ 20 carbon atoms) can be synthesised from the precursor linoleic acid (LA, C18:2n-6) through Δ -6 desaturase, elongation, and Δ -5 desaturase. Arachidonic acid (ARA; C20:4n-6) is an important n-6 LCPUFA as it serves as precursor in the production of eicosanoids, which play important roles in the regulation of numerous physiological functions, including insulin release, sensitivity, and glucose metabolism.

Objective: To review the literature to identify in vitro, animal and human studies relating to ARA and (experimental) diabetes conditions.

Procedure: A systematic search was performed in PubMed using the search terms arachidonic acid and (insulin and/or glucose).

Results: In type-1 or -2 diabetes and several animal models of diabetes, the content of n-6 LCPUFA, particularly ARA, in serum and tissue lipids is decreased compared to healthy controls. Activities of Δ -6 desaturase and Δ -5 desaturase are reduced under conditions of low insulin, high glucose, and high saturated fatty acids and may account for the decreased ARA status in diabetes. Different studies using in vitro and in vivo diabetes models indicate that ARA (and/or its derived eicosanoids) either or not in combination with zinc may beneficially influence glucose disposal, and insulin sensitivity and secretion. In addition, ARA was shown to protect against hyperglycemia-induced abnormal neurodevelopment.

Conclusion: Dietary supplementation of ARA (in combination with zinc) may restore ARA levels, and thereby beneficially influence glucose and insulin metabolism in diabetes. Furthermore, ARA may act neuroprotective by serving as a membrane component required for normal neurovascular structure, formation of neuroprotective eicosanoids, and regulation of nerve conduction. Whether ARA supplementation is beneficial in diabetes conditions, remains to be further elucidated in randomized controlled studies.

P039**Dietary polyunsaturated fatty acids and cytokines in psychological stress**JF Bradbury¹, SP Myers¹, L Brooks¹, B Meyer²¹*Southern Cross University, Lismore, Australia*²*University of Wollongong, Wollongong, Australia*

Objective: To measure the size of the impact of polyunsaturated fatty acids (PUFAs) on stress and distress and to determine the extent of proinflammatory cytokine involvement.

Procedure: 194 adults were recruited into a cross-sectional study, designed for structural equation modelling methodology. Subjective measures: Perceived Stress Scale (PSS-10); Kessler-10 scale (K10); General Health Questionnaire (GHQ-12); Food Frequency Questionnaire (FFQ). Biomarkers: erythrocyte fatty acid levels; ex-vivo stimulated proinflammatory cytokine (IL-1, IL-6 and TNF) production.

Results: The sample age ranged 18-69 years, mean=34.2 years, 74% were female and 56.7% students. The FFQ estimated that 55% of women and 73% of men are not reaching Australian NHMRC Suggested Dietary Targets for dietary LCn3FAs (430mg for women; 610mg for men). According to the omega-3 index biomarker (erythrocyte EPA+DHA in %total FA), 47% of males and 26.6% of females were at high risk of death by CHD, while only 6.29% of females and 1.98% of males had the highest level of protection. Dietary estimates of PUFAs from the FFQ were not good predictors of RBC levels when measured in absolute amounts ($r=.124$ between DietDHA and RBCDHA in ug/ml), but were better when RBC PUFAs were measured as relative ratios ($r=.349$ between DietDHA and RBCDHA in %total FA). In factor analysis the PSS had 3 factors, overwhelm, emotional and coping; the K10 was a second-order factor structure with depression and anxiety the second-order factors; and the GHQ had 3 factors, strain, coping and negative esteem. In structural equation modeling, arachidonic acid positively and n3PUFAs negatively predicted proinflammatory cytokine levels but the cytokines related differentially to the different stress factors.

Conclusions: Most Australians are not actively reducing risk of CHD through meeting dietary targets of LC-n3FAs, reflected in low omega-3 index biomarkers. Erythrocyte PUFAs did not significantly predict stress. Stress is not mediated by this group of cytokines.

P040**Impact of omega-3 polyunsaturated fatty acids in a mouse model of Parkinson's disease**M Bousquet¹, M Saint-Pierre¹, V Émond¹, P Julien², N Salem³, JX Kang⁴, F Cicchetti¹, F Calon¹¹*Centre de recherche du CHUL, Québec, Canada*²*Centre de recherche sur les maladies lipidiques, CHUL, Québec, Canada*³*Nutritional Neuroscience, Laboratory of Membrane Biochemistry and Biophysics, NIH, Rockville, United States of America*⁴*Department of Medicine, Massachusetts General Hospital and Medical School, Boston, United States of America*

Background: We have recently demonstrated that omega-3 polyunsaturated fatty acids (n-3 PUFA) protect mice from dopaminergic denervation induced by the neurotoxin MPTP. Although, the underlying mechanisms of action remain largely unknown, our evaluation of neurotrophic factors (GDNF, BDNF and their receptors) in the corticostriatal projection system revealed a part of implication in the neuroprotective effects observed in the parkinsonian MPTP mouse model. Here we report preliminary results of the evaluation of the capacity of endogenously-produced n-3 PUFA to protect against MPTP neurotoxicity in transgenic *fat-1* mice, expressing n-3 fatty acid desaturase thereby converting n-6 PUFA into n-3 PUFA.

Procedure and Results: Brain fatty acids measurements showed that levels of docosahexaenoic acid (DHA) were significantly increased in transgenic as compared to non-transgenic mice while total n-6 PUFA concentrations were decreased. These fatty acid conversions resulted in an increase of the n-3/n-6 PUFA ratio in *fat-1* mice. Despite noticeable heterogeneity within groups, preliminary data indicates that mice with higher brain DHA concentrations are protected from the nigral neuronal degeneration caused by the MPTP intoxication, as indicated by a strong correlation between DHA levels and nigral neuron counts ($r^2=0.775$; $p=0.0039$).

Conclusion: Additional analyses are currently being performed to confirm the beneficial effect of endogenous n-3 PUFA, as well as to demystify the underlying mechanisms. Taken together, these data reinforce the importance of n-3 PUFA for brain health and more particularly in neurodegenerative disorders such as Parkinson's disease.

P041

Moderate n-3 HUFA levels in salmon diets give the best protection against oxidative damage

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Background: The dietary FA composition strongly influences the FA composition of membrane phospholipids. Consequently, fish fed diets rich in n-3 HUFAs contain membranes rich in EPA and DHA that are highly susceptible to oxidative damage. Mitochondria are among the first responders to various stressors that challenge the homeostasis of cells and organisms. Furthermore, mitochondria are the major site of ROS production. Little is known regarding how the peroxidation of EPA and DHA in phospholipids in fish affects the properties of cellular and mitochondrial membranes in the muscle.

Objective: Our aim was to investigate the effect of HUFAs on muscle and mitochondrial membrane lipids and

their susceptibility to oxidative stress.

Procedure: Atlantic salmon were fed one of four diets with increasing levels of n-3 HUFAs (11-58% of total FAs). Skeletal muscle samples were subjected to biochemical measurements, gene expression and microscopy analysis. **Results:** Cardiolipin and sphingomyelin are phospholipids particularly susceptible to peroxidation. A reduced percentage of these lipids in the muscle membranes in the two high n-3 HUFA groups indicated that these membranes had been damaged by oxidation. The high caspase-3 activity, in addition to high *casp3a* and *bax* gene expression levels in the n-3 DHA group also implies that some degree of oxidative stress occurred. Electron microscopy images showed a higher degree of myofibre-myofibre detachment in fish fed the high HUFA diets than in fish fed the intermediate n-3 HUFA diet. The intermediate n-3 HUFA group had the highest level of mitochondrial integrity and tendencies of lower TBARS level than the other dietary groups.

Conclusion: Our findings show that intermediate levels of n-3 HUFAs in salmon diets give the best protection against oxidative damage of mitochondrial membranes and muscle structure.

P042

The impact of n-3 PUFA enriched beef on the development of atherosclerosis

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Background: N-3 Polyunsaturated fatty acids (PUFA) are protective against cardiovascular disease through their hypolipidaemic and anti-inflammatory properties. Diet has been shown to alter n-3 PUFA content of ruminant meat, with forage-rich diets producing meat with greater n-3 PUFA content compared to cereal-rich diets.

Objective: To determine whether beef produced from animals fed on a forage-based diet, compared to a concentrate-based diet, has beneficial effects on plasma lipids and development of atherosclerosis in the apoE*3 Leiden mouse model.

Procedure: Cattle were fed either forage (grass/white-clover swards) or control (concentrate) diets. Freeze-dried beef (36% w/w) produced from these animals was incorporated into cholesterol-enriched (0.25% w/w) diets of apoE*3 Leiden mice (n=10). After 12 weeks of feeding, serum lipids were quantified and lipid staining of sections through the aorta used to determine the extent of atherosclerosis.

Results: Cattle fed on a forage diet had increased amounts of long chain n-3 PUFA (1.38% vs 0.55% $p=0.001$) and reduced n-6/n-3 ratio (1 vs 7.5) within their muscle

when compared to control. After feeding the beef diets, no difference was seen in serum cholesterol concentration (11.4 vs 12.3mmol/l). However serum triacylglycerol concentration was significantly lower in animals on the forage diet (1.6 vs 2.3mmol/l, $p=0.02$). Quantification of atherosclerotic lesion area within the aorta showed no differences between groups (1.9 vs 1.6mm²).

Conclusion: These findings suggest that forage feeding favourably alters the fatty acid composition of beef, such that it reduces serum triacylglycerol in this mouse model. However, no significant impact was seen in the size of the atherosclerotic lesions in these animals.

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P043

Sustainable production of farmed salmon using vegetable, fish and algal oils: maintaining n-3 LC-PUFA and reducing pollutants

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Background: The benefits of fish and fish oil (FO) consumption for prevention of CHD, inflammatory and neural disorders are well proven. Health benefits are largely due to the efficacy of "Omega 3" LC-PUFA obtained primarily from seafood. However, fish consumption in the developed world is generally low, compared to national and international intake recommendations. This is partly due to conflicting advice on risks and benefits of fish consumption due to concerns regarding persistent organic pollutant levels (POPs).

Objective: To develop sustainable diets for salmon that reduce reliance on FO and meal while maintaining LC-PUFA concentrations and reducing POPs concentrations.

Procedure: Atlantic salmon were cultured, for most of the marine on-growing phase (100g-3kg), using diets with reduced fishmeal and with 100% FO replaced with a vegetable oil blend (VO). These VO diets were compared to commercial FO diets. The blend of VOs used included rapeseed, palm and Camelina oils as well as a DHA-rich algal oil, at two concentrations, derived from *Schizochytrium* sp algal meal. Repletion of LC-PUFA was achieved using decontaminated FO or algal oil.

Results: Most major fatty acid concentrations were significantly affected when FO was replaced with a VO or algal oil diet. When FO was replaced by VO the levels of flesh EPA and DHA were reduced by 70% compared to fish fed FO but these could be restored to ~90% of FO fed-fish values after feeding a decontaminated FO for 16 weeks. EPA & DHA % values in fish fed FO, or 11 or 5.5% algal oil, were 18, 13 and 11%, respectively.

Conclusion: Salmon can be grown on reduced levels of FO for most of the production cycle without significant reduction of LC-PUFA and with POPs levels reduced by ~75%.

P044

Reduced n-6 polyunsaturated fatty acids during postnatal development prevents excessive fat deposition and improves metabolic profile in adult mice

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Objective: Despite the exponential increase in obesity in adults as well as children over the last decades, no major increase in dietary fat or energy intake was found. Since dietary fatty acids have been shown to directly affect adipose tissue development, it is hypothesized that the reported shift towards increased n-6 and decreased n-3 FA intake could underlie the increasing prevalence of childhood obesity and development of metabolic disease. The objective of the present study was to investigate whether reducing n-6 PUFAs during neonatal life has sustained effects on adult metabolic profile and body composition in a mouse model of nutritional programming.

Procedure: Male offspring of C57Bl/6j dams were subjected to either a control diet (CTRL) or a low n-6 PUFA diet, in which linoleic acid (LA) content was reduced by 50% (Low LA) from postnatal day (PN) 2 to 42. Subsequently, mice of both experimental groups were switched to a Western style diet (WSD) until dissection on PN 98. Body composition was measured by dual x-ray absorptiometry at PN 42, 70 and 98. After dissection, plasma lipid profile, glucose, insulin and adipokines were measured. **Results:** Fat accumulation during the WSD challenge was reduced by 27% in mice fed Low LA compared to CTRL ($p < 0.001$). Additionally, lowering LA intake during neonatal development resulted in reduced fasting triglyceride levels, improved insulin sensitivity measured by homeostasis model assessment of insulin resistance (HOMA-IR), and lower fasting resistin and leptin levels.

Conclusion: Reduction of n-6 PUFA intake during infancy and childhood might prevent excessive fat accumulation and an adverse metabolic profile induced by a western style diet during adolescence and adulthood. This study has shown that fat quality of neonatal nutrition plays an important role in early metabolic development and might program adult body composition and metabolic homeostasis.

Biochemistry of Lipids

P045

The PUFA unbound to albumin causes high experimental error in *in vitro* studies: an example of β -oxidation assay

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Background: In the majority of the publications on FA research *in vitro*, authors simply mixed FA and albumin in an aqueous solution assuming the binding of FA and albumin is complete. But is the binding of FA to albumin *in vitro* really complete?

Objective: In the previous *in vitro* studies of β -oxidation using various labeled albumin-bound FAs, we found some non-negligible radioactive survivals in the supernatant after acidic precipitation of albumin-bound FAs, even in the enzyme-free control assays. The present experiment was designed to identify the radioactive survivals and their impact on the biological measuring process.

Procedure: Seven [¹⁻¹⁴C] FAs (16:0, 18:1n-9, 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3, 22:6n-3) were mixed with albumin and assayed for β -oxidation in rat liver homogenates. The initial working media containing FA-albumin compound and the final clarified acidic solution containing oxidation products were analyzed by LCMS/MS. The experimental error induced by the radioactive survivals was also evaluated.

Results: Using LCMS/MS, the radioactive survivals were identified as unbound free FAs. The percentage of unbound FAs recovered in clarified acidic solution was lower than 2% with 16:0 and 18:1n-9, nearly 5% with EPA, 7% with 18:2n-6, 18:3n-3 and 20:4n-6, and dramatically high to 41% with DHA. Various FA/albumin ratios as well as different types of albumin only marginally affected these data. Thus, the big mass of unbound free PUFA led to a high experimental error for the results, particularly unbound DHA causing 60 times higher error than the real value in the procedure of β -oxidation measurement *in vitro*.

Conclusion: High amount of unbound free PUFA in the *in vitro* system will interfere with the results and even cover the designed purpose. In the design of future FA research *in vitro*, the binding capacity of FA to albumin or other proteins must be considered, especially for DHA research.

P046

EPA and DHA differentially modify the organization of non-raft and raft domains of immune cells

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Background: An emerging molecular mechanism by which n-3 PUFAs modify immune cell function is through modification of lipid raft organization. However, the effects of n-3 PUFAs on non-raft formation are poorly defined, despite data from model membrane studies to show that n-3 PUFAs localize to non-rafts and form their own organizationally distinct domains.

Objective: We tested the effects of the n-3 PUFAs eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids on non-raft versus raft organization of EL4 cells, in comparison to other cell types.

Procedure: A combination of quantitative imaging methods on the nanometer and micron length scales were used.

Results: Consistent with model membrane studies, n-3 PUFAs mostly incorporated into non-raft domains of EL4 cells. FRET microscopy showed that EPA and DHA did not modify nanoscale lateral organization of the non-raft protein major histocompatibility complex (MHC) class I in EL4 cells. On a micron scale, live and fixed cell microscopy showed that EPA more than DHA, compared to controls, enhanced non-raft formation of EL4 cells, as measured by binding of the non-raft probe FAST DiI. Similar observations were made with 1153 and primary B220⁺ B cells. The increase in non-raft formation with the n-3 PUFAs also correlated with an increase in EL4 cell size and increased proliferation and survival. EPA and DHA also differentially modified lipid raft organization. DHA, but not EPA, diminished lipid raft clustering, increased raft size, and increased co-localization of the non-raft MHC class I into lipid rafts of EL4 cells.

Conclusion: Taken together, we propose a new model to explain how EPA and DHA differentially modify both non-rafts and rafts on several length scales to trigger changes in protein lateral organization and ultimately immune cell function. Our proposed model on rafts and non-rafts with n-3 PUFAs unifies contradictory data from model membrane and cellular studies.

P047

Metabolism of EPA, DPA and DHA in rats

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Background: The metabolic fate docosapentaenoic acid (DPA) is understudied compared with that of

eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The extent of metabolism of DPA to DHA and beta-oxidation is not known from in vivo studies.

Objective: To investigate the effect of oral administration of pure DPA (unlabelled and C14-labelled) compared with that of EPA and DHA in young male Sprague Dawley rats.

Procedure: Experiment 1: Rats weighing 70-80 g were given 50 mg oral doses of DPA, or EPA, or DHA or oleic acid (OA) for 7 days and tissues examined for DHA levels. Experiment 2: Rats weighing 70-90 g were given a single oral dose of 2.5 μ Ci of C14-labelled DPA, or EPA, or DHA or OA, placed in a metabolic chamber for 6 hrs to collect the CO₂ and then killed at 18 hrs to determine the incorporation of the label into tissues.

Results: In experiment 1, DPA treatment led to a significant increase in DHA in liver; DPA was also retro-converted to EPA in most tissues. In experiment 2, both OA and EPA treatments lead to significantly greater label in the collected CO₂ after 6 hrs compared with DPA & DHA. In liver tissue, label from the omega 3 fatty acids was incorporated between 9-17x more than OA. In the brain, the differential between the omega 3 fatty acids and OA was 5-8x greater, while in heart tissue the differential was >10x. Labelling in the liver was more than 14x that of brain tissue on a per gram basis.

Conclusions: DPA is metabolised to DHA and EPA in young rats. Labelled DPA is significantly less beta-oxidised than EPA and in addition is incorporated into liver & brain tissue similarly to label from EPA & DHA.

P048

Effect of dietary fatty acids on expression of lipogenic enzymes and fatty acid profile in tissues of bulls

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Background: PUFAs, in particular C18:2n-6 and C18:3n-3, provided by diet, regulate gene expression of lipogenic enzymes, like SCD, ACC and Δ 6-desaturase.

Objective: We investigated the long-term effect of C18:2n-6 and C18:3n-3 feeding on lipid metabolism in German Holstein bulls.

Procedure: 14 German Holstein bulls were assigned to two treatment groups. Eight control animals were fed a C18:2n-6 (soybean meal, maize silage) and six experimental animals a C18:3n-3 (linseed oil, rapeseed cake, grass silage) enriched diet. Bulls were slaughtered at a live weight of 621.1 \pm 10.4 kg and 627.7 \pm 12.0 kg. Longissimus muscle and subcutaneous adipose tissue samples were taken directly after slaughter. Fatty acid

methyl esters were analyzed by gas chromatography. Protein expression of SCD, ACC and Δ 6d was measured by Western blotting.

Results: In longissimus muscle of experimental group C18:3n-3 and the sum of n-3 fatty acids are significantly higher than in control group. C18:2n-6 and the sum of n-6 fatty acids are significantly increased in control compared to experimental group. C18:1cis-9 tended to be lower in experimental compared to control group (P=0.09). The sum of total fatty acids, C18:1cis-9, C18:2n-6 and the sum of n-6 fatty acids was significantly reduced in subcutaneous adipose tissue of experimental compared to control group. The ratio n-6/n-3 was significantly lower for both tissues in experimental group.

Protein expression in longissimus muscle of SCD and Δ 6d was significantly reduced in experimental group (37% and 33%, P=0.03). In subcutaneous adipose tissue SCD expression was reduced by 29% (P=0.02). ACC was not affected in both groups and tissues.

Conclusion: A long-term feeding of C18:2n-6 and C18:3n-3 supplemented diets results in an accumulation of these essential fatty acids in tissues. ACC, SCD and Δ 6d are expressed in a tissue-specific way but relatively higher in adipose tissue. The supplementation of C18:3n-3 induced a reduction of protein expression tissue depending.

P049

The changes of membrane lipid microviscosity as affected by alpha-tocopherol in a wide range of concentration

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Background: Vitamin E or alpha-tocopherol (TP) plays important role in cell regulation as inhibitor of lipid oxidation and structural factor in biological membranes. Objective was to study the effect of TP in a wide range of concentration: from high to ultra-low concentrations (ULC) on the structural parameters of membrane lipids. **Procedure:** TP prepared by successive dilutions in ethanol-aqueous solutions. The plasmatic (PL) and endoplasmic reticulum (ER) were isolated from liver cells of mice. The lipid structural parameters were studied by EPR-technique on spectrometer Bruker-200D using two spin-probes: 5- and 16-DSA) localized in the surface and depth of membrane lipid bilayer correspondingly. From EPR spectra obtained it was calculated the rotation correlation time (tc) of 16-DSA - micro-viscosity value of the hydrophobic lipid regions and order parameter (S) of 5-DSA.

Results: It was found three "waves" of micro-viscosity increase (10 in minus 4-10 in minus 9) M (equivalent to physiological concentrations) which can be explained by incorporation of TP in the membrane lipids; in the range

of ULC (10 in minus 9 -10 in minus 18) M, which may be related with the changes or formation of the micro-domains and rafts, and lower than 10 in minus 18 M (ultra-low dilutions - ULD) discussed regarding the role of water structure. It was shown an increase of parameter S in the range at (10 in minus 4 -10 in minus 5) M and its extremely changes in the range of (10 in minus 10 - 10 in minus 18) M TP correlated with activity of protein kinase C (PKC) observed for both membranes (PL and ER). **Conclusion:** The effect of TP in a wide range of concentration is described by the phase mode of the dose-effect curves (obtained in the different lipid membrane regions), which is typical to drugs affecting at ULC.

P050

Phenosan potassium salt can modify lipid domains of liver plasmatic membranes using in ultra low concentraions

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Background: Phenosan potassium salt (PPS), synthetic antioxidant, can effect on a number of normal and pathological processes in cells using in ultra low concentrations (ULC) - 10^{-14} - 10^{-18} M. But the mechanism of the phenomena is still unknown. There is a hint that plasmatic membranes where important control systems are localized could be a targert of PPS in ULC.

Objective: The aim of the paper was to study in vitro effect of PPS in a wide range of concentration (10^{-4} - 10^{-21} M) on structural features of lipid domains of PM isolated from mice liver cells.

Procedure: The structural changes in membranes were detected with EPR-spectrometer "Bruker-200D" (Germany) by spin probe procedure using stable nitroxyl radicals 5- and 16-doxylstearic acids (C5 and C16) to examine the lipid bilayer for changes of rigidity of the surface regions (8Ao) and microviscosity of deep regions (20 Ao) correspondingly.

Results: PPS induced statistically reliable increases both the rigidity of the surface lipid bilayer regions (C5) and microviscosity of deep membrane areas (C16). The dependence effect on concentration had complicated non-linear character with 2 maxima (10^{-6} - 10^{-7} ; 10^{-14} - 10^{-15} M) for the area of C5 localization and 2 maxima (10^{-5} - 10^{-7} ; 10^{-18} - 10^{-19} M) for C16 responsible domain. We also observed the presence of so-called dead intervals on concentration curves, in which effects were absent. The both spin probes gave results which indicate an appearance of additional thermoinduced structural transitions in membranes treated by ultra low doses of

PPS occurring at the temperature (22-26° C) for probe C5 (10^{-14} and 10^{-15} M) and at (40-42°C) for probe C16 (10^{-18} M). **Conclusion:** Plasmatic membrane could be one of the target of PPS effect in ULC.

P051

Assessment of lipid oxidative damage: which is the most reliable method?

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The oxidation of lipids is implicated in aging and the pathogenesis of atherosclerosis, cardiovascular, neurodegenerative and lung diseases, as well as cancer. To date, a limitation has been that most methods that quantify in vivo lipid oxidation lack sensitivity and / or specificity. This presentation will review and evaluate current available methods for assessing lipid oxidation. Although there are a number of assays available for assessing lipid oxidation, to date, the F₂-Isoprostanes (F₂-IsoP) represent the most reliable in vivo biomarkers of oxidative stress and lipid peroxidation. F₂-IsoP are formed in vivo from free radical peroxidation of arachidonic acid and are isomeric in structure to cyclooxygenase-derived PGF_{2α}. Elevated F₂-IsoP have been detected in atherosclerotic plaques and associate with smoking, hypercholesterolaemia, diabetes mellitus, obesity, alcohol consumption, hypertension, hyperhomocysteinaemia and male gender. Several F₂-IsoP are also biologically active, suggesting they may function as pathophysiological mediators of oxidant injury. Gas chromatography-mass spectrometry (GCMS) is the gold standard for measurement of F₂-IsoP.

The F₃-isoprostanes (F₃-IsoP) and F₄-isoprostanes (F₄-IsoP, neuroprostanes) are derived from free radical oxidation of the omega-3 fatty acids EPA and DHA, respectively. Although F₃-IsoP are formed in vitro from oxidation of EPA, they have not consistently been detected in vivo following consumption of omega-3 fatty acids. In contrast, F₄-IsoP are formed from DHA in vitro and in vivo, and likely represent biomarkers of oxidative injury in the brain. F₄-IsoP are present in human cerebrospinal fluid and elevated in Alzheimer's disease. Lipid peroxidation products termed isofurans (IsoF), are formed under conditions of high oxygen tension. It has been suggested that the measurement of IsoF provides a sensitive index of oxidative stress in situations of elevated O₂ tension, whereas IsoP does not.

The combined measurement of both F₂-IsoP and IsoF likely represents the most reliable approach to assess lipid oxidative stress under all circumstances.

P052**Fatty acid desaturase alternative splice variants are widely expressed and localize to the mitochondria**

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Background & Objective: Alternative splicing is the key mechanism for expansion of genomic function in multicellular organisms. We recently described alternative transcripts (AT) of FADS2 and FADS3 and sought to characterize their distribution in neonatal baboon tissues, in other mammals, and in the chicken, and their subcellular localization.

Procedure: PCR primers for the baboon FADS AT were designed and synthesized, and used to amplify cDNA from liver tissue of several vertebrate species. For subcellular localization studies, FADS AT sequences analyzed with Proteome Analyst Specialized Subcellular Localization software indicated that FADS3 and some AT may localize in the mitochondria (mt). GFP-tagged plasmid constructs of FADS classical transcripts and selected AT were used to transfect neuroblastoma cells, and dyes localized the endoplasmic reticulum (ER) and mt. Confocal microscopy revealed the distribution of GFP-protein, ER, and mt.

Results: FADS AT are highly and differentially expressed in 12 different neonate baboon tissues. FADS2 AT1 and FADS3 AT(1,3,4,6,7) were detected in liver of mouse, pig, dog, fox, horse, and chicken. A primer specific for the mouse FADS3 AT5, which includes a section of an intron, was also positive. GFP analysis showed that FADS3AT1 and FADS3AT3, as well as FADS2, all localize to the mt. GFP analysis further revealed that all FADS proteins (1, 2, 2AT1, 3, 3AT1, 3AT3) localize to the ER.

Conclusion: FADS AT are conserved among mammalian species and in the chicken. They are selectively localized in the mitochondria in human cells. Although their function is not yet understood, these studies are further evidence that they play an important metabolic role.

P053**Effects of polyunsaturated fatty acids on the cell cycle and the expression of bHLH transcription factors in neural stem cells**

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Background: Polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) play an important role in neuronal development and function, including neurite outgrowth, signal transduction, and membrane fluidity. We have demonstrated that DHA promotes neurogenesis by regulating basic helix-loop-helix (bHLH) transcription

factors and the cell cycle of neural stem cells (NSCs). However, the effects of EPA and AA on differentiation of NSCs are not known.

Objective: We examined the effect of EPA and AA on proliferation and differentiation in the NSCs, and also investigated the effects of DHA, EPA and AA on the expression of bHLH transcriptional factors and cell cycle in NSCs.

Procedure: NSCs from E14.5 rats were cultured as neurospheres in N2 medium containing basic fibroblast growth factor (bFGF). For the differentiation, the cells were cultured in the bFGF free medium for 1, 4, and 7 days with or without DHA, EPA and AA. mRNA levels of bHLH transcription factors (Hes1, 6, NeuroD) were determined by Real-time PCR. Number of neurons was counted from pictures of immunostaining with Tuj-1. FACS analysis was used for determined the cell cycle.

Results: The number of Tuj-1 positive cells increased in the presence of EPA but not AA. Treatment with EPA increased Hes1 mRNA level on day 1. AA treatment for 4 days decreased the Hes1 mRNA level. The Hes6, an inhibitory factor of Hes1, was increased by EPA treatment but not with AA. DHA and EPA treatments increased the mRNA levels of NeuroD, while AA treatment did not affect. Treatment with EPA or AA decreased the percentage of S phase cells in NSCs.

Conclusion: The present study suggests that EPA affects neuronal differentiation by modulating cell cycle. n-3 PUFA promote neurogenesis of NSCs but that ways may be among substances different each other.

P054**Cultivation of Caco-2 cells in a serum-free medium allows an extensive biotransformation of C18 polyunsaturated fatty acids**

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Background: In human tissues, C18 polyunsaturated fatty acids are partly biotransformed into longer and/or more unsaturated fatty acids. This process may already take place in the intestinal tissue during their absorption.

Objective: The aim of the present study was to compare the biotransformation of three polyunsaturated C18 fatty acids and their distribution in the different lipid classes of human epithelial Caco-2 cells, a well validated model of the intestinal barrier, upon cultivation in absence or in presence of 10% fetal bovine serum (FBS).

Procedure: Confluent Caco-2 cells were incubated for 7 days in the presence of either linoleic acid (C18:2 n-6, LA), α -linolenic acid (C18:3 n-3, ALA) or rumenic acid (CD18:2, RA) at 30 μ M. Cells were cultivated either in DMEM containing 10% FBS or in hormono-defined serum-free BDM.

Results: Upon cultivation in serum-free BDM, the appearance of C20:3 n-6, C20:4 n-6 and C22:4 n-6 from LA and of C20:4 n-3, C20:5 n-3, C22:5 n-3 and C22:6 n-3 from ALA was observed. Upon cultivation in FBS-DMEM, the bioconversion of added fatty acids was limited to an elongation step leading to the formation of C20:2 n-6 and C20:3 n-3 from LA and ALA, respectively. This study also evidenced that RA underwent, to a certain extent, the same metabolic pathway as LA and ALA. It was converted either into CD20:3 or CD20:2, depending on the culture condition. Finally, we observed that, for a same position in the metabolic pathway, LA, ALA and their respective metabolites were similarly distributed between neutral lipids or phospholipids. RA and its metabolites were, as for them, lightly more distributed into the neutral lipid fraction.

Conclusion: This study shows that the metabolic conversion of LA, ALA and RA is more efficient upon cultivation in serum-free BDM than in FBS-DMEM.

P055

Cytoprotective effects of polyunsaturated fatty acids on beta cell viability

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Background and Objectives: Certain monounsaturated fatty acids (MUFA) have been shown to protect pancreatic beta-cells against cytotoxic stimuli such as saturated fatty acids and serum deprivation. The objective of the present study was to investigate whether polyunsaturated fatty acids (PUFA) exhibit similar cytoprotective effects and to consider the mechanisms involved.

Procedure: Clonal rat beta-cells (BRIN-BD11) were treated with fatty acids complexed to serum albumin and cell death analysed by flow cytometry.

Results: Several PUFAs (including gamma linolenic acid (gLA), eicosapentaenoic acid (EPA), arachidonic acid, docosahexaenoic acid (DHA) and adrenic acid) potently and dose-dependently protected beta-cells against cytotoxicity (EC50: gLA - 10µM; EPA - 1µM; arachidonic acid - 1µM; DHA - 2.5µM; Adrenic acid - 1µM). By contrast, two structurally unrelated non-metabolisable analogues of arachidonate, ETYA and AACOCF3, were not cytoprotective. Incubation of BRIN-BD11 cells in the presence of various lipoxygenase or cyclo-oxygenase inhibitors (e.g. 10µM NDGA or 10µM indomethacin) did not alter the cytoprotection exerted by PUFAs. Surprisingly, however, AACOCF3 dose-dependently attenuated the cytoprotection afforded by a range of PUFAs. AACOCF3 has been reported to inhibit PLA2 but two additional

inhibitors of this class of enzyme (bromo-enol lactone and PACOCF3) did not reproduce the blockade achieved with AACOCF3.

Conclusion: Certain PUFAs exert potent cytoprotective actions in pancreatic beta-cells but metabolism of these fatty acids to lipoxygenase or cyclo-oxygenase derivatives is not required for this effect. The arachidonic acid derivative, AACOCF3, did not reproduce the cytoprotective actions of arachidonic acid but, rather, it attenuated the protective response afforded by other PUFAs. This effect is unlikely to reflect the inhibition of PLA2 by AACOCF3 but the data suggest that this agent acts to antagonise a still undefined site of action of PUFAs in pancreatic beta-cells.

P056

Investigation of the substrate- and stereoselectivity of adipose triglyceride lipase

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Background Diacylglycerols (DAGs) and free fatty acids (FFAs) are intermediary products in lipid metabolism. More recently, they have been recognized as important signaling molecules, activating protein kinase C (PKC) and specific classes of nuclear receptors, respectively. DAGs and FFAs may derive from lipolytic breakdown of phospholipids and TAGs. DAGs, are chiral molecules with three different stereospecific conformations (sn-1,2 DAG, sn-2,3 DAG and sn-1,3 DAG). FFAs occur physiologically as heterogenous group differing in chain-length and saturation.

Objective The aim of this study was to investigate the FFA species and stereospecific conformation of DAGs generated by adipose triglyceride lipase (ATGL)-mediated hydrolysis of TAGs.

Procedure Chiral-HPLC analysis as well as GC/MS-analysis were used to determine chirality of DAGs and FFA-species, respectively.

Results ATGL specifically hydrolyzed the second ester bond of TAG leading to the formation of sn-1,3 DAG. Furthermore, ATGL stimulated by its coactivator CGI-58 hydrolyzed the first and second ester bond, resulting in the formation of sn-2,3 DAG or sn-1,3 DAG, respectively. Analysis of FFA release from fat pads of wildtype, hormone-sensitive lipase (HSL)-deficient, and ATGL-deficient mice indicated that ATGL catalyzes predominantly the cleavage of unsaturated FFA.

Conclusion In summary, our data suggest that ATGL hydrolyzes TAG in a stereospecific as well as fatty acid species specific manner. Thus, we speculate that these ATGL-derived lipolytic intermediates may effect a variety of cellular signaling pathways.

P057**Alpha-synuclein association with lipids modulate its conformation and aggregation process**

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Background: Parkinson's Disease is a progressive neurodegenerative pathology present in more than 1% of the population older than 65 years. This disease is characterized by amyloid aggregates of proteins known as Lewy bodies, alpha-Synuclein (AS) being the most abundant component. AS is also of particular interest because it belongs to the group of natively unfolded proteins.

Objective: To investigate the role of the lipids representative of neuronal tissue on the putative conformational changes that may trigger the pathological aggregation of AS we conducted experiments evaluating the interactions of monomeric and different stages of aggregated AS with fatty acids (FA) as well as with phospholipidic membranes.

Procedure: We employed AS labeled at positions 18, 90 or 140 with a solvatochromic fluorescent dye of 3-hydroxyflavone family (which reports local environmental polarity and H-bonding) to characterize the binding of monomeric AS to membranes. The aggregation process of AS was followed in parallel by standard ThioflavinT assay, vesicle leakage of ANTS/DPX complexes, Static and Dynamic Light Scattering and Atomic Force Microscopy in the presence of saturated (SFA), monounsaturated (MUFA) and polyunsaturated FA (PUFA).

Results: Membrane charge and curvature, phospholipid phase and the degree of unsaturation of the FA are important factors that modulate AS conformation and binding to lipids. In particular, AS seems to preferentially bind to negatively charged membranes or neutral phospholipids in the gel phase, and its conformation in the interface depends on the properties of the membrane and the protein/lipid ratio.

Conclusions: The fluorescent probes employed can clearly discriminate monomeric AS bound to membranes of different composition, and the labels at different positions also allowed us to estimate the relative immersion of different regions of the protein into the membranes. The presence of FA have different effects on the aggregation kinetics of AS.

P058**The menstrual cycle and use of oral contraceptives have significant effects upon circulating long chain polyunsaturated fatty acids**

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Background: There are gender differences in the ability to convert α -linolenic acid to long chain (LC) n-3 polyunsaturated fatty acids (PUFA), reflected by higher circulating docosahexaenoic acid (DHA) levels in women, with evidence that sex hormones have a role in the up-regulation of LC PUFA biosynthesis pathways.

Objective: This study investigated the effect of the menstrual cycle and oral contraceptive use on LC PUFA in plasma phosphatidylcholine (PC) in young women (18-40yr) consuming < 2 portion/week oily fish. **Procedure:** Fasted blood samples were collected at mid- and end-cycle for women not using oral contraceptives (n = 30) and on day 21 of the pill phase for women using the contraceptive pill (n = 21). The fatty acid composition of plasma PC was analysed by gas chromatography.

Results: γ -linolenic acid (GLA) content of plasma PC was significantly higher mid-cycle than end-cycle among women not using oral contraceptives (P = 0.045). Women using the contraceptives pill had a significantly higher plasma PC content of GLA (P < 0.05), di-homo-GLA (P < 0.001) and arachidonic acid (P < 0.001) than women not using oral contraceptives, and significantly lower plasma PC linoleic acid (P = 0.048) than end-cycle samples from women not using oral contraceptives. Women using the contraceptive pill had significantly lower plasma PC docosapentaenoic acid (DPA) content than mid-cycle samples from women not using oral contraceptives (P = 0.025). No significant differences in plasma PC DHA levels was found due to the menstrual cycle or oral contraceptive use.

Conclusion: There is a significant effect of the menstrual cycle and use of oral contraceptives upon circulating LC PUFA status. This provides further evidence that variations in female sex hormone status, either due to the menstrual cycle or the use of oral contraceptives, can influence LC PUFA status.

P059**Cultured human intestinal cells take up and metabolize branched chain fatty acids**

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Background & Objective: We recently showed differences in the distribution of branched-chain fatty acids (BCFA) in the vernix and meconium of healthy newborns. BCFA with 12 to 26 carbons were detected in vernix but those with less than 16 carbons were not found in meconium. In this study we tested the hypotheses: 1. BCFA are taken up and metabolized by the enterocyte; 2. BCFA affect genes expression involved in fatty acid (FA) metabolism. Caco-2 cells in culture were studied.

Procedure: For the uptake study, Caco-2 cells were grown on 6 well plates for 14 days. Cells were incubated with graded levels of 4 BCFA or control for 4 hours, and were harvested for determination of lipid classes and for FA analysis. For gene expression studies, Caco-2 cells were grown in flasks and incubated for 24 hours with a mixture of 5 BCFA or a control fatty acid at 7 and 14 days post seeding. RNA was reverse-transcribed into cDNA which was subjected to semi-quantitative PCR.

Results: BCFA were taken up by Caco-2 cells and were differentially incorporated into cellular lipid fractions. Small amounts of elongation products were detected. The expression of ELOVL3 (very long chain fatty acid elongase) and BCAT (branched-chain amino transferase) was higher at BCFA-treated cells at 7 days compared to 14 days post seeding, and there was no notable changes in gene expression for the control cells.

Conclusion: Caco-2 cells take up BCFA and differentially incorporate them into lipid classes. Selectivity in lipid class deposition indicates that they play some metabolic role and are not completely burned for energy. The time dependence of BCFA-related genes indicates that handling of BCFA is under developmental control.

P060

The methylene-interrupted cis double-bonded PUFA (homoallylic) motif may form a double helix with transmembrane protein alpha-helices

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Background & Objective: The methylene-interrupted, all-cis (Z) bond configuration is overwhelmingly favored in PUFA of vertebrate membranes, particularly the highly unsaturated membranes of electrically-active neural tissue. There is no coherent theory to explain this dominance. We investigated the hypothesis that hexaene and pentaene homoallylic groups are sterically possible using energy minimization and molecular dynamics simulations with the GROMOS96 force field.

Procedure: Two phosphatidylcholines PC226345(ω 3) and PC225(ω 6)345(ω 3) were used to simulate n-3 and n-6 PUFA within relevant molecules. Helix 2 of rhodopsin (Protein Database: 1L9H) was used with the lipids configured as a hetero-double helix for initial conditions. Molecular dynamics simulations were performed with the GROMACS package. The steepest descent algorithm was used for energy minimization. The time step used in molecular dynamics was 2 fs, and the particle-mesh Ewald method was used for the calculation of long-range electrostatic interactions.

Results: The double-bonds of the helical homoallylic group forms an inclined planar structure along the α -helix groove. The α -helix groove spacing is 2.8-5.3 Å between hard sphere atoms in consecutive turns. The sp² HCCH is

planar, while the maximum distance required to accommodate the doubly allylic H's 2.4 Å, using hard sphere diameters. The doubly allylic H's point directly inward and interact most closely with the α -helix, while the planar sp² HCCH assumes an inclined plane running along the α -helix groove and point outward. Homoallylic (unsaturated) regions tended to maintain their positions within the groove for a longer distance than saturated regions.

Conclusion: These results confirm and extend preliminary results (ISSFAL-Cairns) indicating that a previously unreported interaction between membrane PUFA and transmembrane helices is possible. This interaction may play a role in anchoring active proteins to the membrane and explain the favorability of moderately and highly unsaturated homoallylic hydrocarbon chains.

P061

DHA decreases endothelial free radical production via inhibition of sPLA2

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Background: Inflammation plays a relevant role in the onset and development of atherosclerosis and cardiovascular disease. Long-chain n-3 (or omega 3) fatty acids, namely docosahexaenoic acid (DHA, 22:6n-3), have been attributed cardioprotective properties; yet, the extent and precise nature of such activities are to be elucidated.

Objective: To unveil part of the intracellular machinery responsible for the anti-inflammatory and antioxidant effects of DHA.

Procedure: We investigated the vascular anti-inflammatory and antioxidant activities of DHA by evaluating its modulation of the two enzymes most involved in vascular inflammation, i.e. endothelial secreted phospholipase A2 (sPLA₂) and NADPH oxidase 4 (NOX) 4.

Results: A 48-h exposure of human aortic endothelial cells (HAEC) to DHA led to its preferential incorporation into outer leaflet phospholipids. Pre-treatment with DHA abolished HAEC stimulation induced by A23187 and Ang II, whereas the effects on IL-1 β treatment were less pronounced, though still statistically significant. Group V sPLA₂ RNA was similarly modulated by DHA supplementation. In addition, DHA decreased Nox 4 expression and activity; this effect was associated with reduced production of reactive oxygen species. Further, the use of specific inhibitors allowed demonstrating that group V sPLA₂ is involved in the downregulation of Nox 4 expression and activity by DHA; notably, inhibition of sPLA₂ blocks the effects of DHA on Nox 4 expression. This interplay is mediated by ERK and PKC, whose inhibitors

downregulated Nox 4-mediated induction (both activity and expression) of group V sPLA₂.

Conclusion: These studies provide novel mechanistic insights into the anti-inflammatory and vasculo-protective effects of DHA.

Lipids and Nutrition

P062

Long chain polyunsaturated fatty acids stimulate cellular fatty acid uptake in human placental trophoblasts through acyl-CoA synthetases

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Background: Supplementation of long-chain polyunsaturated fatty acids (LCPUFAs) is advocated during pregnancy in some countries although very little information is available on their effects on the uptake ability of placenta for LCPUFAs.

Objectives: To identify the effects of LCPUFAs on placental fatty acid uptake, we examined the effects of LCPUFAs on the uptake of fatty acids and expression of fatty acid transport/metabolic genes in placental trophoblast cells (BeWo).

Procedures: Following 24h incubation of M of LCPUFAs (AA, 20:4n-6, EPA, 20:5n-3, or DHA, 22:6n-3) mBeWo cells with 100 and oleic acid, OA, 18:1n-9, the cellular uptake of different [¹⁴C]fatty acids (EPA, AA, DHA and OA) and their incorporation into different cellular lipid fractions was determined. In parallel, expression of relevant lipid uptake/metabolic genes was measured under similar experimental conditions by Taq Man Gene Expression assay.

Results: Preincubation of these cells with LCPUFA stimulated fatty acid uptake by 20-50% and accumulated fatty acids were preferentially incorporated into phospholipid fractions. OA, 18:1n-9, on the other hand, could not stimulate fatty acid uptake. LCPUFAs and OA increased the gene expression of lipid droplet gene, ADRP whilst decreased the expression of ACSL3, ACSL4, ACSL6, LPIN1, and FABP3 in these cells. However, LCPUFAs but not OA increased expression of ACSL1 and ACSL5. Since acyl-CoA synthetases are involved in cellular uptake of fatty acids via activation for their channelling to lipid metabolism and/or for storage, the increased expression of ACSL1 and ACSL5 by LCPUFAs may be responsible for the increased fatty acid uptake.

Conclusions: Our data demonstrate that LCPUFA may function as an important regulator of general fatty acid uptake in placental trophoblast cells and may thus have impact on fetoplacental growth and development.

P063

Effect of dietary n-6 PUFA on tissue levels of arachidonic acid and docosahexaenoic acid in adult humans: a systematic review

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Background: Linoleic acid (LA), with a dietary reference intake of 11-17g/d, is the most commonly consumed polyunsaturated fatty acid (PUFA) in the Western diet. The concern is that LA is the metabolic precursor of arachidonic acid (AA) and its consumption may enrich tissues with AA and contribute to chronic and overproduction of bioactive eicosanoids derived from AA. Similarly, there is a concern that dietary LA may interfere with the production and incorporation of the long chain n-3 PUFA into tissues, in particular, docosahexaenoic acid (DHA).

Objective: To determine if changing dietary LA levels modifies tissue AA or DHA levels in adult humans.

Procedure: In this study, we systematically reviewed the human literature that reported changes in dietary LA and its subsequent impact on changing tissue AA and/or DHA in erythrocytes and plasma phospholipids of adults.

Results: Decreasing dietary LA up to 90% failed to modify tissue AA levels (p=0.39). When dietary LA levels were increased 6 fold, no changes in tissue AA levels were observed (p=0.72). Similarly, decreasing (by 90%) or increasing (6 fold) dietary LA failed to modify tissue DHA levels (p=0.39 and p=0.55, respectively). However, when dietary AA was supplemented to diet, dietary AA significantly increased tissue AA levels, suggesting conversion of dietary LA to AA and not the absolute levels of AA in the tissues is rate-limiting (within the context of doses outlined in this study).

Conclusion: Our results suggest that modifying current intakes of dietary LA have no effect on changing tissue levels of AA or DHA in adults.

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P064

The effects of EPA, DHA, and aspirin ingestion on plasma lysophospholipids and autotaxin

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Background: Lysophosphatidylcholine (LPC) and lysophosphatidic acid (LPA) are potent lysolipid mediators increasingly linked with atherosclerosis, neoplasia, and inflammation. LPA can be produced from LPC by the action of autotaxin, but it is not known if this is the major source of LPA in human plasma.

Objective: The primary goal is to characterize the effects that eicosapentaenoic acid/docosahexaenoic acid (EPA/DHA), and aspirin have on plasma LPA, LPC, and autotaxin in healthy adults.

Procedure: We conducted an open-label, four-week, sequential therapy trial of EPA/DHA and aspirin: Day 1, baseline blood sampling followed by single oral dose of 650 mg aspirin; Day 2, blood sampling followed by initiation of daily EPA/DHA (4 g/d, Lovaza; n-3 long-chain polyunsaturated fatty acid {LCPUFA}); Day 29, blood sampling followed by 650 mg aspirin; Day 30, blood sampling. Plasma LPC and LPA species were determined using modified Bligh and Dyer extraction and direct infusion electrospray MS/MS. Autotaxin activity was measured with an assay using a fluorescently labeled LPC analog, with a “dequenching” motif, as substrate.

Results: The distribution of LPC and LPA acyl species differed significantly in all samples, suggesting that LPC is not the major precursor of LPA. Of 11 major acyl LPC and LPA species measured, only LPC-EPA and LPC-DHA concentrations increased significantly with LCPUFA supplementation, while LPC-adrenic acid decreased, and autotaxin was unaffected. Aspirin ingestion had no significant effect on LPC, LPA, and autotaxin. EPA and DHA LPC, but not LPA concentrations, respond to EPA and DHA ingestion, suggesting that the majority of LPA is either derived from acyl selective hydrolysis of LPC, or by mechanisms other than direct conversion from LPC.

Conclusion: Our data demonstrate that plasma LPC, but not LPA, species can be dynamically regulated by dietary supplementation, and argue against a simple model of LPA generation via LPC hydrolysis.

P065

Association of higher omega-6/omega-3 fatty acids in the diet with higher prevalence of metabolic syndrome in North India

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Background: The epidemic of obesity, over the last two decades, in the middle and high income countries is associated with marked rise in the incidence of metabolic syndrome

Objective: To measure the prevalence of metabolic syndrome (MS) and determine its association with ratio of omega-6/omega-3 fatty acids in the diet.

Design and methods: Cross-sectional surveys were conducted in 20 urban streets in the city of Moradabad, India. We contacted 2422 urban subjects aged 25 yrs and above, of which 220(9.08%) refused to participate and rest 2002 (1016men and 986 women) volunteered to be included in the study. Randomly selected subjects with MS aged 25 years and above were evaluated and graded according to omega-6/omega-3 ratio in the diet. Physical examination, sphygmomanometer, questionnaire and blood tests were done.

Results: The overall prevalence of MS was 19.3% (n=387) without any gender difference. The prevalence of MS, type 2 diabetes, CAD and hypertension showed a higher rate, in relation to omega-6/omega-3 ratio in the diet. Subgroup analysis showed that subjects eating low omega-6/omega-3 ratio (<5.0) diets had significantly lower prevalence of MS, and related components compared to higher ratio diets, among both sexes. Multivariate logistic regression analysis after adjustment of age showed, that hypertriglyceridemia (odds ratio 0.90 in men, 0.76 in women) was strongly (P<0.01) associated with MS. Hypertension, HDL-C, and central obesity were weakly associated with MS in both sexes. Hypercholesterolemia was weakly associated with MS only in women.

Conclusion: MS has become a public health problem in India. Higher omega-6/omega-3 ratio is a major risk factor of MS and CAD. It is possible that a low omega-6/omega-3 ratio in the diet (<5.0) may be protective against MS and related components.

P066

Association of dietary ω-6/ω-3 fatty acid ratio and inflammation with risk of hip fracture

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Objectives: There is evidence that ω-3 fatty acids intakes are inversely associated with risk of osteoporosis and hip fractures. We examined the association of food consumption pattern and ω-6/ω-3 fatty acid ratio of the diet with hip joint fractures.

Methods: Sixty cases having fracture neck of femur and 95 control subjects above 50 years of age were included in this case study. Dietary intakes were obtained by 3 days assessment of food intakes by questionnaires among patients with fractures(n=50) and in 95 controls.

Cytokines were measured by chemoluminescence enzyme immunometric assay (immulite automated analyzer) kit (DPC Los Angeles, CA, USA).

Results: Among 60 cases, the fracture was more common in male than female. Fruits, vegetables and legume (165 ± 12.6 vs. 205 ± 15.8 g/day, $P < 0.03$) as well as milk products (milk, curd, butter etc) consumption (205 ± 25.8 vs. 318 ± 31.5 g/day, $P < 0.05$) were significantly lower and ω -6 rich oils intake was significantly higher among patients with fractures compared to controls. respectively. Omega-3 fatty acids intakes were significantly lower among patients with fractures (0.45 ± 0.74 g/day, $P < 0.05$). Osteoporosis (92.0%), trivial trauma (92.0%), physical inactivity (80.0%), diabetes mellitus (21.6%) were common among patients with hip fracture. Multivariate logistic regression analysis showed that the intakes of fruit, vegetable and legume (odds ratio 1.12, confidence interval (CI) 1.02-1.21, $P < 0.05$), physical activity (OR 1.36, CI 1.22-1.52, $P < 0.05$), ω -3 fatty acids (OD 1.05, 0.92-1.17, $P < 0.01$) intake were inversely associated with fracture, whereas ω -6/ ω -3 ratio (OD 1.33, CI 1.18-1.47, $P < 0.01$) interleukin-6, (OD 1.11, CI 1.02-1.19, $P < 0.01$), tumor necrosis factor- α (OD 1.09, CI 1.01-1.17, $P < 0.01$) were positively associated with fracture.

Conclusion: This study showed that increased consumption of fruit, vegetable and legume, milk products and ω -3 fatty acid and low ω -6/ ω -3 ratio diet as well as physical activity may be protective against hip joint fractures.

P067

Animal feeding regime and affects on meat fatty acid content: a health perspective

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Background: Lifestyle diseases such as cardiovascular disease and diabetes are often linked to animal fat intake. However little attention is paid to the fatty acid composition of meat from varying regions where animal feeding regimes can be vastly different. Historically in an evolutionary sense our ancestors consumed wild game animals, low in fat but enriched in long chain (LC) PUFA (particularly: 20:4n6, 20:5n3 and 22:5n3) for several millennia, resulting in higher intakes of LC n3 PUFA than in modern western societies.

Objective: To investigate the difference in fatty acid composition between domesticated animal meats that result from grain feeding versus pasture feeding and relate these fatty acid levels (particularly LC PUFA) to those found in wild game animals. Then to evaluate the effect of consumption of pasture fed meats on human tissue levels of LC PUFA.

Procedures: Analyses of various cuts of pasture fed and grain fed Australian beef were carried out and compared

for LC PUFA content, then compared to US beef and various wild game meats. Secondly the effect on tissue levels of LC PUFA in human subjects was investigated following intake of pasture fed animal products.

Results: Australian pasture fed beef had almost double the n3 LC PUFA levels of grain fed animals (120 versus 70 mg/100g meat respectively), compared with no recordable levels in US grain fed beef. Total n3 LC PUFA levels in Australian beef and lamb were comparable or higher than some wild game animals and white fish. Australians consuming pasture fed beef and lamb had tissue levels of n3 LC PUFA and tissue n3:n6 ratio significantly higher than corresponding age matched male vegetarians.

Conclusion: Pasture feeding beef and lamb retains levels of n3 LC PUFA similar to wild game animals and ensures higher status of these fatty acids in human consumers.

P068

Age dependence of cardiovascular risk, dietary habits and intake of omega-3 fatty acids: results from the Lipid Study Leipzig (LSL)

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Background: A population - based lipid screening study - Lipid Study Leipzig (LSL) was initiated in the city of Leipzig, Germany, and included more than 30,000 subjects.

Objective: To evaluate the cardiovascular risk profile and its dependence on age, dietary and lifestyle factors.

Procedure: LSL data were obtained from subjects recruited at community centres in the city of Leipzig. The study program included measurement of parameters of lipid metabolism, anthropometric measures and blood pressure. Seven-day diet diaries were used to assess the dietary intakes of 489 subjects (age 14 - 87 years) and analysed with the computer program PRODI 4.5 expert.

Results: LSL shows an age-dependent increase of measurable cardiovascular risk factors, which may have been partly preventable. The mean total supply of dietary fatty acids nearly corresponds to guiding values; however, their composition is improvable. The ratio of saturated fatty acids : monounsaturated fatty acids : polyunsaturated fatty acids is 1 : 0.83 : 0.35. The mean ratio of omega-6 fatty acids : omega-3 fatty acids is above 5 : 1. Depending on fish consumption the mean supply of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in young age ranges from only 0.09 to 0.19 g/d followed by an age-dependent increase.

Conclusion: In all age groups of population the average composition of dietary fats is improvable. Providing

insight into dietary habits, LSL may help to develop effective prevention strategies on population basis.

P069

Immune programming by maternal dietary fatty acids

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Background: Polyunsaturated fatty acids (PUFAs) are important immune modulating compounds. They are precursors of prostaglandins and leukotrienes and PUFAs can alter immune cell function via various mechanisms, including alteration of transcription factor activity.

Objective: In the westernized world, there has been a marked change in dietary fatty acid intake over the last decennia. Since most PUFAs are acquired from the diet and immune development occurs mainly perinatally, the maternal diet may influence fetal and neonatal PUFA status and thereby immune function. Therefore, we investigated the effect of maternal dietary ω -3 and ω -6 PUFAs on the programming of the immune system in her offspring.

Procedure: Dams were fed diets varying in C18:3 ω -3/C18:2 ω -6 ratio during gestation and/or lactation. After weaning, pups were transferred to a Western-style diet. At the age of 12 weeks, the effects of maternal PUFA-diet were examined in the adult offspring using the ovalbumin-induced allergic asthma model

Results: Significant differences in the acute allergic skin response (ASR) were observed between different diet groups and different feeding periods; all PUFA-diets lowered the ASR compared to control diet, but the high C18:2 ω -6 diet attenuated the ASR most when fed to pregnant dams, while the high C18:3 ω -3 diet was most effective when fed during lactation.

Conclusion: Both the maternal ω 3- and ω 6-PUFA-diets lowered the allergic skin response in the adult offspring, indicating a long lasting, i.e. programming, effect of the maternal diet. The mechanisms by which these diets alter offspring immune function are currently under investigation.

P070

Effects of a convenience drink fortified with omega-3 fatty acids on the omega-3 index

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Background: There is strong evidence that the intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid

(DHA) reduces the risk of adverse cardiac events. Fish and fish-oil capsules are not necessarily an ideal source of EPA and DHA for every individual.

Objective of the present study was to evaluate the effect of a convenience drink enriched with 500mg EPA and DHA on the omega-3 index, a biomarker for an individual's status in EPA and DHA.

Procedure: Of 190 subjects with atherosclerotic disease screened between February and June 2009, 50 were recruited, based on an omega-3 index <5%. The 50 participants were randomly assigned to receive either convenience drink supplemented with omega-3- (n=40), 200mg EPA and 300mg DHA, or with omega-6 fatty acids (n=10), 1.1g linoleic acid, from corn oil, daily for 8 weeks. The primary endpoint was a change in omega-3 index. Analysis was by intention-to-treat. The study was conducted according to Good Clinical Practice, and registered at clinicaltrials.gov (NCT00886704).

Results: After 8 weeks of daily intake of 200mg EPA plus 300mg DHA the omega-3 index increased significantly ($p < 0.001$) from mean $4.37 \pm 0.51\%$ to $6.80 \pm 1.45\%$. The control group showed no significant change in the omega-3 index. There was a high interindividual variability in response (Coefficient of variation of the delta=0.21) between 4.37% and 11.80%. Dose and the preparation used were safe, well tolerated and highly palatable.

Conclusion: Daily intake of a convenience drink supplemented with omega-3 fatty acids lead to a significant increase of the omega-3 index with a high interindividual variability in response.

P071

Effects of omega-3 PUFA and their oxygenated derivatives on cytokine secretion

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Background: Diets rich in long-chain n-3 polyunsaturated fatty acids, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), protect against insulin resistance and obesity in rodents and increase insulin sensitivity in humans.

Objective: The aim of this study was 1) to determine the kinetics of DHA incorporation into different mice tissues and the effects of DHA supplementation on adiponectin and leptin secretion 2) to evaluate the effects of EPA and DHA and their respective derivatives, PGD3 and PDX, an isomer of protectin D1, on adiponectin secretion by 3T3-L1 adipocytes.

Procedure: Mice were fed either a control diet or a DHA-rich diet and were sacrificed on day 0, 4, 8, 16 or 32. Some mice were fed the DHA-rich diet for 16 days and then maintained on the control diet for 16 more days

(wash out period). Additionally, 3T3-L1 cells were treated with either EPA or DHA (1, 10 and 100 μ M), or with 1 μ M PGD3 or 1 μ M PDX.

Results: DHA supplementation increased plasma adiponectin secretion by 2.4-fold as early as 4 days after initiation of the DHA-enriched-diet. Plasma leptin levels were significantly lowered after 4 day feeding with DHA. These effects were associated with a significant increase in DHA incorporation in PE and PC of all analyzed tissues (liver, heart and white adipose tissues). Increased adiponectin secretion was also observed in 3T3-L1 adipocytes incubated with DHA, EPA and their metabolites, PDX or PGD3.

Conclusion: The present data show that DHA rapidly improved the profile of secreted adipokines in mice and that these protective effects were long lasting. EPA was more efficient than DHA to increase adiponectin secretion by 3T3-L1. The effect of n-3 PUFA on adiponectin secretion may be mediated by their respective metabolites.

P072

Antioxidant activity and phenolic acids of *Rosmarinus officinalis* and *Origanum dictamnus* extracts from Greece

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Background: Oxidative stress, induced by oxygen radicals, is believed to be a primary factor in various degenerative diseases, such as cancer, atherosclerosis and other pathological events. Natural antioxidants from plant sources can protect the human body from free radicals and retard the progress of many chronic diseases as well as the lipid oxidative rancidity in food, cosmetics and pharmaceutical materials.

Objective: In the current study two Lamiaceae plants, *Origanum dictamnus* (dittany) and *Rosmarinus officinalis* (rosemary) grown in the island of Crete and widely used in the Greek folk medicine were examined for their antioxidant activity and phenolic phytochemicals.

Procedure: The methanol as well as the hot aqueous extracts (infusions) were first screened for their free radical scavenging and ferric reducing antioxidant power properties using the DPPH and the FRAP assays. Standard antioxidants such as BHT, ascorbic acid and a number of phenolic acids were also used for comparison reasons. In a second step, the identification and quantification of phenolic phytochemicals which belong to hydroxybenzoic and hydroxycinnamic acids were performed using a RP-HPLC method.

Results: The results showed that the examined plant extracts were capable free radical scavenging (IC50 values ranged from 0.412 to 0.263mg/ml) and ferric reducing antioxidants (IC50 values ranged from 0.044 to 0.026mg/ml). The presence of the total identified phenolic acids (rosmarinic, caffeic, ferulic, gallic and protocatechuic acids) at high levels in the methanol (from 8.9 to 9.5 mg/g dry plant) and hot aqueous extracts (from 8.8 to 10.4 mg/g dry plant) may contribute significantly to the overall antioxidant activity of these plants.

Conclusion: These studies may prove beneficial in the exploitation of natural antioxidant sources, for use as functional foods or nutraceuticals.

P073

Egg yolk & lecithin: the second-to-none alternative to breast milk

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Background: Egg yolk (Columbus Concept) & lecithin (OvoLife Concept) are designed to mimic breast milk composition, digestion and bio-availability.

Objective: To feed laying birds rations that translate into egg yolk & lecithin compositions similar to that of breast milk in terms of essential nutrients.

Procedure: Laying birds were fed designed rations to test for feed / yolk / lecithin transfer of various water- and lipid-soluble that are essential nutrients to human newborns. Egg yolks, and their lecithins extracted with an aqueous / alcoholic isotropic mixture to exclusively exclude the protein fraction, were then analyzed for their composition and response to changes in feed composition. Mathematical models were established to predict egg yolk & lecithin make-up of essential nutrients from compositional data of birds feeding regimes.

Results: Egg yolk & lecithin can be readily designed to mimic breast milk composition in most if not all essential & conditionally-essential nutrients to human neonates, including essential fatty acids, xanthophylls, water- and lipid-soluble vitamins, and minerals. Additionally, the specific compartmentalization of those essential nutrients in egg yolk & lecithin makes them chronobiologically available through digestion, absorption and distribution to tissues. Providing the omega-6/3 ratio is taken care off (Columbus Concept), non-allergenic egg yolk can be designed by taking advantage of the perfect fit of egg yolk essential amino acid patterns to infant tissue needs.

Conclusion: Egg yolk (Columbus Concept) & lecithin (OvoLife Concept) can be positioned as the second-to-none alternative to breast milk from the evolutionary and biological standpoints.

P074**A review of dietary fatty acid intakes in children and adolescents in different regions of the world**

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Background: Cardiovascular risk factors are now increasingly observed in children. Dietary fatty acid composition is known to influence cardiovascular risk factors in children; however global intake data on fatty acids in children are lacking.

Objective: To review international dietary fatty acid intake in children and adolescents and compare these with the World Health Organization 2003 population nutrient intake goals for prevention of chronic diseases.

Methods: Data on fat and fatty acids intake were collected from national dietary surveys and population studies (N>100) published after 1995. Mean intakes were reported separately for children (~2-10y) and adolescents (11-18y).

Results: We found intake data for 28 countries; mainly from Europe, North America, Australia and New Zealand, with data for only 4 countries from other regions. Mean total fat intakes ranged from 23-40 percentage of total energy (%E) for children and from 25.4-39.9%E for adolescents. Mean saturated fatty acid (SAFA) intakes ranged from 6-16.5%E in children and 10-15.8%E in adolescents and were higher than recommended (>10%E) in 26 out of 28 countries. Mean polyunsaturated fatty acid (PUFA) intakes ranged from 3.5-9.7%E for children and from 3.6-11.2%E for adolescents; where 21 out of 28 countries had intakes below recommended (<6%E). Intake data were only available for 8 countries for linoleic acid (LA) and α -linolenic acid (ALA); these intakes ranged from 2.9-8.2%E and 0.2-1%E respectively. Intakes were lower than recommended in 7 out of 8 countries for ALA and in 5 out of 8 countries for LA.

Conclusions: This review shows that the fatty acid composition of the diet of children and adolescents is of concern because SAFA intake is higher and PUFA intake is lower than current recommendations for the prevention of cardiovascular diseases. Public health initiatives should focus on improving dietary fatty acid composition of the diet starting in early childhood.

P075**Altered metabolism of ^{13}C -docosahexaenoic acid during healthy aging**

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Objective: To evaluate whether the incorporation and \hat{I}^2 -oxidation of ^{13}C -docosahexaenoic acid (DHA) differs in healthy elderly compared to young adults.

Procedure : Six young between 18-29 years-old and six elderly between 70-79 years-old were given a single dose of 50 mg of uniformly labelled ^{13}C -DHA. Blood and breath samples were collected to follow incorporation of ^{13}C -DHA into blood lipids and its \hat{I}^2 -oxidation after 4h, 24h, 7d and 28d of the intake. Total lipids were extracted from the plasma and thereafter separated into triglycerides, free fatty acids, cholesteryl esters and phospholipids. Gas chromatography-combustion-isotope ratio mass spectrometry was used for the analysis of ^{13}C -DHA in plasma lipids while enrichment of ^{13}C -CO₂ in the breath was done by isotope ratio mass spectrometry.

Results : At baseline, young and elderly had similar DHA concentration in plasma total lipids suggesting similar fish and seafood intake. After ^{13}C -DHA intake, incorporation and \hat{I}^2 -oxidation reached a maximum within 4h hours in both groups. However, ^{13}C -DHA was 3.5 times higher in plasma total lipids of the elderly 4h after the tracer intake (p=0.03). The difference was more specific to the triglycerides (p = 0.006) and the free fatty acids (p = 0.02) subclasses. During the same period, ^{13}C -DHA was 1.9 time higher \hat{I}^2 -oxidized in the elderly compared to the young. In the follow-up period, ^{13}C -DHA was 2-3 times more incorporated in cholesteryl esters of the elderly after 7 and 28 days.

Conclusion: Our results support that DHA metabolism is altered with aging and whether it results from lower needs or lower capacity of the elderly to incorporate DHA in tissues still needs further investigations.

P076**Effect of dietary arachidonic acid (ARA) levels on growth and clinical chemistry in domestic piglets**

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Background: ARA and docosahexaenoic acid (DHA) are long chain PUFA commonly supplemented in infant formulas. The optimal ratio of ARA:DHA in terms of functional outcomes associated with ARA remains to be determined.

Objective: The objective of the present study was to determine the effect of dietary ARA on growth and clinical chemistry in domestic piglets when DHA is constant and near the high end of human breastmilk levels.

Procedure: On day 3 of age, artificially-reared (AR) piglets were assigned to one of six milk replacer diets (n = 8 per diet) and fed at 80% ad libitum intake. Diets

ARA:DHA was measured as follows (%w/w): 0.1:1.0; 0.53:1.0; 0.69:1.0; 1.0:1.0; 0.67:0.62; 0.66:0.33. A seventh group of maternal reared piglets served as a reference treatment. Body weights were measured weekly. On day 28, blood was collected and serum harvested. Piglets were then euthanized and organs weighed.

Results: Total milk replacer intake averaged 51.6 ± 0.1 L and did not differ among AR treatments. There were minor differences in AR body weights (range of means, 2.60-2.74 kg) on day 7 with the 0.1:1.0 group significantly lower than one of five other AR groups. AR piglets weighed more than MR piglets on days 21 and 28. Absolute brain weights did not differ among treatments. There were very few differences in clinical chemistry among AR piglets. Lymphocyte count in the 1% ARA group was elevated. Serum iron and several hematological values differed for MR than AR piglets likely reflecting growth differences.

Conclusion: There is a negligible effect of dietary ARA levels on growth and clinical chemistry when DHA is constant and near the high end of human breastmilk levels.

P077

Accumulation of n-3 LC-PUFA in human lipids by supplementation with vegetable n-3 fatty acids: α -linolenic acid and stearidonic acid

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Background: The dietary intake of n-3 fatty acids (FA) is generally below the recommendation. To improve the supply of n-3 LC-PUFA in human nutrition new vegetable oils containing potential n-3 LC-PUFA precursors have to be tested.

Objective: A human pilot study was done to clarify the tolerable dose of a vegetable oil rich in n-3 FA and the accumulation of LC-PUFA after a short-term supplementation.

Procedure: Subjects (4 male & 4 female) consumed daily 16.0 g of the test oil (Echium oil, 45% ALA & SDA, CRODA). After 7&14 days of intervention the FA distribution of plasma, erythrocytes and peripheral blood mononuclear cells (PBMC) were determined. For isocaloric adjustment and for baseline values the subjects consumed daily 16.0 g of a baseline oil (n-9 & n-6 rich) before the intervention (14 days). Subjects consumed no further n-3 rich oils and fish products (4 wks).

Results: The test oil was well-tolerated. The supplemented ALA and SDA increased from day 0 to 14 (FA stated as % of fatty acid methyl esters). *Plasma:* ALA 0.49 to 1.58, SDA 0.03 to 0.25; *erythrocytes:* ALA 0.17 to 0.37, SDA 0.02 to 0.04; and *PBMC:* ALA 0.20 to 0.35, SDA 0.03 to 0.09. Their possible conversion products C20:4 n-3, C20:5

n-3 (EPA) and C22:5 n-3 were significantly higher (day 14) compared to baseline; e.g. EPA: *plasma* 0.48 to 1.33, *erythrocytes:* 0.70 to 1.03, *PBMC:* 0.34 to 0.65. The C22:6 n-3 (DHA) was unchanged.

Conclusion: Already after short-term consumption of n-3 rich Echium oil the lipids of erythrocytes and PBMC had increased n-3 levels (C18 n-3 by their consumption only and C20 & C22 n-3 most likely by endogenous conversion of their precursors). This outcome could reduce cardiovascular risk, especially in subjects with increased risk of CVD. This should be proved in a long-term intervention study.

P078

Inflammatory mediators are modified by soybean (ω -6) or fish (ω -3) oil-rich diets: anti-inflammatory effect upon the development of experimental asthma

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Objectives: Asthma studies have been shown a pro-inflammatory effect of ω -6 rich diet versus an anti-inflammatory effect of ω -3. To understand this mechanisms, this study analyzed the effect of soybean or fish oil-rich diets on the release of bradykinin (BK), nitric oxide (NO), corticosterone (CT), Leukotriene B4 (LTB4) and Lipoxin A4 (LXA4).

Procedures: Male Wistar rats (28-30 days) were fed for 7 weeks with chow diet (Control), chow diet enriched with 15% of fish (Fish) or soybean oil (Soy). At the 4th week of feeding rats were sensitized on days 0 and 7 by i.p. injection of ovalbumin-alumen in saline. At 14th and 21st days after the 1st immunization the animals were challenged by exposure to an albumin aerosol 2.5% for 20 min. Animals were killed 24 hours after the 2nd aerosol challenge for BAL and lungs collection. Lung concentrations of CT, LTB4 and BK were detected by ELISA and HPLC, respectively. BAL and lung levels of LXA4 and NO were detected by ELISA and NO analyser, respectively. Statistical analysis was performed by Kruskal-Wallis ANOVA and Dunn test with $p < 0.05$.

Results: Both diets efficiently decreased BK lung release while increased NO levels in BAL. Only the Soy group was able to increase CT and LXA4 levels in relation to the Control group.

Conclusions: The soybean oil-rich diet had an anti-inflammatory effect as the Fish oil-rich diet controlling the release of BK, a bronchoconstrictor mediator in asthma and increasing the release of NO. Furthermore, only the Soy group showed an increase in CT and LXA4. Being LXA4 an important mediator for the resolution phase of inflammation, this is an important effect of this diet rich in ω -6.

P079**Fish oil or soybean oil-rich diet in experimental asthma: immunodulatory and anti-inflammatory effect**

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Procedures: Male Wistar rats (28-30 days) were fed for 7 weeks with chow diet (Control), chow diet enriched with 15% of fish (Fish) or soybean oil (Soy). At the 4th week of feeding rats were sensitized on days 0 and 7 by i.p. injection of ovalbumin-alumen in saline. At 14th and 21st days after the 1st immunization the animals were challenged by exposure to an albumin aerosol 2.5% for 20 min. Animals were killed 24 hours after the 2nd aerosol challenge for BAL and lungs collection. BAL was used for total cell count, the percentage of eosinophil (EOS), neutrophil (NEUT) and mononuclear cells (MONO), and the intensity of inflammation by histological analyzes and score of inflammation. BAL and lung Th1 (TNF- α , IFN- γ) and Th2 (IL-4) cytokines were detected by ELISA and Th2 (IL-5) by immunohistochemistry. Statistical analysis was performed by Kruskal-Wallis ANOVA and Dunn test with $p < 0.05$.

Results: Both diets efficiently decreased the inflammation score, but only fish decreased total cells. The BAL percentage of EOS and NEUT were lower in Soy and fish but levels in BAL were not only MONO cells were increased in fish group. INF- detectable in any group. Fish or Soy diets didn't change TNF- α levels in BAL, as and TNF. BAL and lung IL-4 were significantly decreased by lung levels of INF- Soy and Fish oil rich-diets. IL-5 was also lower in Soy and Fish groups.

Conclusions: We demonstrated that soybean oil-rich diet diminishes the migration of inflammatory cells to the lung and does not have a bad effect under asthma development. Both diets significantly decreased Th2 cytokines (IL-4 and IL-5) and do not change Th1 cytokines levels. This work suggests that Fish and Soybean rich-diets have an anti-inflammatory effect and avoid the Th2 profile upon the experimental asthma process.

P080**The DHA content of rat liver phospholipids are significantly higher when an α -linolenic acid rich diet is provided during pregnancy**

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Background: In both rats and humans, females have higher liver and/or plasma docosahexaenoic acid (DHA)

content than males. In rats, higher α -linolenic acid (ALA) intake results in higher eicosapentaenoic acid and docosapentaenoic acid content in plasma and liver phospholipids in non-pregnant females compared to males. Pregnant females have higher plasma and liver DHA content than virgin females maintained upon the same diet.

Objective: We investigated whether changes to total dietary fat content or the linoleic acid to ALA ratio of the diet during pregnancy influence pregnancy-associated increases in maternal plasma or liver DHA content.

Procedure: Female Wistar rats aged 10 wk were fed experimental diets 20 d ad libitum (virgin females, $n=6$ per diet) or from conception to day 20 gestation ad libitum (pregnant females, $n=6$ per diet). The experimental diets were a low-fat soybean oil based diet (LFS, 27.1g fat/kg, 1.6g ALA/kg), high-fat soybean oil based diet (HFS, 130.9g fat/kg, 9.1g ALA/kg) or high-fat linseed oil based diet (HFL, 130.9g fat/kg, 50.2g ALA/kg). Fatty acid composition of plasma and liver lipids were determined by gas chromatography.

Results: Pregnancy was associated with significantly higher liver and plasma phospholipid DHA content across all dietary groups. The DHA content of liver phosphatidylcholine (PC) differed significantly between the three dietary groups among pregnant females (mean \pm SD %: LFS 14.6 \pm 1.5, HFS 17.3 \pm 1.8, HFL 20.4 \pm 1.5), but not virgin females, with significant pregnancy x diet interactions on liver PC ($p < 0.001$) and phosphatidylethanolamine ($p < 0.001$), and a similar trend in plasma PC ($p = 0.056$).

Conclusion: Higher ALA intake increases the DHA content of maternal liver phospholipids in pregnant, but not virgin, females. ALA supplementation may therefore be a useful tool in ensuring adequate DHA supply to the fetus among pregnant women who are unable to or do not wish to consume oily fish.

P081 **$\Delta 6$ - and $\Delta 9$ -desaturase polymorphisms affect fatty acid profile of San Daniele dry cured hams**

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Background: San Daniele ham is a typical dry-cured ham (prosciutto) produced in northeast Italy. In Italy, more than 80% of pig production is destined to the traditional ham market. Fatty acid profile is of main importance for the dietary quality of hams but as well for the production of the typical dry-cured flavour. $\Delta 9$ -desaturase (SCD) is a microsomal enzyme required for the biosynthesis of unsaturated fatty acids and $\Delta 6$ -desaturase (FADS2) is the rate limiting enzyme in the production of arachidonic acid from linoleic acid. **Objective:** The aim of this study was to determine if SNPs in these genes can explain the

differences observed in the MUFA and arachidonic acid content in meat and fat.

Procedure: Through sequencing, a SNP was observed in the SCD promoter and in the third exon of FADS2. One hundred and six animals were genotyped by PCR-RFLP for these mutations and fatty acid profiles were determined by gas chromatography for backfat, leg fat cover, longissimus dorsi and biceps femoris. Single locus genotype association analyses using the General Linear Mixed Model in SPSS were carried out.

Results: The ratio of stearic to oleic acid was significantly associated with SCD polymorphism in the 4 tissue analysed ($P < 0.01$), as well as the ratio of palmitoleic to palmitic acid ($P < 0.05$). Significant associations were found between FADS2 polymorphism and the ratio of arachidonic to linoleic acid in backfat ($P < 0.01$) and in L.dorsi ($P < 0.05$) and with the concentrations (g/100g of total fat) of arachidonic acid in backfat, leg fat cover and L.dorsi ($P < 0.0001$; $P < 0.01$; $P < 0.05$) respectively.

Conclusion: Our findings suggest that the SCD and FADS2 polymorphisms are associated with dietary quality of dry cured San Daniele ham. **Acknowledgments:** The technical assistance of Angela Sepulcri and Massimo Lanzoni is gratefully acknowledged. This work was funded by the Region FVG, LR 26/2005, art. 23.

P082

Dietary $\omega 3$ lipids change fatty acid pattern of dog erythrocyte ghosts within 1 week

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Background: Increasing the dietary level of $\omega 3$ fatty acids (FA) has the potential to benefit a variety of medical conditions, such as arthritis, atopic dermatitis or cancer. $\omega 3$ FA are important in those diseases as fish oil (FO) influences the properties of membranes, the epidermal lipid barrier, the release of inflammatory mediators and even gene expression. Data from time course studies with mice and humans suggest that diet-induced changes in immune cell fatty acid profiles occur within a few weeks. This appears to be sufficient to alter immune cell fatty acid composition and in vitro functions.

Objective: Studies of the incorporation of FA into erythrocyte membranes may be suitable to determine the kinetics of the cellular fatty acid status.

Procedure: Venous blood was drawn from 30 dogs in three dietary groups (control, $\omega 3$ additive, FO diet) to examine the FA composition of the erythrocyte ghosts for 12 weeks (w). The lipids were extracted and their FA were determined by gas liquid chromatography.

Results: Both $\omega 3$ diets significantly increased ghost $\omega 3$

FA after 1 w already, apparently reaching a plateau for DHA within 12 w. The absolute and relative response to dietary supplementation with DHA seems to be larger than the response to EPA. In comparison to the control group, $\omega 6$ FA are significantly reduced after 8 w.

Conclusion: These data are important in order to define the optimal duration of clinical trials and treatments with $\omega 3$ FA in dogs.

P083

Inverse association between the proportion of oleic acid in serum phospholipids and metabolic syndrome traits in a dyslipidemic Spanish population

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Background: There is an increasing worldwide incidence of obesity and associated metabolic alterations, namely the metabolic syndrome (MetS) and diabetes, related to visceral fat accumulation. Evidences suggest that consumption of saturated fatty acids and polyunsaturated fatty acids relate to an increased and decreased risk of MetS, respectively, while data on oleic acid are inconsistent. Epidemiological and clinical studies in Mediterranean countries suggest that oleic acid as a nutrient and olive oil as a food protect against MetS.

Objective: To assess associations between the proportion of oleic acid in serum phosphatidylcholine and the prevalence of MetS and its components in 451 Spanish subjects (53% men, mean age 45 y) with primary dyslipidemia well phenotyped for cardiometabolic risk factors who were recruited in two lipid clinics in North-East Spain.

Procedure: MetS was defined per ATPIII criteria. The fatty acid composition of serum phosphatidylcholine, as a proxy of intake and enrichment in inner membranes, was determined by gas-chromatography.

Results: 127 subjects (28.2%) had the MetS. The average (\pm SD) proportion of oleic acid for the study group was $11.7 \pm 2.0\%$. After adjusting for age, gender, centre, smoking status and other fatty acids known to relate to MetS (saturated fatty acids, linoleic acid and n-3 long-chain polyunsaturated fatty acids), the proportion of oleic acid in serum phosphatidylcholine showed inverse associations with visceral obesity (OR [95% CI] 0.83 [0.71-0.98]), low HDL-cholesterol (0.84 [0.72-0.98]) and MetS (0.84 [0.71-0.99]). Serum phosphatidylcholine oleic acid also related inversely to the number of MetS components (p for trend 0.010).

Conclusion: Higher phospholipid proportions of oleic acid as a result of high olive oil intake relate to a lower cardiometabolic risk in a Mediterranean population. However, whether oleic acid enrichment in phospholipids

is beneficial per se or is a surrogate of the consumption of bioactive compounds found in olive oil deserves further research.

P084

Comparative metabolic fate of α -linolenic acid from natural oil (flaxseed oil) and structured lipids in rats

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Background: the Human health benefits of the omega-3 (ω 3) fatty acids are well established whether it's on cognitive, cardiovascular, immune and inflammatory disease prevention. Although total lipids intake is elevated, epidemiological studies in Western countries have demonstrated an insufficiency in the consumption of the alpha-linolenic acid (ALA, 18:3n-3), precursor of the ω 3 family. Thus, improving ALA bioavailability without increasing lipid intake is a real challenge even though lipid consumption should be controlled.

Objective: the objective consists in increasing ALA bioavailability while maintaining or even decreasing the total amount of ingested oil by controlling its physical and/or chemical form input.

Procedure: two parameters known to affect the fatty acid metabolic fate were tested : i) physical form, linolenic oil in emulsion versus bulk phase, ii) ALA position on the glycerol backbone using structured triacylglycerols (STAG). These studies were performed in thoracic lymph duct-cannulated rats after intragastric feeding of oil in emulsion or not, and STAG.

Results: emulsification favored ALA recovery in lymph since, ALA absorption was greater (+ 135 %) than with the bulk phase. Moreover, maximal ALA absorption appeared earlier (2 hours before) and was significantly ($p < 0.05$) greater (+ 55 %) in the emulsion group compared to the bulk phase group. On the other hand, assays with structured TAG demonstrated that ALA intestinal absorption rate was comparable regardless of the STAG used. However, ALA position into the lymphatic TAG was different from that of ingested TAG.

Conclusion: the intestinal recovery of ALA was improved by the emulsification of a natural oil. On the other hand, although the lymphatic absorption of ALA was similar regardless of the TAG structure, these data not exclude the influence of the ALA position in the ingested TAG on its metabolic fate in the organism.

P085

Effect of fish oil and olive oil supplementation on cardiovascular system and biochemical profile of adults Spontaneously Hypertensive Rats (SHR)

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Objective: To evaluate the effect of supplementation with fish or olive oil on systolic blood pressure (SBP), Angiotensin Converting Enzyme (ACE) catalytic activity and Nitric Oxide (NO), cholesterol HDL and glucose.

Procedures: Male SHR adults (12 weeks) received olive oil (OO - 1.5 g/kg), fish oil (FO - 1.5 g/kg) or vehicle (C) supplementation by gavage for 8 weeks, once a day. SBP was measured once a week by tail cuff plethysmography method. Blood samples were collected for biochemical analysis. NO cardiac and renal tissue was analyzed by Nitric Oxide Analyzer (NOATM). ACE catalytic activity was determined fluorimetrically.

Results: The supplementation with FO or OO decreased SBP in relation to Control Group. Heart weight in OO group was lower compared with FO and C groups. Glucose levels were increased in FO group in relation to the C group. Cholesterol HDL was decreased in FO in relation to C and OO groups. Both oils decreased ACE activity in serum. NO levels in the cardiac tissue were reduced in FO, while in the OO group, lowers values were found in the renal tissue.

Conclusion: FO and OO supplementation decreased the SBP associated with a reduction on ACE activity in Adults SHR. However, FO induced alterations in HDL (dyslipidemia) and glucose levels suggesting insulin resistance, which could promote cardiovascular muscle cell proliferation.

P086

Study on the mechanism of improved swimming endurance in adult mice fed Erabu sea snake lipids

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Objective: In order to clear the mechanism regarding the improved swimming endurance in mice fed Erabu sea snake lipids, the susceptibility to lactic acid in adult mice fed sea snake lipids and the effect of strain difference on endurance of adult mice following this diet have been investigated.

Procedure: Experiment 1. ICR mice aged 27 weeks were divided with similar swimming time, and were fed diets containing 6% lard, fish oil or sea snake lipids. At the end of 16 weeks of feeding trial, intraperitoneal injections of lactic acid and glucose (25 mg/kg body weight) were given following injection of saline. Swimming times of mice with 1% loads were measured 30 min after each injection. Experiment 2. Four strains (ddy, ICR, C57BL and

BALB) of mice aged 18 weeks were divided into two groups with similar swimming time, and were fed 6% lard or sea snake lipid diets. Swimming times after 16 weeks of feeding were measured.

Results: Ratios of swimming times of lactic acid to saline injected were significantly higher than those of glucose to saline injected in ICR mice fed sea snake lipids ($p < 0.05$) but not in those fed lard or fish oil diets. Their ratios for lactic acid to saline injected were greater in ICR mice fed sea snake lipids than in those following lard or fish oil. In addition, greater swimming endurance in mice fed sea snake lipids than those following lard, was only observed in ddy and ICR mice, but not in C57BL and BALB mice.

Conclusion: These results revealed that dietary lipids showed different susceptibility to lactic acid in adult ICR mice, and Erabu sea snake lipids may contain factors that regulate lactic acid metabolism effectively during exercise; and swimming endurance of adult mice fed sea snake lipids was different by strain difference.

P087

Inverse association between the proportion of α -linolenic acid in serum phospholipids and advanced atherosclerosis in spanish subjects with primary dyslipidemia

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Background: Epidemiological, clinical and forensic pathology evidences suggest that there is a delay in onset of clinical coronary heart disease (CHD) in Spain. Intake of alpha-linolenic acid (ALA) appears to protect from CHD, an effect that might be mediated by delayed atherosclerosis development. The main sources of ALA in the Spanish diet are walnuts and, to a lesser extent, olive oil.

Objective: To assess associations between the proportion of ALA in serum phospholipids, as a proxy of intake, and carotid plaque, as a measure of advanced atherosclerosis, in Spanish subjects at high cardiovascular risk well phenotyped for risk factors.

Procedures: Cross-sectional study of 201 asymptomatic subjects (38% men, mean age 46 y) with primary dyslipidemia, with determination of fatty acid composition of serum phosphatidylcholine by gas chromatography and plaque outcomes (prevalence, number, score and maximum height) in six pre-specified carotid segments by high-resolution ultrasound.

Results: 106 subjects (53%) had carotid plaques. The mean (\pm SD) proportion of ALA for the whole group was $0.16 \pm 0.10\%$. In multivariate regression analyses after adjusting for age, gender, lipid genotype, BMI, smoking (pack-years score), prior treatment with statins and

antihypertensive agents and other fatty acids known to relate to atherosclerosis (oleic acid, linoleic acid and n-3 long-chain polyunsaturated fatty acids), the proportion of ALA showed an inverse association with plaque prevalence, with OR (95% CI) 0.69 (0.50 to 0.97) for one SD-increase, and a nearly significant inverse association with plaque number, with regression coefficient B -0.19 (-0.42 to 0.03), $p = 0.085$.

Conclusions: Higher phospholipid proportions of ALA related to less advanced atherosclerosis in a Mediterranean population with primary dyslipidemia. This might explain in part delayed CHD occurrence in Spain. However, whether ALA enrichment in phospholipids is beneficial per se or is a surrogate of the consumption of bioactive compounds in parent foods deserves further research.

P088

Does supplementing pregnant women with DHA improve visual acuity in infancy? A nested follow-up study of DOMInO trial infants

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Background: Docosahexaenoic acid (DHA) accumulates in retinal tissues during fetal development. Observational studies demonstrate that higher fish consumption during pregnancy is associated with better visual development in childhood.

Objective: To determine whether supplementing pregnant women with DHA improves visual development in infancy.

Procedure: Infants whose mothers were enrolled in the DOMInO trial (DHA to Optimize Mother and Infant Outcomes) were enrolled in a nested follow-up study. The DOMInO trial is a large scale ($n = 2399$), double blind, randomised controlled trial in which pregnant women between 18-21 weeks gestation were randomly assigned to consume capsules containing ~ 1 g/d of DHA (treatment group) or a blended vegetable oil (control group) until birth. Sweep visual evoked potential (VEP) acuity was assessed at 4 months of age on a subset of the DOMInO trial infants who were born >37 weeks gestation and >2.5 kg with no known visual pathology. VEP latencies and anthropometric measures (weight, length and head circumference) were collected as secondary outcomes.

Results Data collection began June 2007 and ended August 2008. Of the 249 infants invited to participate in the follow-up study, 186 were enrolled and 184 (99%) returned for visual assessments. Half of the infants assessed were girls ($n = 92/184$, 50%) and 57% were breastfed at the time of the visual assessment. The age at

assessment (mean \pm SD) was 4.0 \pm 0.2 months and weight (kg) was 6.8 \pm 0.8. Mean VEP acuity was 8.4 \pm 2.0 cycles per degree which was within the Australian norms for this age. Mean latencies to checkerboards eliciting 69, 48 and 20 minutes of arc were 116 \pm 8, 121 \pm 11 and 132 \pm 14 milliseconds, respectively.

Conclusion: This nested follow-up will be un-blinded when the primary outcome assessments of all children at 18 months are complete (March 2010). Final results will be presented.

P089

Metabolic fate of purified dietary docosapentaenoic acid (22:5n-3) in the rat

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Background: DPA (docosapentaenoic acid, 22:5n-3) is a significant constituent of the Inuit diet via the fat of marine mammals. It has recently been reported that short-term DPA supplementation can increase the level of DHA (22:6n-3) in rat liver and EPA (20:5n-3) in liver, heart, and muscle by retroconversion (Kaur et al., BJN 103:32 (2010)).

Objective: The purpose of the present work was to assess the effect of oral DPA administration using a rat model on the absolute fatty acid concentrations (and relative %) of various lipid fractions (total lipid, total phospholipid, total triglyceride, free fatty acid, cholesterol ester and individual phospholipids (PC, PE, PS, PI, SPH) in liver, heart, and kidney as well as blood serum.

Procedure: Following ten days of daily oral gavage with corn oil (controls) or corn oil plus purified DPA (at 2.5% of total fat intake), the tissue/serum samples were extracted, subjected to thin-layer chromatographic separations, and the derived fatty acid methyl esters of the lipids were analyzed by gas-liquid chromatography.

Results: After ten days, the level of DPA rose markedly in the phospholipid, triglyceride, and free fatty acid fractions of serum, heart, liver, and kidney as did that of EPA (in contrast to DHA). Heart phospholipid exhibited a pronounced accumulation of DPA with supplementation (12.1 wt.% vs 1.3% in control). EPA levels rose to particularly high levels in kidney phospholipid. Interestingly, in direct contrast to the major phospholipids (PC and PE) of heart and liver following DPA supplementation, kidney PC and PE exhibited overall ratios of EPA:DPA of 2:1 and 5:1, respectively. Wide differences were found in the enrichment patterns (DPA and the derived EPA) across individual phospholipids in the tissues studied.

Conclusion: These results suggest marked heterogeneity for the incorporation of DPA into various tissue lipids (including individual phospholipids) and apparent retroconversion to EPA.

P090

Remodeling of DHA-containing phospholipid molecular species during impaired neurogenesis following n-3- α linolenic acid deprivation

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Background: We showed recently that dietary alpha-linolenic acid (ALA, 18:3n-3) deficiency causes transient aberrations of cell migration around birth in several regions of the postnatal brain including frontal cortex and hippocampus.

Objectives: Examine the ontogeny of cortical phosphoglycerides (PG) and determine changes in molecular species profile induced by ALA deficiency.

Procedure: Pregnant rats were fed from conception to 4 weeks after birth an ALA-adequate or ALA-deficient diet. Lipid extracts prepared from frontal cortex of off-springs were analyzed by gas chromatography and by quadrupole/time-of-flight mass spectrometry combined with electrospray ionization.

Results: Different proportions of molecular species were generated in the course of postnatal cortical development the most significant being a net increase in 18:0/22:6 in both ethanolamine and serine containing PG species. At this time 16:0/22:6 and 18:1/22:6 levels of 1,2-diacyl ethanolamine were reduced whereas those of 1-alkenyl-, 2-acyl (plasmalogen) remained unchanged. Dietary ALA deficiency induced a remarkable decrease in 18:0/22:6 ethanolamine PG that was counterbalanced by the appearance of 18:0/22:5n-6. Likewise, 16:0/22:6 and 18:1/22:6 were replaced by 16:0/22:5 and 18:1/22:5 species. Similar substitutions took place in serine PG. Arachidonic acid (20:4n-6)-containing species were reduced during brain development but were not affected by dietary manipulation.

Conclusion: The changes in the profile of cortical PG molecular species by dietary means appear very specific such that 22:6n-3 is substituted by 22:5n-6 fatty acids and the relative proportions of PGs throughout development are maintained. While these substitutions are highly preserved with respect to polar head group and sn-1 position within the PG, they nevertheless result in impaired neuronal migration and affect a variety of cognitive, behavioral and motor functions in the brain. The importance of individual PG molecular species in regulating brain development at these critical periods remains to be elucidated.

Supported by Gulston Foundation (USA) and Mother and Child and Letten Foundations (UK).

P091**Levels of very long-chain fatty acid in whole blood are associated with cardiovascular risk factors in children**

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Background: The composition of fatty acids in blood and tissue affects various important physiological functions related to the development of cardiovascular diseases. Recent studies demonstrated that absolute levels of saturated very long-chain fatty acid (hexacosanoic acid; C26:0) in whole blood appeared to be associated with metabolic syndrome in adults.

Methods: Eighty-eight children (47 male, 41 female; average age, 10.9 years) participated in this study. Waist circumference was measured at the level of the umbilicus, and the waist to height ratio (WHtR) was obtained. Levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL) and triglyceride were measured by enzymatic methods. Low-density lipoprotein cholesterol (LDL) levels were obtained using the Friedewald formula. Levels of C26:0 in whole blood were analyzed using gas chromatography-mass spectrometry.

Results: Compared with reported levels in the whole blood of adults, children had lower C26:0 levels, which correlated positively with waist circumference ($r=0.223$, $p=0.0363$), WHtR ($r=0.217$, $p=0.0424$), systolic blood pressure ($r=0.301$, $p=0.0044$), diastolic blood pressure ($r=0.389$, $p=0.0002$), TC concentration ($r=0.227$, $p=0.0355$) and LDL concentration ($r=0.229$, $p=0.0316$). C26:0 levels increased with increasing the number of cardiovascular risk factors.

Conclusions: Elevation of C26:0 levels may be one of the metabolic features of children with cardiovascular risk factors.

Abstracts - Monday May 31

Plenary: Diet and Nutrition : Maternal and infant nutrition

08.30 - 09.15

Essential fatty acid metabolism in mothers and their children and some functional associations

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Pregnancy is associated with increased absolute amounts of plasma phospholipid (PL)-associated essential fatty acids and their derivatives, the LCPUFA (together called essential PUFA, ePUFA). Since ePUFA intake hardly changes during pregnancy, this indicates active ePUFA mobilization from body stores and/or metabolic rerouting. Paradoxically, increasing amounts of ePUFA status markers, indicate a *lowering* of the biochemical ePUFA status during pregnancy. Because of the strong relationship between maternal and fetal ePUFA concentrations, this may imply a sub-optimal neonatal ePUFA status, which could have functional consequences.

ePUFA concentrations in vascular tissue from umbilical veins (V) and arteries (A) represent the longer-term ePUFA status of blood entering and leaving the fetus, respectively. Consequently, V-A differences in ePUFA concentrations can be considered proxies for the net balance between ePUFA consumption and production. For most ePUFA, the V-A difference is positive, illustrating net ePUFA consumption by the fetus. For DHA, however, the V-A difference is *negative*, suggesting net fetal *production*. Nonetheless, the biochemical DHA status is significantly lower in umbilical arteries compared to veins.

ePUFA supplementation of pregnant women not only affects their own ePUFA status, but that of their neonates also. Thus, supplementation with marine or terrestrial n-3 fatty acids increases n-3 fatty acid concentrations at the expense of n-6 LCPUFA, whereas linoleic acid supplementation increases n-6 fatty acid concentrations at the expense of n-3 LCPUFA. This illustrates that the well-known competition between both ePUFA families operates during pregnancy also.

Observational studies with data from the Maastricht Essential Fatty Acid Birth (MEFAB) birth cohort demonstrate fatty acid specific tracking of ePUFA concentrations over time. In addition, the mother-child relationship, known to exist at delivery/birth, appeared to persist until at least 7 years later.

Finally, evidence will be presented to suggest that the fetal growth promoting potential of DHA, recently shown to be limited to early pregnancy, may result from n-3LCPUFA-mediated placental growth.

09.15 - 10.00

Consequences of FA supplementation

I Helland

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Docosahexaenoic acid (DHA, 22:6 n-3) and arachidonic acid (AA, 20:4, n-6) are the major polyunsaturated fatty acids (PUFAs) in the central nervous system. The parent n-3 and n-6 fatty acids are essential fatty acids and must be present in the diet. Data from non-human primates indicate that preformed DHA is necessary for normal brain development. Epidemiological studies report an association between higher levels of fish consumption and later developmental outcomes. Several studies indicate that DHA supplementation during pregnancy, lactation or childhood plays an important role in childhood neurodevelopment. In a double blind randomized trial performed in Norway, pregnant women were allocated to receive either 10 ml of cod liver oil containing DHA and eicosapentaenoic acid (EPA, 20:4 n-3), or 10 ml of corn oil containing linoleic acid (LA, 18:2 n-6) and ARA. The mothers took supplements from week 18 of pregnancy until 3 months after delivery. The infants were scheduled for assessment of cognitive function at 6 and 9 months, and at 4 and 7 years of age. At 4 years of age, the children in the cod liver oil group had a 4-point advantage on the Kaufmann ABC test. In a multiple regression model, maternal intake of DHA during pregnancy was the only variable of statistical significance for the children's mental processing scores at 4 years of age. Studies on preterm infants have also shown beneficial effects of DHA supplementation on cognitive function and visual resolution.

Controlled trials have also shown that supplementation with DHA and EPA may help in the management of childhood psychiatric disorders, and improve visual and motor functions in children with phenylketonuria.

Concurrent Session - 7**Lipids and Health : Metabolic syndrome - obesity/diabetes****10.30 - 11.00*****Invited Contribution*****Ectopic fat storage and insulin sensitivity**M Hesselink*Maastricht University, Maastricht, The Netherlands*

Post-receptor defects in insulin signalling are pivotal in the development of insulin resistance, the hallmark in development of type 2 diabetes. Skeletal muscle is responsible for as much as 80% insulin stimulated glucose uptake in the postprandial phase. Hence, myocellular insulin resistance is a major contributor to the development of type 2 diabetes. It has firmly been established that in subjects with blunted insulin sensitivity the amount of fat stored in the muscle correlates negatively with muscle insulin sensitivity as determined in hyperinsulinemic euglycemic clamp studies. Data in trained subjects and athletes, who store fat abundantly in their muscles whilst being very insulin sensitive, indicate that this relationship is unlikely to be causal. Rather, it appears that the balance between fat storage and the capacity of the muscle to liberate this ectopically stored fat for oxidation is a determinant of insulin sensitivity, rather than ectopic fat storage *per se*. This highlights the importance to improve our fundamental understanding of the processes involved in fat storage and controlled ectopic fat degradation. The last decade new lipases have been identified in muscle along with a series of lipid droplet coating proteins which are crucial to store and degrade ectopically stored fat in a controlled manner.

The present lecture will show data on the putative role of these proteins in human skeletal muscle of obese and type 2 diabetic subjects as well as the response of these proteins to a 12 week exercise training program.

11.00 - 11.15**Dietary linoleic acid promotes hyperactive hepatic 2-arachidonoyl-glycerol and contributes to diet-induced obesity**A Alvhheim¹, D Osei-Hyiaman², P Robert², L Madsen¹, K Kristiansen³, L Frøyland¹, J Hibbeln²¹*NIFES (National Institute of Nutrition and Seafood Research), Bergen, Norway*²*National Institute on Alcohol Abuse and Alcoholism, Bethesda, United States of America*³*Department of Biochemistry and Molecular Biology, Odense, Denmark*

Background: Excessive activity of the endocannabinoid system increases the risk of obesity and associated complications of diabetes and cardiovascular disease through multiple metabolic systems. Both anandamide and 2-arachidonoyl-glycerol, the two main endocannabinoids are synthesized from a common precursor, arachidonic acid (20:4n-6 -PL) which is a component of membrane phospholipids.

Objective: We sought to determine if increasing dietary linoleic acid (LA, 18:2n-6) alters the endocannabinoid precursor pool of 20:4n-6 -PL and causes excessive endocannabinoid system activity and obesity.

Procedure: Linoleic acid was isolated as an independent variable to reflect the dietary increase in linoleic acid from 1% energy to 8% energy occurring in the US during the 20th century. Mice were fed diets containing 1% energy LA, 8% energy LA and 8% energy LA + 1% energy EPA+DHA in medium fat diets (35% energy fat) and high fat diets (60% energy fat) for 14 weeks from weaning,

Result: The increase in dietary linoleic acid from 1% energy to 8% energy significantly increased the endocannabinoid precursor 20:4n-6 -PL and elevated hepatic 2-AG. Compared to diet of 1% energy LA, 8% energy LA induced appetite, significantly increased feed efficiency and caused greater adiposity. The use of 1% energy LA prevented the expected increases in adiposity. Addition of 1% energy EPA and DHA to an 8% energy LA diet reduced both the 20:4n-6 -PL pool and 2-AG and was accompanied by reduced both feed efficiency and adiposity.

Conclusion: We conclude that dietary linoleic acid increases tissue 20:4n-6 -PL, and subsequently elevates the endocannabinoid 2-AG levels in the liver leading to the development of diet-induced obesity. Furthermore, the adipogenic effect of linoleic acid can be prevented by consuming sufficient dietary EPA and DHA to reduce the 20:4n-6 -PL pool and normalize endocannabinoid tone.

11.15 - 11.30

N-3 fatty acids augment beneficial effects of mild calorie restriction in mice fed high-fat diet - role of SIRT1

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Objective: n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, act as natural hypolipidemics and reduce risk of cardiovascular disease. In rodents, n-3 LC-PUFA prevent obesity and insulin resistance induced by high-fat diet. We investigated additive benefits of n-3 LC-PUFA and caloric restriction (CR) in the prevention of obesity and glucose intolerance in mice.

Procedure: Two-month-old singly-caged C57BL/6J male mice were habituated to a corn oil-based high-fat diet (cHF; lipid content 35% wt/wt) for two weeks and then assigned for five or fifteen weeks to various dietary treatments (n = 9-11): (1) cHF-diet, ad libitum; (2) cHF-diet with EPA/DHA concentrate (EPAX 1050 TG; EPAX, a.s., Lysaker, Norway) replacing 15% of dietary lipids, ad libitum (cHF+F); (3) cHF-diet, 10% CR (cHF+CR); or (4) cHF+F-diet, 10% CR(cHF+F+CR).

Results: Compared with cHF-diet, all the treatments reduced body weight gain and adiposity (cHF > cHF+F > cHF+CR > cHF+F+CR). Induction of insulin resistance by cHF-diet was counteracted in the cHF+F mice, and the cHF+F+CR mice exerted even less impaired sensitivity. Accumulation of triglycerides in muscle and liver was strongly reduced by the combination treatment. The above effects correlated with the rate of palmitate oxidation in white fat. Gene expression analysis revealed synergistic effects of n-3 LC-PUFA and CR on activation of PPAR α /PGC-1 α signaling pathways in white fat, but not in liver, brown fat and skeletal muscle. Our preliminary results on the transgenic SIRT1^{+/-} mouse suggest that NAD⁺-dependent deacetylase SIRT1 is required for PPAR α mediated induction of gene expression (ACOT2, CPT-1 α) in white fat by n-3 LC-PUFA.

Conclusion: Dietary intake of n-3 LC-PUFA combined with calorie restriction results in additive/synergistic stimulation of conversion of white adipocytes into fat-burning cells. This approach may represent a perspective strategy for the treatment of metabolic syndrome.

11.30 - 11.45

Reduced hepatic and serum fatty acid amides in L-FABP^{-/-} mice alter feeding behavior and protect against obesity

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Liver fatty acid binding protein (L-Fabp) facilitates intracellular movement of fatty acids (FA) in both liver and intestine. Female L-Fabp^{-/-} mice fed a high saturated fat (SF) diet are protected against obesity and hepatic steatosis vs C57BL/6 (WT) mice, without evidence of altered FA absorption, oxidation or impaired utilization.

Objective: Does deletion of L-Fabp, either germline or liver-specific, alter feeding behavior? Is production of a satiety factor altered in L-Fabp^{-/-} mice?

Procedure: WT or C57BL/6 congenic L-Fabp^{-/-} mice were fed chow or SF diet. WT mice were administered L-Fabp antisense-oligonucleotides (ASO) to reduce hepatic L-Fabp expression.

Results: SF diet fed L-Fabp^{-/-} mice exhibit a trend toward decreased daily food consumption (WT, 2.73 \pm 0.09 g/day; KO, 2.50 \pm 0.09; p=0.09). L-ASO treated mice (liver-specific L-Fabp KO) demonstrate reduced obesity and hepatic steatosis after 4 weeks compared to control ASO mice, and reduced food consumption (C-ASO, 2.88 \pm 0.15g/d; L-ASO, 2.33 \pm 0.06; p=0.002). To examine feeding behavior, food consumption was determined 6h after mice were refed following a 24h fast. Female L-Fabp^{-/-} mice fed chow diet exhibit reduced food consumption at 6h (WT, 1.96 \pm 0.09g; KO, 1.63 \pm 0.09;p=0.021). Decreased food consumption was also observed in SF fed L-Fabp^{-/-} mice (WT, 1.80 \pm 0.05g/6h; KO, 1.60 \pm 0.05; p=0.011). To understand the basis for this response, we examined levels of orexigenic (AEA, 2-AG) or anorexic (OEA, PEA) FA amides in serum and tissues of L-Fabp^{-/-} mice. OEA, PEA and 2-AG were reduced in livers of free fed L-Fabp^{-/-} mice, with no difference in intestine. Serum OEA and 2-AG levels were also reduced in L-Fabp^{-/-} mice.

Conclusion: L-Fabp deletion alters production and/or trafficking of liver-derived FA amides, which in turn modulate food consumption, resulting in protection against obesity and steatosis.

11.45 - 12.00

Creatine depletion hampers glucose and lipid metabolisms of AGAT^{-/-} mice

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Background: Arginine:glycine-amidino transferase (AGAT) catalyzes the rate-limiting step of creatine (Cr) biosynthesis. Scarce uptake of Cr matched with deficient expression of AGAT causes disarrangements in brain and muscle. AGAT^{-/-} mice maintained on a Cr-free-diet have permanent reduced body-weight despite of doubled food intake compared to WT. We hypothesized that AGAT^{-/-} have impaired glucose and lipid metabolisms due to improved insulin action.

Objective: Determine if Cr depletion in AGAT^{-/-} mice hampers hepatic triglyceride (HTG) pool and whole-body glucose tolerance. We combined new MR measurements to determine HTG and its relative sources, de-novo-Lipogenesis (DNL) and dietary fatty acids, which were correlated to glucose and insulin levels.

Procedure: AGAT^{-/-} and WT mice were kept in Cr-free-diet until depletion of Cr/PCr levels confirmed by ³¹PMRS. After glucose challenge, glucose and insulin levels were quantified over 2h. 2H₂O was administered to fed mice, to reach 3% enrichment of body-water. 2H-enrichment of body-water and HTG were determined by 1H-decoupled-2HNMR on plasma and hepatic extracts. DNL contribution to HTG was estimated as the HTG 2H-methyl/2H-body-water. Dietary contribution is 100-DNL(%), assuming no other source contribute to HTG, during feeding.

Results: 5-months of Cr-free-diet depleted Cr/PCr from AGAT^{-/-}. AGAT^{-/-} had lower body-weight (19.3±2.2g vs 33.9±5.6g, p<0.0001), lower blood glucose (889±59 vs 1396±89 AUC p<0.0001) and insulin levels (34.0±3.7 vs 65.5±5.9, AUC p<0.01) after glucose load, compared to WT. AGAT^{-/-} had lower HTG pool, 106±19 μmol/gww vs 195±20 μmol/gww, p=0.03, and doubled DNL contribution for the HTG, 16.9±2.7% vs 7.1±1.2%, p=0.002, compared to WT mice. Hence, dietary fatty acids was source of 83.1±2.7% HTG in AGAT^{-/-} and 92.9±1.2% in WT mice.

Conclusion: The data demonstrate that AGAT^{-/-} have an insulin sensitive phenotype, increased DNL contribution to a reduced HTG pool. Dietary fatty acids contribute less to HTG, suggesting a re-direction of dietary fatty acids to high-energy demanding tissues as muscle.

Concurrent Session - 8

Biochemistry of Lipids: Lipid signaling/lipid mediators

10.30 - 11.00

Invited contribution

Multiple mechanisms of action of n-3 fatty acids on metabolism - possible impact for the treatment of metabolic syndrome

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Development of diseases associated with obesity, like type 2 diabetes, dislipidaemia and cardiovascular disease (metabolic syndrome) could be delayed by lifestyle modifications, while both dietary and pharmacological interventions are required for the therapy. Naturally occurring n-3 long-chain (LC) PUFA, EPA and DHA, act as hypolipidaemic factors, reduce cardiac events and decrease the progression of atherosclerosis. In animal studies, n-3 LC-PUFA efficiently prevent the development of obesity, hepatic steatosis and dyslipidaemia, as well as impaired glucose tolerance. However, in diabetic patients, n-3 LC-PUFA appear to have little effect on glycemic control.

Physiological effects of n-3 LC-PUFA and their active metabolites, eicosanoids and other lipid mediators, are mediated by multiple mechanisms in peripheral tissues, including the key intracellular regulators of transcription, AMP-activated protein kinase, as well as cannabinoid receptors and other mechanisms. Our experiments in C57BL/6 mice fed obesogenic high-fat diet revealed that adipose tissue is specifically linked to the beneficial effects of n-3 LC PUFA, as indicated by (1) the prevention of adipose tissue hyperplasia and hypertrophy, (2) the induction of mitochondrial biogenesis in adipocytes, (3) the induction of adiponectin, and (4) the amelioration of low-grade tissue inflammation in response to the n-3 LC PUFA

supplementation. Activation of a metabolic switch toward lipid catabolism and suppression of lipogenesis in adipose tissue, liver and small intestine was probably responsible for counteraction of dyslipidaemia and ectopic deposition of lipids, thus contributing to the improved insulin signaling. Importantly, n-3 LC-PUFA in combination treatment with thiazolidinediones, specific PPAR- α agonists, exerted additive effects on both the prevention and reversal of obesity, dyslipidaemia and insulin resistance, while inducing adiponectin and counteracting obesity-associated low-grade inflammation of adipose tissue in mice.

Our results suggest that combined treatment using n-3 LC-PUFA in addition to pharmacological agents, as well as calorie restriction, may improve therapy for patients with type 2 diabetes and other diseases clustered in metabolic syndrome.

11.00 - 11.15

Inhibition of platelet aggregation by a new class of fatty acid oxygenated products called poxotrins, including a protectin d1 isomer

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Background: In spite of results regarding leukotriene B4 and its isomer 5(S),12(S)-dihydroxyeicosatetraenic acid, few data are available on the effect of dihydroxylated triene fatty acids on platelet aggregation.

Objective: The goal of this study was to compare different dihydroxylated fatty acids with the E/Z/E, Z/E/E or E/E/E conjugated triene geometry on human platelet aggregation.

Procedure: Different derivatives with different triene geometry were prepared by soybean lipoxygenase treatment of polyunsaturated fatty acids with 18 to 22 carbons, and tested on platelet aggregation triggered either by collagen, arachidonic acid or U46619, a PGH2 analogue and thromboxane receptor agonist.

Results: We observed that only compounds with E/Z/E conjugated triene geometry equally inhibited collagen-, or arachidonic acid, or U46619-induced platelet aggregation at submicromolar concentrations. In addition, platelet cyclooxygenase but not lipoxygenase was inhibited. The localisation of the conjugated triene had no effect on the inhibitory activity. In contrast, molecules with either Z/E/E or E/E/E conjugated triene geometry had no effect on platelet aggregation. Moreover, neither the presence of double bonds out of the conjugated triene on the fatty chain, nor the stereochemistry of carbons having the hydroxyl groups, were important for such an activity.

Conclusion: It is concluded that dihydroxylated fatty acids with E/Z/E conjugated triene geometry, called poxotrins, are potent anti-aggregatory agents acting at both cyclooxygenase activity and thromboxane receptor site levels.

11.15 - 11.30

Accumulation of two atypical sphingolipids underlies the pathology in hereditary sensory neuropathy HSAN1

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Background: HSAN1 is an autosomal inherited neuropathy, characterized by severe sensory loss, distal muscle wasting and ulcers. HSAN1 is caused by missense mutations in the SPTLC1 subunit of serine palmitoyltransferase (SPT) which catalyzes the initial step in the de novo sphingolipid synthesis; the condensation of serine and palmitoyl-CoA.

Objective: Effect of the HSAN1 mutation on SPT activity?

Procedure: Analysing SPT activity and sphingoid base composition in mutant overexpressing cells, HSAN1 mouse model and in plasma of HSAN1 patients

Results: We found that the HSAN1 mutations induce a shift in the substrate specificity of SPT which enables the enzyme to metabolise, besides serine, also alanine and glycine as alternative substrates. This leads to the formation of two atypical deoxy-sphingoid bases (DSB) Deoxy-sphinganine and Deoxymethyl-sphinganine. Both metabolites lack the C1 hydroxyl group of sphinganine and can neither be converted to complex sphingolipids nor degraded. High DSB levels were found in lymphoblasts of HSAN1 patients and also in the plasma of HSAN1 patients. The metabolites show pronounced neurotoxic effects on cultured sensory neurons impairing neurite number and length. Transgenic mice expressing the HSAN1 mutant develop a peripheral neuropathy at the age of 14 month and show also highly elevated DSB levels in plasma and PNS tissue. Interestingly, we found that the DSB generation can be suppressed in-vivo by increasing L-serine levels. HSAN1 mice which

received an L-Serine enriched diet showed a marked decrease in plasma DSB levels and did not develop neuropathic symptoms. The efficacy of an L-Serine treatment was confirmed in a intervention study with 14 HSAN1 patients. We observed an up to 80% reduction in plasma DSB levels within six weeks of treatment

Conclusion: 1. A neurotoxic accumulation of DSBs is the pathophysiological background of HSAN1

2. A supplementation with L-serine could be a therapeutic approach for treating HSAN1

11.30 - 11.45

Identification of different isoforms of PLA2 in mouse CD36-positive lipid gustatory cells: implication in calcium signalling

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Background / Objective: We have previously demonstrated that the cells expressing CD36, localized apically on the taste buds of mouse lingual circumvallate papillae (CVP), act as lipid gustatory cells. We have also shown that long chain fatty acids (LCFA) induce increases in $[Ca^{2+}]_i$ by recruiting calcium from endoplasmic reticulum, followed by calcium influx via opening of store operated calcium (SOC) channels. However, the mechanisms, implicated in the opening of SOC channels are not well-understood.

Procedures: The experiments were performed on CD36-positive cells, isolated from the lingual CVP of the mouse. We detected different isoforms of PLA2 by RT-qPCR. The increases in $[Ca^{2+}]_i$ were determined by using Fura-2/AM.

Results: We observed that CD36-positive and CD36-negative cells express different isoforms of sPLA2 (secretory), cPLA2 (calcium-dependent) and iPLA2 (calcium-independent). However, the CD36-positive cells expressed, in high quantities, two isoforms of sPLA2, i.e., type V and type IIa, and one isoforme of iPLA2, i.e., type IV. We incubated cells with $[^3H]$ -arachidonate and assessed its release towards the extracellular medium in the presence of a LCFA or other activators like thapsigargin (TG). We observed that TG induced the release of $[^3H]$ -arachidonate in both CD36-negative and CD36-positive cells though the action of these agents was more efficient in the latter. Furthermore, LCFA induced the release of this fatty acid only in CD36-positive cells. By using PLA2 inhibitors in the calcium-free calcium-reintroduction (CFCR), we observed that SOC influx, triggered by TG and LCFA, is inhibited by sPLA2 and iPLA2.

Conclusion: Our results show that lingual CD36-positive cells possess different isoforms of PLA2 which may catalyse the release of free fatty acids into the mouth cavity. Later on, the released free fatty acids acid may be implicated in the modulation of calcium signalling, involved in gustatory perception of lipids, and also in the regulation of other taste modalities.

11.45 - 12.00

The cytoprotective actions of oleoylethanolamide in pancreatic beta-cells require fatty acid amide hydrolase activity and are not mediated via GPR119

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Background & Objective: Selected unmodified mono-unsaturated fatty acids (MUFAs) can protect pancreatic beta-cells against cytotoxicity in vitro, however the molecular mechanisms are unclear. Oleoylethanolamide (OEA), a naturally occurring MUFA derivative, has been recently identified as an endogenous agonist of the G-protein coupled receptor, GPR119, and was shown to stimulate insulin secretion from pancreatic beta-cells. However, it is unknown whether activation of GPR119 by OEA leads to cytoprotection in beta-cells and we have investigated this.

Procedure: Cultured rat beta-cells (BRIN-BD11 and INS-1) were treated with fatty acids complexed to bovine serum albumin in the presence or absence of fatty acid amide hydrolase (FAAH) inhibitors. Cell viability was assessed by vital dye staining or flow cytometry. The effects of synthetic GPR119 agonists on cell viability were also studied. Expression of FAAH was determined by RT-PCR.

Results: Both oleate, and OEA protected beta-cells against the cytotoxic actions of either palmitate (250 μ M) or serum-withdrawal. OEA was more potent than oleate (OEA: EC50-5 μ M; oleate EC50- 25 μ M) although both exerted complete protection at higher concentrations. FAAH mRNA expression was readily detected in BRIN-BD11 and INS-1 cell lines and the cytoprotective response to OEA was abolished by incubation of the cells with either of two irreversible FAAH inhibitors, URB597 (p<0.001) or URB532 (p<0.001) or by the competitive inhibitor JNJ-1661010 (p<0.001). These inhibitors had no

effect on the cytoprotective actions of oleate. The cytoprotection afforded by OEA was not reproduced by any of several synthetic GPR119 agonists (including AR231453, PSN119-1, PSN375967 and PSN632408).

Conclusions: The results demonstrate that OEA exerts a cytoprotective response in pancreatic beta-cells only under conditions when FAAH activity is functional. The results imply that cytoprotection is not mediated by OEA per se and that it does not derive from agonism of GPR119. Rather, the generation of free oleate is required to maintain cell viability.

Concurrent Session - 9

Lipids and Nutrition : Maternal and Infant nutrition II - Mental development

10.30 - 11.00

Invited contribution

LC-PUFA supplementation and infant development

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Long-chain polyunsaturated fatty acids (LCPUFA) have in recent years been purported as important for infant nutrition. DHA, in particular, has been found to have specific structural and functional roles within the central nervous system including signal-transduction, neurotransmission, neurogenesis and anti-inflammation.

Most preterm formula is now supplemented with 0.3% DHA despite limited evidence of a benefit. In the updated Cochrane Review of the 17 randomised clinical trials (RCT), visual acuity over the first year was measured by Teller or Lea acuity cards in eight studies, by VEP in six studies and by ERG in two studies. Most studies found no significant differences in visual assessment between supplemented and control infants. Three out of seven studies reported some benefit of LCPUFA on neurodevelopment in different populations at different postnatal ages. Meta-analysis of Bayley Scales of Infant Development (BSID) of four studies at 12 months (n=364) and three studies at 18 months (n= 494) post-term showed no significant effect of supplementation (Simmer et al 2008). Other meta-analyses of supplemented preterm formula have reported similar results: Smithers et al (2008) reviewed 7 trials which reported neurodevelopment and found no significant effect of supplementation. Beyerlein et al (2010) performed a individual patient data (IPD)-metanalysis of four trials in preterm and term infants, and found no difference between supplemented and control groups, nor in any of the subgroups. Doses in preterm formula trails ranged from 0.2 to 0.64% fatty acids.

LCPUFA supplementation of term formula has not been demonstrated to have an effect on global development at doses equal to breastmilk levels in Western countries. The Cochrane review of LCPUFA supplemented formula for term infants included 14 RCT (n=1719), most of which showed no beneficial effects of LCPUFA supplementation of formula on visual and neurodevelopmental outcomes (one group of researchers demonstrated some beneficial effects on VEP acuity and two groups of researchers showed some beneficial effect on mental development) (Simmer et al 2008). DHA doses in term formula trails ranged from 0.1 to 0.35% fatty acids. Direct DHA supplementation of term infants at low dose (20mg DHA/day) had no clinical benefit in a large Italian RCT (n=1160, Agostini et al 2009).

A dose-response relationship has been proposed and may explain the lack of effect seen in many trials (Lauritzen 2001, Uauy et al 2003, Simmer et al 2008). Henriksen et al (2008) conducted a RCT of 'high' v 'low' dose DHA (enteral 60 v 30mg/d) in preterm infants with birthweight <1500g (n=141) in Norway. At 6m, the 'high' dose group performed better on problem solving subscore of Ages and Stages Questionnaire. The ERP data (electrophysiological measure, event related potential) revealed that infants in the 'high' dose group had lower responses to a standard image and no difference in response to novel image indicating better recognition memory.

In a large Australian trial, preterm infants < 33 weeks (n=657) were randomised to DHA 1% v 0.3% fatty acids (enteral feeds). The data demonstrated a significant benefit for preterm girls in the primary endpoint BSID MDI with posthoc analysis demonstrated that there were fewer children overall with significant cognitive delay in the 'high' dose group (Makrides et al 2009). *'High' dose relates to 60mg and the approximate equivalent 1% fatty acids enteral feeds for preterm infants and is based on inutero accretion and is usually compared with 'low'dose based on breastmilk composition.*

Birch et al(2010) published a randomised dose-response study in term infants which compared sweep VEP visual acuity of infants fed 0, 0.32, 0.64 or 0.96% DHA. Infants fed the unsupplemented formula had significantly poorer visual acuity at 12 m than any of the groups fed DHA supplemented formula but there were no differences in visual acuity between the 3 supplemented groups.

In addition to dose, it is possible that genetic differences may contribute clinical outcomes reported in RCTs. Current research includes analysis of subgroups based on FADS 1 and FADS 2 candidate genes which have a role in the modification of dietary fatty acids, by encoding the delta-5 and delta-6 desaturase enzyme and therefore DHA and AA status. In summary, despite a large number of RCT of LCPUFA-supplemented formula and some trials of direct LCPUFA supplementation, over the past two decades, results are still inconclusive. Further studies using high doses of DHA and sensitive and specific tests of functional outcomes, and analysing genetic subgroups are needed to guide recommendations.

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11.00 - 11.15

Effect of prenatal supplementation with docosahexanoic acid on child size and development at 18 mo: randomized placebo-controlled trial in Mexico

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Background: The n-3 fatty acid docosahexanoic acid (DHA) is important in the development of neural and visual function. Little is known however about the effects of prenatal DHA supplements on child growth and development.

Objective: We evaluated the effects of prenatal DHA supplementation on child size and development at 18 mo of age.

Procedure: We followed up the offspring (n=739; 76% of birth cohort) of women who had participated in a randomized double-blind placebo-controlled trial of 400 mg/day of algal DHA during the latter half of pregnancy. We measured length, weight, head circumference (HC) and development (using the Spanish version of Bayley Scales of Infant Development-II) at 18 mo of age. We calculated standardized psychomotor development (PDI) and mental development indices (MDI).

Results: At 18 mo, intent-to-treat differences (DHA-control) were: length -0.21 cm (95% Confidence Interval: -0.58, 0.15); weight -0.03 kg (95%CI:-0.19, 0.13); HC 0.02 cm (95%CI:-0.18, 0.21); PDI 0.90 (95%CI: -0.55, 2.35) and MDI 0.26 (95%CI: -1.10, 1.63) (all p>0.05 by t-test). Following our previous finding of selective effects by parity on birth size (Ramakrishnan et al., FNB 2010) we tested for interactions by parity. The treatment by parity interaction had an associated p<0.1 for length, weight and HC. Among first-borns, estimates (95% CI) were: length 0.72 cm (0.11, 1.33); weight 0.18 kg (-0.09, 0.44); HC 0.26 cm (-0.11, 0.62); among later-borns, estimates (95% CI) were: weight -0.08 kg (-0.29, 0.12); length -0.13 cm (-0.59, 0.32); HC -0.17 cm (-0.40, 0.05). The heterogeneity remained significant for length (p< 0.05) even after adjusting for birth size. There was no heterogeneity by parity for PDI and MDI.

Conclusion: Prenatal DHA supplementation in a population with low intakes of DHA did not improve global development scores but was associated with improved linear growth in first-borns at 18 mo of age.

11.15 - 11.30

Cognitive function in 18-month-old term infants of the Diamond study: a DHA dose-response randomized, controlled clinical trial

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Background: Studies investigating the cognitive effects of docosahexanoic (DHA) supplementation yield conflicting results, perhaps due to inadequate dietary DHA concentrations.

Objective: To assess the optimal concentration of DHA supplementation to term infant formula for enhanced maturation of cognitive function at 18-months of age.

Procedure: This was a double-masked, randomized, controlled, prospective trial. A total of 139 infants were enrolled at 1-9 days of age and randomly assigned to infant formula with one of four levels of DHA: Control (0% DHA; n=28), 0.32% DHA (n=32), 0.64% DHA (n=32), or 0.96% DHA (n=28). All DHA-supplemented formulas contained 0.64% arachidonic acid. Infants were fed assigned formulas to 12 months of age when blood fatty acids were analyzed. Cognitive function was assessed at 18 months using the Bayley Scales of Infant Development II (BSID II).

Results: There were no diet group differences on the Mental Development Index (MDI), the Psychomotor Development Index (PDI), or the Behavior Rating Scale (BRS) of the BSID II. However, when the MDI scores of children who received DHA-supplemented formulas were combined (mean=104.1) and compared to control children (98.4), a significant difference emerged ($p=0.02$). Children fed the control formula were more likely to show delayed performance on the MDI ($p=0.05$). MDI scores at 18 months and the blood DHA level at 12 months (intervention termination) were not correlated overall, likely due to the ceiling effect observed for higher levels of DHA; indeed, a significant correlation was found when the two highest supplementation levels (which conferred no additional cognitive benefit) were omitted from the analysis ($p<0.05$).

Conclusions: These results suggest that dietary supplementation of DHA during the first year of life leads to enhanced cognitive development at 18 months of age. DHA levels greater than 0.32% in term infant formula did not further enhance cognitive function at this stage of infant development.

11.30 - 11.45

LC-PUFA supplemented to mothers during pregnancy and breast-feeding improves cognitive performance in their children four years later—an rct study

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Background: n-3 fatty acids may be related to cognitive development, including intelligence, executive skills and social cognition. However, it is not known if this relation is due to genetics of fatty acid metabolism, or if it can be influenced by means of PUFA supplementation.

Objective: The main purpose of this study was to investigate the possibility that PUFA supplementation from the third trimester through the first three months of breastfeeding influenced subsequent cognitive performance. A secondary purpose was to assess the relationship between n-3 and n-6 PUFA plasma levels and performance.

Procedure: Placebo controlled randomized double-blind study. Mothers received daily supplementation with 1.6g eicosapentaenoic acid (EPA) and 1.1g docosahexaenoic acid (DHA) (Pharma Nord, Vejle, Denmark) or placebo starting in the 25th gestational week and continuing through 3.5 months of breastfeeding. Blood samples from the children were taken at birth, 3, 12, and 24 months of age. A battery of neuropsychological tasks (assessing language, memory, spatial and executive skills) was administered when the children were 46 months old.

Results: PUFA supplementation resulted in significant improvement as to errors in the executive task ($P=.01$) and the visuospatial block design task ($P=.04$); memory and language remained unchanged. When performance at 46 months was correlated with plasma PUFA, executive task errors were related to the AA/EPA and AA/DHA ratios (significant correlations ranged between .34 and .49 for varying time points; suggestive of a weak-to-moderate strength of association).

Conclusion: PUFA supplementation during latter parts of gestation and the first month of breastfeeding resulted in improved performance on a visuospatial task and an executive task involving behavioral inhibition. The outcome gives further support to the notion that PUFAs are involved in intelligence and cognitive processes related to social skills and demonstrate that the associations are apparent between three and four years of age.

11.45 - 12.-00

Effects of EPA versus DHA on literacy and behaviour in children with ADHD and learning difficulties

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Background: Previous studies indicate that supplementation with omega-3 polyunsaturated fatty acids (n-3 PUFA) may improve symptoms in children with ADHD and learning difficulties. However, the relative benefits of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) supplementation are unknown.

Objectives: To compare effects of supplementation with DHA-rich and EPA-rich oils versus safflower oil placebo on attention literacy and behaviour in children with ADHD symptoms and learning difficulties.

Procedure: Ninety children were recruited for a 12-month double-blind placebo-controlled three-way crossover trial. Supplements high in EPA (1000 mg EPA + 120 mg DHA/day), DHA (240 mg EPA + 1000 mg DHA/day) and n-6 PUFA (safflower oil) were each consumed in random order for four months. Data were gathered on erythrocyte PUFA status, assessments of attention, cognition and literacy and Conner's Parent Rating Scales (CPRS) at 0, 4, 8 and 12 months.

Results: Fifty four volunteers completed the trial. Comparison of outcomes within each child following each treatment showed no differences overall following EPA or DHA supplementation compared with placebo. There were also no differences in responses to the supplements in a parallel comparison over the first 4 months. However, in 45 children with erythrocyte PUFA data, increases in DHA content over 4 months were associated with improved CPRS scores on oppositional behaviour ($r=.38$, $p<.05$), anxiety/shyness ($r=.33$, $p<.05$), divided attention ($r=.32$, $p<.05$) and reading ($r=.50$, $p<.01$). In a subgroup with learning difficulties ($n=16$), increased DHA was associated with improved oppositional behaviour ($r=.78$, $p<.01$), hyperactivity ($r=.70$, $p<.01$), restlessness/impulsivity ($r=.71$, $p<.01$), divided attention ($r=.71$, $p<.01$), word reading ($r=.67$, $p<.01$) and spelling ($r=.56$, $p<.01$).

Conclusion: DHA consumption may improve ADHD symptoms and literacy in children with learning difficulties. Further studies with larger samples are required to investigate this further.

Concurrent Session - 10

Lipids and Health : Brain function II - Other aspects

14.00 - 14.15

The effects of arachidonic acid and docosahexaenoic acid on neural stem/progenitor cells

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Background: We have previously shown that arachidonic acid (ARA) promotes postnatal neurogenesis in the rat hippocampus. In addition, another group has shown that docosahexaenoic acid (DHA) promotes adult neurogenesis in the hippocampus in rats fed with a fish-oil deficient diet over three generations.

Objective: To further elucidate functions of these fatty acids in neurogenesis, we analyzed effects of ARA and DHA on maintenance and differentiation of neural stem/progenitor cells (NSPCs) using in vitro neurosphere assays.

Procedure: Here, we used primary neurospheres as neurogenic NSPCs and tertiary neurospheres as gliogenic NSPCs. To examine effects on maintenance of NSPCs, neurospheres were dissociated and incubated with various concentrations of ARA and/or DHA. After 7 days in culture, the number of neurospheres was counted. To examine effects on differentiation of NSPCs, neurospheres were dissociated and cultured attached to dishes with medium containing various concentrations of ARA or DHA. After 4 days in culture, cells were stained with antibodies specific for cell types, and the proportion of neurons and astrocytes was calculated.

Results: Regarding effects of ARA and DHA on neurogenic NSPCs, ARA at 10^{-7} and 10^{-6} M increased the number of neurospheres and so did DHA at 10^{-7} M, whereas both ARA and DHA had no detectable effect on differentiation of NSPCs. As for effects of ARA and DHA on gliogenic NSPCs, DHA at 10^{-10} to 10^{-8} M increased the number of neurospheres, while ARA had no such effect. On the other hand, ARA at 10^{-5} M increased the proportion of astrocytes, whereas DHA at 10^{-5} M increased that of neurons.

Conclusion: ARA promotes maintenance/proliferation of neurogenic NSPCs and induces glial differentiation of gliogenic NSPCs. DHA promotes maintenance/proliferation of both neurogenic and gliogenic NSPCs and induces neuronal differentiation of gliogenic NSPCs.

14.15 - 14.30

Effects of dietary n-3 fatty acids and brain DHA content on activity and response to novelty in the developing rat

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Background: Insufficient availability of n-3 fatty acids during pre- and neonatal development decreases accretion of docosahexaenoic acid (DHA, 22:6n-3) in the developing brain. Low tissue levels of DHA are associated with neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD).

Objective: The goal of this project was to determine the effects of inadequate dietary n-3 fatty acids, and the resulting changes in brain DHA content, on activity and responses to novelty across development in a rat model.

Procedure: First- and 2nd-litter male Long-Evans rats were raised from conception on either a control diet containing α -linolenic acid or a diet deficient in α -linolenic acid. Activity, habituation, and response to spatial change in a familiar environment were assessed in a single-session behavioral paradigm at ages between postnatal days 28 and 70 inclusive, using a between-groups design.

Results: The deficient diet resulted in a decrease in brain phospholipid DHA of 44% in 1st-litter pups and 62% in 2nd-litter pups at postnatal day 28. Activity and habituation varied by age with younger rats exhibiting higher activity, less habituation, and less stimulation of activity induced by spatial novelty. During the first and second exposures to the test chamber, which assessed activity, habituation, and response to a familiar environment, 2nd-litter-deficient pups exhibited higher levels of activity that persisted later into peri-adolescence than control or 1st-litter-deficient pups, but were not more active after introduction of a novel spatial stimulus.

Conclusion: The higher level of activity observed in periadolescent 2nd-litter-deficient pups in familiar, but not novel, environments is consistent with clinical observations in ADHD and suggests that the observed behavioral effects are likely due to differences in brain DHA accumulation during development, rather than dietary n-3 PUFA content.

Supported by NIH MH067938, NIH HD02528, and NIH RR016475

14.30 - 14.45

The relationship between measures of depression, anxiety and anger/disruptive behaviour and omega-3/6 fatty acids in adolescents with and without ADHD

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Background: Inadequate fish consumption and low levels of omega-3 fatty acids have been persistently related to mood disorders and symptoms of depression. The World Health Organisation has predicted a global rise in mental ill-health, along with a 50% increase in child mental ill health by 2020. ADHD is a neurodevelopmental disorder associated with a number of complex symptoms including inattentiveness, hyperactivity, restlessness, and impulsivity. It is also comorbid with mood disorders, such as anxiety and depression. This study employed the Becks Youth Inventories (BYI-II) which to date has not been examined in relation to essential fatty acids in red blood cells of children/adolescents with and without ADHD. The BYI-II comprises of 5 self-report scales and assesses a child's experience of depression, anxiety, anger, disruptive behaviour and self concept.

Objective: In light of previous research it was hypothesised that the long-chain ω 3 fatty acids, EPA and DHA would be negatively associated with scores of depression, anxiety and positively associated with self concept in children/adolescents with and without ADHD. Furthermore and based on findings with young offenders, a secondary hypothesis predicted that blood levels of ω -3 will be inversely related to scores of anger and disruptive behaviour.

Procedures: Fifty children were assessed for total lipid fractions in their red blood cells and measures of BYI-II.

Results: Preliminary blood analyses (n = 28) support some but not all of the hypotheses. However, data from the full sample will be available and presented in May.

Conclusion: In light of the WHO predictions concerning increases in child mental ill health, it is important to determine whether specific polyunsaturated fatty acids as measured in erythrocyte membranes are associated with the behaviour and ideation of children/adolescents with and without ADHD. Such investigations may also have implications for the focus and development of less costly, dietary interventions.

14.45 - 15.00

Omega-3 fatty acids for secondary prevention of posttraumatic stress disorder following accidental injury: an open-label pilot study

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Background: Animal studies have revealed that augmentation of dietary omega-3 fatty acids relative to omega-6 fatty acids up-regulated adult neurogenesis. Recently, Kitamura et al. have shown that hippocampal neurogenesis contributes to the clearances of artificially induced long-term potentiation, putative cellular model of learning and memory, in the dentate gyrus of hippocampus (Cell, 2009). We hypothesized that promoting adult neurogenesis by omega-3 fatty acids might facilitate clearance of fear memory in humans.

Objectives: We assessed the efficacy and safety of omega-3 fatty acids in the secondary prevention of posttraumatic stress disorder (PTSD) following accidental injury.

Procedure: A prospective, open-label trial of omega-3 fatty acid supplementation was conducted in accident-injured patients (n = 15; 80% men; mean age of 34 years) consecutively admitted to an intensive care unit. The median number of hours between the time when the emergency medical-care system recognized the accident and when written consent was obtained was 74 hours. Patients received omega-3 fatty acid capsules containing 1,470 mg docosahexaenoic acid and 147 mg eicosapentaenoic acid daily for 12 weeks. The primary efficacy variable was total score on the Clinician-Administered PTSD Scale (CAPS).

Results: Omega-3 fatty acid supplementation was well tolerated and resulted in a significantly increased docosahexaenoic acid in erythrocytes. Compared with the hypothetical mean in our previous cohort study, omega-3 fatty acid supplementation resulted in significantly reduced mean CAPS total scores (11 vs. 25, p = 0.03). There were significant differences in erythrocyte DHA concentrations between weeks 0 and 12 (mean % total fatty acids: 5.9 [SD = 1.4] vs. 8.4 [SD = 1.7, p < .001]).

Conclusion: Omega-3 fatty acid supplementation in patients with accidental injury might be efficacious in attenuating PTSD symptoms.

Trial Registration: ClinicalTrials.gov Identifier. NCT00671489

Support: This study was supported by CREST, Japan Science and Technology Agency.

15.00 - 15.15

Arachidonic acid (ARA)-enriched triacylglycerol supplementation was more effective in improving cognitive function of elderly men with low serum ARA level

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Background & Objective: In a previous study, we have shown that supplementation of arachidonic acid (ARA)-enriched triacylglycerol improved cognitive function in elderly men employing measurement of P300 components, which are the most intensively investigated event-related potentials (ERPs) measure. In order to clarify the relationship of serum ARA level to cognitive function, and the effect of ARA-enriched triacylglycerol on cognitive function, stratified analyses were performed.

Procedure: The study used a double-blind crossover design. The subjects (20 healthy Japanese men, 62.70 ± 3.18 years) were administered 600 mg/day of ARA-enriched triacylglycerol (containing 240 mg ARA) in capsules or the same amount of olive oil in capsules as an inactive placebo for one month. ERPs were measured before capsule administration and after one month of administration, and P300 latency and amplitude were measured. Before and after the administration periods, fatty acid composition of serum phospholipids was determined.

Results: After one month supplementation, ARA-enriched triacylglycerol shortened P300 latency significantly, and increased P300 amplitude significantly. Stratified analyses performed for the content of ARA in serum phospholipids revealed that P300 latency of the subjects with lower content of ARA (Low-ARA group) was significantly longer, and P300 amplitude of Low-ARA group tended to be lower than those with higher content of ARA (High-ARA group). Furthermore, the

stratified analyses suggested that ARA-enriched triacylglycerol supplementation was more effective in Low-ARA group than in High-ARA group. In Low-ARA group, ARA-enriched triacylglycerol shortened P300 latency significantly, and increased P300 amplitude significantly, although no significant differences were found in High-ARA group.

Conclusion: It can be concluded from these data that aging-associated decrease in cognitive function was mainly found in Low-ARA group, and ARA-enriched triacylglycerol supplementation was more effective in improving cognitive function in those Low-ARA subjects.

15.15 - 15.30

Fish fatty acids and mental health in older people

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Background: Higher intakes of fish and marine n-3 polyunsaturated fatty acids are suggested to be protective against age-related cognitive decline and impaired mental well-being. However, results from observational studies are inconclusive and data from randomized controlled trials in older people without clinical dementia or depression are scarce.

Objective: We investigated the effect of daily supplementation with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on cognitive performance and mental well-being in an older non-clinical population. Furthermore, we assessed the association of fish and EPA+DHA intake with mental health in different aging populations.

Procedure: The effect of low and high doses of EPA+DHA (400 and 1,800 mg per day, respectively) on cognitive performance and mental well-being was examined in a 26-week randomized controlled trial involving 302 individuals aged 65 years or older. The cross-sectional association between fatty fish and EPA+DHA intake with cognitive change during 6 years of follow-up was assessed in 1,025 aging US men. The associations of EPA+DHA and fish intake with depressive symptoms and dispositional optimism were assessed in 644 Dutch subjects with a history of myocardial infarction.

Results: Daily intake of low or high doses of EPA+DHA did not affect cognitive performance or mental well-being after 13 or 26 weeks of intervention. In elderly US men we found no association of fatty fish or EPA+DHA intake with 6-year cognitive change. Intake of EPA+DHA was positively associated with dispositional optimism, but not with depressive symptoms in subjects with a history of myocardial infarction.

Conclusion: Supplemental intake of EPA+DHA is unlikely to have a short-term impact on cognitive performance or mental well-being of older people without a clinical diagnosis of dementia or depression. Whether long-term intake of EPA+DHA and fish could be beneficial to the maintenance of cognitive performance or mental well-being of older people still needs to be established.

Concurrent Session - 11

Biochemistry of Lipids: Cardiobiochemistry

14.00 - 14.15

Fish oil feeding induces nutritional preconditioning in the rat heart by upregulating endogenous antioxidants and reducing oxidative damage

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Background: Dietary intervention with fish oil increases incorporation of long-chain n-3 polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA) into cardiac and other muscle membranes. The consequent increase in unsaturation of the membranes together with their high metabolic rates predicts a high risk of oxidation damage in these tissues. However, this contradicts the reputed cardioprotective effects of dietary fish oil.

Objective: This study investigated the effects of n-3 PUFA on oxidative stress markers and endogenous antioxidants in the heart at rest and after ischaemia and reperfusion.

Procedures: Rats were fed diets rich in fish oil (n-3 PUFA); sunflower seed oil (n-6 PUFA); or saturated fat-rich beef tallow (SF) for 6 weeks. Isolated perfused hearts (37°C) were reperfused for 120min after 30min occlusion of the left anterior descending coronary artery. Lipid peroxidation products and antioxidant concentrations were measured in normoxic hearts and in ischemic and non-ischemic regions of the hearts.

Results: The concentrations of lipid hydroperoxides (LPO) and malondialdehyde (MDA) and antioxidant manganese superoxide dismutase (MnSOD) were higher in n-3 PUFA normoxic perfused hearts at rest. Ischaemia and reperfusion induced large increases in LPO and MDA in the ischaemic region of SF and n-6 PUFA hearts, but not in the n-3 PUFA hearts. Infarct size was reduced in the n-3 PUFA hearts and was inversely associated with MnSOD concentration across all diets ($r^2=0.851$; Slope: $p < 0.001$, $N=18$). The n-6 PUFA diet was associated with elevated α -tocopherol with no reduction in infarct size.

Conclusions: Fish oil feeding increased n-3 PUFA concentration and predicted risk of peroxidation in myocardial membranes but paradoxically, ischaemic damage was reduced. The results of this study confirm that dietary fish oil is cardioprotective and suggest that n-3 PUFA-induced nutritional preconditioning of the heart is in part mediated by upregulating endogenous anti-oxidant systems.

14.15 - 14.30

The cardioprotective effect of docosahexaenoic acid occurs through myocardial physiological and metabolic changes in rat heart on digitalis drug treatment

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Background: Cardioprotective effects have been observed with fish oil containing both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In particular, fish oil promoted positive inotropy of ouabain without inducing toxicity and changes in cardiac metabolism (Maixent *et al.*, Am. J. Physiol. 1999).

Objectives: We tested the hypothesis that DHA is mainly responsible for these effects and that an enrichment of the diet with DHA alone could confirm these previous results.

Procedure: Four groups of 13 adult male rats were respectively fed during 4 weeks a diet supplemented with regular egg yolk powder (10 mg DHA/kg body weight, DHA10) or DHA-enriched egg yolk powder (35 or 60 mg DHA/kg, DHA35 or DHA60) or standard rat chow (control, C). Fatty acid profile of heart membranes was determined by gas chromatography. The responsiveness to ouabain (10^{-7} M to $3 \cdot 10^{-4}$ M) was evaluated in Langendorff perfused rat hearts. The relative variations in cardiac energetic metabolism and intracellular pH were obtained during the cumulative ouabain dose-response curves by phosphorus-31 magnetic resonance spectroscopy. All data were expressed as means \pm SEM.

Results: Supplementation led to a dose-dependent increase in cardiac membrane incorporation of DHA resulting in significantly increased contents of phosphocreatine at baseline for DHA35 and DHA60 groups (12.4 ± 1.8 and 15.8 ± 0.9 vs. 8.6 ± 1.1 mM for C group, $p < 0.001$). Positive inotropy (10^{-4} M ouabain dose) achieved with ouabain was significantly increased in DHA groups vs control group (percent above the control values: 126 ± 46 , 135 ± 93 , $136 \pm 105\%$ respectively for DHA10, DHA 35 and DHA60 vs $40 \pm 19\%$ in control group, $p < 0.05$). Alteration of function and energetic metabolism in response to ouabain toxic dose ($3 \cdot 10^{-4}$ M) was delayed.

Conclusion: This study demonstrates that dietary supplementation with DHA at the dose of 60 mg/kg improves ouabain efficiency on myocardial contractility and delays toxicity.

14.30 - 14.45

Effect of dietary omega-3 fatty acids on the endogenous CYP-eicosanoid profile

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Background: Cytochrome P450 (CYP)-dependent arachidonic acid (AA) metabolites such as epoxyeicosatrienoic acids (EETs) and 20-hydroxyeicosatetraenoic acid (20-HETE) serve as second messengers in the regulation of cardiovascular function.

Objective: This study was performed to test the hypotheses that eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) are alternative substrates of AA metabolizing CYP-enzymes.

Procedure: Fatty acid metabolism by recombinant CYP1A, CYP2C, CYP2J, CYP4A and CYP4F isoforms was analyzed by HPLC. Rats (n=6 per group) received diets supplemented with either omega-6 fatty acids (5 % sunflower-oil; SF-group) or additionally with EPA/DHA (2.5 % OMACOR®, Solvay Arzneimittel GmbH; OM-group) for 3 weeks. Fatty acid and CYP-eicosanoid profiles were determined by gas chromatography and LC-MS/MS.

Results: The CYP-isoforms tested displayed equal or even higher activities when converting EPA or DHA instead of AA. Most of the epoxigenases converting AA to EETs preferentially attacked the omega-3 double bond and produced 17,18-epoxyeicosatetraenoic acid (17,18-EEQ) and 19,20-epoxydocosapentaenoic acid (19,20-EDP) from EPA and DHA. The hydroxylases that produced 20-HETE from AA metabolized EPA to 20-hydroxyeicosapentaenoic acid (20-HEPE) and DHA to 22-hydroxydocosahexaenoic acid (22-HDoHE). Compared to SF-rats, OM-feeding resulted in a partial and tissue-specific replacement of AA by EPA and DHA in membrane phospholipids. Concomitantly, the EET:EEQ:EDP-ratios were significantly shifted, namely from 95:1:4 to 38:56:6 in the kidney and from 87:0:13 to 27:18:55 in the left ventricle. Moreover, 17,18-EEQ and 19,20-EDP became the predominant epoxy-metabolites in these organs. Similar profound changes occurred in the plasma, liver, lung and pancreas but were less pronounced in the brain. High endogenous 20-HETE levels in the organs of SF-fed rats were 60-90% reduced upon OM-feeding and compensated by strongly increased 20-HEPE- and 22- HDoHE-levels.

Conclusion: EPA and DHA are efficiently converted by CYP enzymes to novel epoxy- and hydroxy-metabolites that may mediate some of the beneficial cardiovascular effects of dietary omega-3 fatty acids.

14.45 - 15.00

Balanced substrate supply is essential for cardiac protection of the compromised heart

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Background: Unbalanced glucose and fatty acid levels are considered to contribute towards development of metabolic cardiomyopathy.

Objective: To investigate if cardiac stress by pressure overload after thoracic aortic constriction (TAC), on top of metabolic stress by exposure to a Western-type diet (WTD), aggravates TAC-induced cardiac dysfunction and inappropriate remodeling, and if CD36 ablation protects against detrimental dietary effects.

Procedure: Wildtype (WT) and CD36^{-/-} mice received regular chow or WTD for 10 weeks, and then underwent sham-operation or TAC. Diet was continued for 6 weeks, and then cardiac function was assessed.

Results: In TAC, but not in sham mice, both WTD (-26%, p<0.05) and CD36^{-/-} (-35%, p<0.05) reduced fractional shortening, in comparison to WT chow. Both interventions increased left ventricle (LV) hypertrophy after TAC (LV/bodyweight: +1.83 and 1.75-fold, p<0.001), while only CD36^{-/-} altered ANF expression after TAC (+9.14-fold, p<0.01). Surprisingly, the combination of WTD and CD36^{-/-} restored cardiac functional and structural parameters after TAC. WTD increased intramyocardial lipid content in WT (+1.66-fold, p<0.001). CD36^{-/-} reduced cardiac lipid content relative to WT (-1.47-fold, p<0.001), whereas combined WTD and CD36^{-/-} resulted in similar lipid levels as WT-chow. WTD, CD36^{-/-} or surgery did not affect protein-expression of other fatty-acid transporters, mRNA expression of Cpt1 and PGC1, or expression of β^2 -oxidation proteins. WTD induced an increase in basal Akt-phosphorylation in WT-TAC (p<0.05). CD36^{-/-} increased gene-expression of PPAR α (p<0.01) and decreased Pdk4 expression (p<0.05).

Conclusion: In contrast to the effects observed in WT, exposure of CD36^{-/-} to WTD prevented adverse effects of pressure overload on cardiac function and remodeling, in association with decreased intramyocardial lipid accumulation. These data show the metabolic roots of cardiac dysfunction and imply that treatment strategies using metabolic manipulation may be beneficial in the compromised heart.

Dutch Diabetes Research Foundation, Grant: 2006.00.044.

15.00 - 15.15

Myocardial lipid accumulation and diabetic cardiomyopathy status studied in vivo in a diabetic mouse model using ¹H-MRS and MRI

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Background: The preference towards fatty acid (FA) metabolism in the diabetic heart may lead to an elevated level of myocardial lipids when FA uptake exceeds the rate of oxidation. This metabolic disturbance is thought to be partly responsible for the development of diabetic cardiomyopathy (DCM), *i.e.* the reduction of cardiac performance without any evidence of coronary artery disease.

Objective This study aims to assess the relationship between myocardial lipid accumulation and the status of DCM using ¹H MRS and MRI.

Procedure: Four 7-week old diabetic (db/db) and six non-diabetic (db/+) mice underwent MR measurements at a 9.4T scanner. Fasting blood glucose level was measured prior to the MR measurements. Cardiac metabolite spectra were acquired in a 4 μL interventricular septal voxel using localized ¹H MRS to quantify the myocardial lipid level, which was calculated as the CH₂-lipid to unsuppressed water peak ratio. Cinematic FLASH MRI was performed to calculate ejection fraction (EF) and early-to-late ventricular filling (E/A) ratio as a measure for systolic and diastolic function, respectively. The resulting cardiac movies were also analyzed to calculate the left ventricular (LV) mass. Data are presented as means±SD for db/db vs db/+ mice.

Results: The db/db mice were hyperglycemic, whereas the db/+ mice had normal blood glucose levels (15.45±2.30 mM vs 5.85±0.75 mM, p<0.001). The db/db mice exhibited early-stage DCM as indicated by a significant reduction in diastolic function (E/A: 0.92±0.18 vs 1.27±0.22, p=0.025), but no alteration in systolic function (EF: 72.13±1.17% vs 72.39±0.87%, p=0.715). In addition, the db/db mice showed a trend for hypertrophy (LV mass/tibia length: 3.54±0.08 mg/mm vs 3.37±0.18 mg/mm, p=0.110). At this stage, a significant increase in myocardial lipid level was observed in the db/db mice (lipid/water: 2.01±0.43% vs 1.34±0.21%, p=0.045).

Conclusion: Our observation suggests that myocardial lipid accumulation might be associated with the early-stage development of DCM.

15.15 - 15.30

Myocardial sphingolipid metabolism in Zucker diabetic fatty rats

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Background: Myocardial lipid accumulation and consequently lipotoxicity have been proposed to contribute to the development of metabolic cardiomyopathy in Zucker diabetic fatty (ZDF) rats. Diabetes-related lipid accumulation increases ceramides, which are known to induce insulin resistance, inflammation, and apoptosis in skeletal muscle. Ceramides can be synthesized by *de novo* synthesis from fatty acids and by hydrolysis of sphingomyelin. Glycosphingolipids are suggested to mediate the link with insulin resistance, whereas sphingosine-1-phosphate has been reported to protect against ischemia/reperfusion. However, detailed sphingolipid metabolism has not been studied in the metabolic compromised heart.

Objective: We performed lipodomic analyses in hearts of ZDF rats to examine the involvement of sphingolipid metabolism in the development of metabolic cardiomyopathy.

Procedure: Left ventricular tissue from ZDF and Zucker lean (ZL) rats were used for lipodomic, protein, mRNA and histological analyses.

Results: Consistent with a diabetic phenotype, ZDF rats were obese, hyperglycemic, hyperinsulinemic, hypertriglyceridemic and insulin resistant (all p<0.05). Increased triacylglycerol, diacylglycerol and ceramide content were found in ZDF hearts (all p<0.05), which was paralleled by increased TNFα protein expression (p<0.0001) and TUNEL-positive cells (p=0.09). Sphingomyelin was 1.4-fold increased (p<0.05) in ZDF hearts, combined with a 0.4-fold decreased phosphatidylcholine content (p<0.01) compared to ZL hearts. No differences were found in myocardial sphingosine and

sphingosine-1-phosphate content. Further, despite the unchanged lipid content of glucosylceramide and lactosylceramide and mRNA levels of glucosylceramide synthase and GM3 synthase, ganglioside GM3 was decreased ($p < 0.0001$) in ZDF hearts compared to controls.

Conclusion: Increased lipid accumulation in the diabetic rat heart was paralleled by signs of inflammation and apoptosis. Moreover, changes in sphingolipid metabolism pointed forwards to augmentation of sphingomyelin, impaired ganglioside metabolism, whereas sphingosine-1-phosphate seems unaltered. Further studies are needed to investigate the role of glycosphingolipid metabolism and ceramide synthesis via hydrolysis of sphingomyelin in the association between myocardial lipid accumulation and metabolic cardiomyopathy.

Concurrent Session - 12

Lipids and Nutrition : Standardizing protocols

14.00 - 14.30

Invited contribution

Methodological issues and inconsistencies in omega-3 fatty acid research

S Dyll

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There is growing interest into researching the physiological and biochemical roles of omega-3 fatty acids; however, there is considerable variability in the basic methodological rigour employed in the design of many studies. For example, many studies typically involve the addition of oil based preparations to standard animal feed and it is well known that under ambient conditions polyunsaturated fatty acids (PUFA) are particularly unstable and prone to autoxidation and peroxidative damage. This has the potential to not only decrease the available dose of PUFA, but also introduce toxic aldehydes into the experimental diets. It is therefore important to take specific precautions with the stock preparations and when preparing the experimental diets.

In addition, there are many other methodological complications inherent to omega-3 PUFA research that need to be considered when designing and conducting studies. For example, fish oil preparations typically contain between 30 to 50% omega-3 PUFA, leaving a range of other ingredients such as other fatty acids, cholesterol and antioxidants added to experimental diets, which cannot always be considered innocuous. For this reason in our fish oil enrichment studies there are typically two control diets, a basal diet and a basal diet supplemented with the other major ingredients found in the fish oil preparations. Other confounding factors include the type and dose of antioxidant to add to high purity oil preparations and experimental diets and importantly selecting the most appropriate control/placebo diets. Until there is general agreement as to what constitutes good experimental design in omega-3 PUFA research there will always be concerns over results being artefacts of the experiment. The purpose of this presentation is to illustrate how we have tried to address some of these issues in our studies and discuss the foundations of future recommendations.

14.30 - 14.45

Participating in a controlled dietary fat study, rather than the nature of intervention, regulates the plasma proteome in healthy men

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Background: Consumption of industrial trans fatty acids raises LDL and lowers HDL cholesterol, and raises the risk of coronary heart disease. It is unclear whether consumption of trans fatty acids from dairy products - like cis-9, trans-11 conjugated linoleic acid (CLA), a trans fatty acid derived from biohydrogenation in ruminant animals - modulates disease development.

Objective: We investigated the effect of industrial trans fatty acids or cis9, trans11 CLA, compared with oleic acid, on the plasma proteome in healthy men.

Procedure: Twelve men were sequentially fed each of three diets for three weeks, in random order, for a total of nine weeks. Diets were identical except for 7% of energy (approximately 20 g/day) which was provided either by industrial trans fatty acids, cis9, trans11 CLA or oleic acid. Plasma was collected at the end of each 3-week intervention period, depleted of its 12 most abundant proteins and analysed by 2-D gel electrophoresis.

Results: Principal component analysis of protein spot intensity values revealed that the intervention provided in the 1st period produced a significant treatment effect compared with the interventions being provided in the 2nd and 3rd period. There was also a significant subject effect, but the nature of the dietary intervention did not significantly affect the plasma proteome. These findings were confirmed by the distribution of p-values of tests of treatment, period and subject effects. We are now in the process of identifying the proteins responsible for the period and subject effects using mass spectrometry.

Conclusion: The initial participation in this controlled dietary intervention study markedly affected the plasma proteome, whereas the nature of the dietary intervention, i.e. 7% of energy provided by industrial trans fat or cis9,trans11 CLA, did not. This finding may affect the interpretation of results of such controlled dietary intervention studies.

14.45 - 15.00

Long term adherence following advice to increase eicosapentaenoic and docosahexaenoic acid intakes using different dietary strategies

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Background: Canadian eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) intakes are lower than levels associated with protection from cardiovascular disease.

Objective: To examine one year adherence to different strategies to increase EPA and DHA intakes.

Procedure: Males and females (ages 35-50, n=45) with low EPA and DHA status were randomized to one of four dietary advice strategies targeting an EPA and DHA intake of 1g/d. The advice was based on seafood, fish oil supplement, omega-3 enriched food, or combined strategies. Adherence was determined by 3-d dietary records and fatty acid composition analyses of fasting blood at 0, 1, 2, 3, 6 and 12 months. Dietary fat intake was also determined by 3-d duplicate food analyses at 1 month.

Results: The percentage of n-3 highly unsaturated fatty acids (>20 carbons, >3 carbon-carbon double bonds, HUFA) in total HUFA in erythrocytes of all participants was $27.5 \pm 3.9\%$ at baseline. Erythrocyte n-3 HUFA in total HUFA% peaked at 3 months with significant increases from baseline in fish oil ($35.6 \pm 4.4\%$), combined ($35.0 \pm 3.8\%$) and seafood ($34.5 \pm 2.7\%$) but not omega-3 enriched food ($30.6 \pm 5.0\%$) strategies ($p < 0.05$). At 12 months, these values remained significantly higher than baseline for fish oil ($32.2 \pm 4.0\%$), but combined ($32.6 \pm 4.2\%$), seafood ($29.5 \pm 3.2\%$) and enriched food ($29.1 \pm 4.1\%$) were similar to baseline ($p < 0.05$). Intake of EPA and DHA as determined by 3-d dietary record at 3 months was estimated to be 1.3 ± 0.9 g/d for seafood, 1.1 ± 0.1 g/d for fish oil, 0.7 ± 1.1 g/d for omega-3 enriched food and 1.5 ± 1.1 g/d for combined.

Conclusion: Dietary advice to increase EPA and DHA intakes can significantly increase erythrocyte levels of EPA and DHA. Adherence to dietary advice strategies by Canadians may be the highest with fish oil supplementation.

15.00 - 15.15

Percentage of n-3 in HUFA - a simple measure of HUFA balance

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Background: Epidemiological studies among different populations show significant differences in n-3 HUFA levels which correlate with health outcomes. Ischaemic heart disease, depression and even homicides are linked to seafood consumption and n-3 HUFA levels. N-3 HUFA status has been expressed as Arachidonic acid/Eicosapentaenoic acid (AA/EPA) ratio and as the Harris Schacky Index (HS Index). The latter combines EPA and DHA levels but excludes docosapentaenoic acid (22:5n-3) which can be a significant contributor to n-3 HUFA levels.

Objective: The percentage of n-3 in HUFA is a simple expression of HUFA balance, using the seven most clinically significant HUFA in the analysis: 20:3n-6, 20:4 n-6, 22:4n-6, 22:5n-6, 20:5n-3, 22:5n-3 & 22:6n-3. We call the percent of total HUFA that is n-3 HUFA “The Ideal Omega Index”.

Procedure: We compared RBC polar lipid fatty acids with those from finger tip whole blood samples from 51 subjects.

Results: The percentage of n-3 in HUFA was closely correlated in both types of sample ($r^2 = 0.946$), showing isolation of RBC is not needed for valid HUFA assay in health risk assessments. Also, 36 Scottish patients eating diets with no fish oil supplements had values ranging from 12% to 30% n-3 in HUFA (average = 19.7%). Another group of 131 subjects taking varied amounts of supplements had %n-3 in HUFA ranging from 14% to 64%, showing the wide diversity of HUFA balance that people can maintain when motivated.

Conclusion: This convenient method of testing and interpreting results offers an opportunity for widespread testing of HUFA status in the population and could motivate significant lifestyle change with positive health consequences.

15.15 - 15.30

Whole blood fatty acids, not only n-3, are associated with dietary habits and cardiovascular risk factors in an Italian population

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Background: The association between dietary habits and risk of various non-communicable diseases has been confirmed by a large number of epidemiological studies and intervention trials, which have convincingly shown that some cardiovascular risk factors are strictly related to the consumption of specific foods or nutrients. Moreover, several data indicate that circulating fatty acids (such as n-3 PUFA) reflect both the nutritional status and physiological/pathological conditions.

Objective: Aim of this study was the evaluation of nutrient intake levels, fatty acid profiles, anthropometric and biochemical parameters associated with cardiovascular disease risk, and the assessment of the possible relationships among these data.

Procedure: Blood samples were obtained from 460 Italian subjects living in Milan, aged 40-79, randomly recruited by general practitioners. Subjects underwent a clinical evaluation and a dietetic interview. Dietary consumptions were estimated by seven-day self-reported diaries and the fatty acid profiles were analyzed by fast gas chromatography of whole blood samples.

Results: Significant correlations were observed between the total dietary PUFA intake and blood linoleic acid and between the n-3 PUFA intake and circulating DHA. Significant relationships were also identified between BMI, abdominal fat, systolic blood pressure, triglycerides, glycemia and both blood oleic acid (positive) and linoleic acid (negative). These associations were stronger in women than in men. On the opposite, HDL plasma cholesterol levels resulted to be negatively related to oleic and positively to linoleic acid concentrations. Among the other PUFA, DHA showed to be also negatively associated with BMI, blood pressure and triglyceridemia.

Conclusion: These data suggest that blood fatty acids, not only n-3 PUFA, also depending upon the amount and quality of dietary fats, are associated, in different ways, with cardiovascular risk factors and may represent additional parameters useful in the cardiovascular risk assessment.

Plenary: Lipids and Health 2 : Brain function

16.00 - 16.45

Imaging brain arachidonic acid metabolism in aging and the neuroinflammation of Alzheimer's disease and HIV-1 infection: from bench to bedside

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Regional incorporation of intravenously injected radiolabeled unesterified arachidonic acid (AA, 20:4n-6) into brain phospholipid can be imaged in unanesthetized rodents using quantitative autoradiography, or in humans using positron emission tomography (PET). Incorporation compensates for AA metabolic loss from brain, since AA cannot be synthesized *de*

novo in vertebrates and is minimally converted (<1%) in brain from its circulating linoleic acid (18:2n-6) precursor. In a rat model of neuroinflammation, caused by infusing bacterial lipopolysaccharide into the cerebral ventricles for 6 days, brain AA incorporation was widely increased in relation to activated increased expression of cytosolic phospholipase A₂ (cPLA₂), secretory sPLA₂ and cyclooxygenase (COX)-2 and increased unesterified AA, PGE₂ and TXB₂ concentrations, evidence of upregulated AA metabolism. Accordingly, we predicted that AA incorporation would be increased in Alzheimer disease (AD) patients, whose postmortem brain shows proinflammatory cytokines and increased cPLA₂, sPLA₂ and COX-2 expression. Whereas clinical PET studies using intravenous [1-¹¹C]AA found no change in brain AA incorporation (after atrophy correction) in healthy volunteers with regard to age, PET studies showed significantly increased AA incorporation in AD compared with control subjects, while brain blood flow measured with [¹⁵O]H₂O was reduced (reaffirming the independence of AA incorporation of blood flow). Thus, PET AA neuroimaging might be used to detect neuroinflammation in patients with neurodegenerative, viral or other brain diseases. One of these diseases is HIV-1 associated dementia. We find with quantitative autoradiography markedly upregulated brain AA incorporation in a non-infectious transgenic rat model expressing part of the HIV-1 virus, including the glycoprotein120 that activates microglia. We now plan to use PET to test for increased brain AA incorporation in relation to disease progression in human HIV-1 infection.

16.45 - 17.30

DHA and cognitive decline during aging

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Docosahexaenoic acid (DHA) has deservedly received wide attention as a nutrient supporting both optimal brain development during infancy and childhood and cardiovascular health in adults. Cognitive decline in the elderly, particularly Alzheimer's disease (AD), is a major socio-economic and healthcare concern. Prospective studies generally show that low intake of fish and ω 3 fatty acids significantly increase the risk of AD. However, biological studies, including blood and brain DHA analyses are much less clear. In the literature as a whole, there is no difference in plasma DHA in AD versus age-matched controls, but there is wide variability between studies, often with opposite changes in DHA depending on the plasma lipid class. There are fewer published data on DHA in AD brain but these too show variable results, especially in the cortical regions. Methodological issues may explain some of the inconsistency in plasma and brain DHA levels reported for AD. In particular, careful selection of age-matched controls is critical because we have found that healthy aging itself has a significant effect on DHA metabolism which is much more apparent when followed with a tracer (¹³C-DHA) than with plasma DHA levels alone. Deteriorating brain glucose metabolism is the earliest defect thus far reported in AD. In animal models and *in vitro*, brain glucose transport is sensitive to brain DHA and to omega-3 fatty acid intake so, assuming DHA is linked to protecting brain function in the elderly, it may be through a mechanism that helps maintain optimal brain glucose transport.

Financial support was provided by INSERM, the Canada Research Chairs, CFI, CIHR, FRSQ, and Université de Sherbrooke.

Posters - Monday May 31

Lipids and Health

P092

Lipidomic analysis of bioactive lipids in the orthotopic C6 glioma model in the presence or absence of α -linolenic acid

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Background: Gamma-linolenic acid (GLA) is an inhibitor of glioma cell proliferation both in vitro and in vivo, although its effects on eicosanoid metabolism are poorly understood.

Objective: The aim of this study was to identify the eicosanoid and hydroxy-fatty acid profile of the orthotopic C6 glioma model in control conditions and after GLA treatment.

Procedure: Lipidomic analysis was performed using LC/ESI-MS/MS lipidomic assays in order to identify the principal lipid mediators produced by the C6 glioma in vivo. mRNA and protein expression profiles of cyclooxygenases, lipoxygenases, prostaglandin (PG) E synthases and PGE receptors were determined in the C6 glioma by RT-PCR and Western blotting. Lipidomic analysis, mRNA and protein expression analysis were also performed on tumour samples after 14 days treatment with 5mM GLA in artificial CSF by Alzet osmotic pump infusion.

Results: The C6 model expressed cyclooxygenases, lipoxygenases, prostaglandin (PG) E synthases and PGE receptors in vivo. Lipidomic analysis identified the presence of PGD₂, TXB₂, 5-HETE, 8-HETE, 11-HETE, 12-HETE, 15-HETE, 9-HODE and 13-HODE as the major prostanoids and monohydroxy-fatty acids present in the C6 glioma model. The 14-day treatment of tumours with 5mM GLA significantly increased the production of both 12-HETE ($p < 0.045$) and 15-HETE ($p < 0.003$). The infusion of 5mM GLA caused a significant decrease in C6 tumour growth in comparison with CSF alone ($p < 0.001$).

Conclusion: The C6 glioma produces several bioactive lipids which can play a role in stimulating both tumour cell proliferation and endothelial cell proliferation. The marked effects of GLA on 12- and 15-HETE suggests that they may be important in controlling glioma cell proliferation and further studies are currently underway to investigate this in human gliomas.

Financial support: FAPESP; Royal Society International Joint Project Award.

P093

Lowering levels of n-6 polyunsaturated fatty acids in the early postnatal diet reduces adipocyte number in adult rats

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Objective: Compared to lean counterparts, obese adults have a higher number of adipocytes from early age onward suggesting that preadipocyte proliferation and differentiation during early development is a major determinant of fat mass in adulthood. PUFAs have been shown to directly affect adipogenesis. The current study investigated whether reducing n-6 PUFAs in the early postnatal diet has sustained effects on development of white adipose tissue (WAT), and hence, affects adult body composition and metabolism in rats.

Procedure: From postnatal day (PN) 2 to 42 male offspring of WU dams were subjected to either a control diet (CTRL) or a diet with a 50% lower linoleic acid content (Low LA). Subsequently, rats of both experimental groups were switched to a moderate Western style diet (WSD) until dissection on PN 98. Body composition was measured by dual x-ray absorptiometry at PN 42, 70 and 98. Glucose homeostasis was evaluated by an intravenous glucose (GTT) and insulin tolerance test (ITT) at PN 82 and 90, respectively. After dissection at PN 98, adipocyte number and cell size distribution were analyzed in visceral and subcutaneous WAT.

Results: Adipocyte number in both WAT depots was decreased in the Low LA compared to the CTRL group ($p < 0.001$). Additionally, lowering LA intake during neonatal development resulted in a shift in cell size distribution towards increased number of large cells. This effect was more pronounced in visceral than subcutaneous WAT. Lowering dietary n-6 PUFAs did not affect body composition in the current study. However, Low LA rats did show an improved glucose tolerance.

Conclusion: Reduction of n-6 PUFA intake during early life reduces adipocyte number and improves glucose tolerance in adulthood. Hence, improved fat quality of postnatal diet affects WAT development and might prevent disturbances in metabolic homeostasis due to an obesogenic environment in later life.

P094

Inhibition of cyclooxygenase activity, prostaglandin E2 synthesis and cell proliferation in rat and human glioma cells by a diruthenium-ibuprofen complex

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Background: Malignant gliomas have poor patient prognosis due to their highly invasive nature and their resistance to conventional radiotherapy and chemotherapy. Cyclooxygenase 2 expression is up regulated and prostaglandin concentration is increased in gliomas, these changes being correlated with increasing histological grade and poor prognosis. Previous studies from our group have shown a novel diruthenium-ibuprofen complex (Rulbp) has inhibitory effects on C6 glioma cell proliferation, although the mechanisms of action have not yet been clearly determined [1].

Objective: The aim of this study was to identify the effects of Rulbp upon proliferation and prostaglandin metabolism in glioma cells of human and rat origin.

Procedure: Cell proliferation was determined at varying concentrations and times of exposure to Rulbp. mRNA and protein expression profiles of cyclooxygenases, lipoxygenases, prostaglandin (PG) E synthases and PGE receptors were determined in the C6 and T98G glioma cell lines by RT-PCR and Western blotting. PGE₂ synthesis and cyclooxygenase activity were determined by ELISA.

Results: The C6 and T98G cells expressed cyclooxygenases, lipoxygenases, prostaglandin (PG) E synthases and PGE receptors in vitro. Rulbp had a dose-dependent effect on the proliferation of C6, T98G and U138MG which was most effective after a 72-hour period of treatment. Rulbp inhibited cyclooxygenase activity ($p < 0.01$) in both rat and human glioma cells and inhibited basal PGE₂ production ($p < 0.001$) in these cells.

Conclusion: The novel ruthenium compound Rulbp has significant effects on both glioma cell proliferation and capacity to produce PGE₂ in vitro. These findings support the hypothesis that Rulbp may act intracellularly via both inhibition of prostaglandin synthesis and inhibition of cell proliferation. Further work is ongoing to determine specific targets of action in glioma cells.

Acknowledgements: Santos RLSR. **Financial support:** FAPESP, CNPq, CAPES.

Reference: [1] Ribeiro G, Benadiba M, Colquhoun A, Silva DO. *Polyhedron* (2008) 27, 1131-1137.

P095

Safety of docosahexaenoic acid (DHA) - the MIDAS trial findings

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Background: DHA plays an important role in neural function. MIDAS, a study that evaluated the benefit of DHA on cognitive function was recently completed and demonstrated benefit on some cognitive measures in adults aged ≥ 55 years with subjective memory complaints but having normal Mini-Mental State Examination scores.

Objective: This report considers the safety data obtained from the MIDAS study.

Procedures: All subjects received DHA 900mg/day or matched placebo for six months in a randomized double blind method. Safety data was collected on routine visits.

Results: A total of 485 subjects were enrolled in the study, 242 on DHA and 243 on placebo. Of these, 90% completed the study in each group. Compliance was over 82% in each group. Equal numbers in each group withdrew prematurely. Blood pressure at the end of the study did not differ between groups (48% on antihypertensive medications), but there was a significant decrease in heart rate in the DHA group, -3.2 bpm vs -1 bpm ($p < 0.03$). There was no change in body weight. There was no difference in non fasting total cholesterol (36 % of subjects on statins). No differences were seen between groups for levels of serum creatinine or liver enzymes. Adverse experiences (AE) spontaneously reported did not demonstrate any differences, 7 subjects in each group reported serious AE. 12 subjects (5%) in the DHA group and 7 subjects (2.9%) in the placebo group withdrew from participation because of an AE. Overall, 12.4% of subjects reported gastrointestinal disorders compared to 16.9 % on placebo (n.s.); there were no differences including eructation. No bleeding issues were reported.

Conclusion: DHA 900mg /day for 6 months was well tolerated in a >55 year old population with memory complaints but who are otherwise healthy. The decreased heart rate with DHA alone provides cardiovascular benefit to this population

P096

Replacement of hydrogenated fat by palm oil has unfavorable effects on venous thrombosis propensity

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Background: It has been proposed an association between dietary lipid intake and the occurrence of thrombotic events, being controversial the influence of a specific fatty acid type in these processes. **Objective:** We tested whether diets containing partially hydrogenated fat (PHVO, rich in trans fatty acids or palm oil (PO, rich in saturated fat - C16 palmitic fatty acid) had different effects on the propensity for venous thrombosis, a marker of haemostatic cardiovascular risk.

Procedure: Female Wistar rats were fed normolipidic

diets containing PHVO or PO during lactation, and their young male pups were fed the same diets from weaning until the 180th day of life. We evaluated platelet fatty acid composition, serum lipids, platelet aggregation, clotting time and venous thrombus formation.

Results: A significant and cumulative incorporation of trans fatty acid was observed only in the platelet lipids from the PHVO group, associated with an increased sensitivity to ADP and venous thrombus formation in vivo. Platelets from rats raised on the palm oil diet also exhibited platelet aggregation induced by ADP and an increase in venous thrombus weight, with a concomitant increase in serum triglycerides.

Conclusion: The prolonged replacement of dietary hydrogenated fat by palm oil impaired platelet aggregability and venous thrombosis, suggesting an increased risk of thromboembolic diseases.

P097

Impaired leptin's role to suppress stearoyl-CoA desaturase activity in abdominal obesity

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Objective: Leptin is a major suppressor of stearoyl-CoA desaturase (SCD), which has a central role for mediating the metabolic effects of leptin and contributes to the development of obesity. Thus we investigated the relationship between SCD activity and serum leptin levels in children to evaluate leptin resistance in abdominal obesity.

Procedure: One hundred seventy-nine children (97 male, 82 female), including 58 abdominal obesity defined by having the waist to height ratio (WHtR) over than 0.5, were included. Serum leptin was determined and fatty acid composition in plasma phospholipids was analyzed by gas chromatography after overnight fast. The SCD activity was estimated by the 18:1/18:0 ratio.

Results: Leptin concentration had a positive correlation with the WHtR ($r = 0.749$, $p < 0.0001$). In addition, in non-abdominal obese children, the WHtR correlated inversely with SCD activity ($r = -0.289$, $p = 0.0006$), while in abdominal obese children a positive association between SCD activity and WHtR was demonstrated ($r = 0.328$, $p = 0.0386$).

Conclusion: These results suggest that leptin's role to suppress SCD activity might be impaired in abdominal obese children. This characteristic metabolic feature suggests leptin resistant state.

P098

An omega-3 index for obesity?

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Background: The effect of long-chain omega-3 polyunsaturated fatty acid (LCn-3 PUFA) intake on body weight is uncertain, with epidemiological evidence for direct and inverse associations in women and men respectively. The Omega-3 Index (O3I), the relative content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in erythrocytes, is a reliable indicator of an individual's LCn-3 PUFA status and is inversely related to cardiovascular risk (Harris & von Schacky, *Prev Med* 2004;39:212).

Objective: To investigate relationships between O3I, body mass index (BMI) and body composition.

Procedure: BMI and the O3I were measured at baseline in 476 intervention trial participants (291 women, 185 men). In most cases, waist circumference (WC) and DEXA assessments of body composition were also obtained.

Results: Means \pm SD of assessments for women and men respectively were as follows: BMI = 34 ± 7 and 31 ± 5 kg/m²; WC = 105 ± 16 and 110 ± 13 cm; percentage body fat (BF%) = 48 ± 5 and 35 ± 6 %; O3I = 5.3 ± 1.3 and 5.1 ± 1.2 %. Both O3I and DHA alone correlated inversely with BMI, WC and BF% in women and with BF% only in men. Curvilinear regression revealed stronger relationships ($P < 0.0001$) between O3I and all 3 measures of adiposity, with a plateau around the mean O3I and a detrimental influence at lower O3I levels. For example, there was a 3% difference in BF% between women in the lowest quartiles of O3I but no difference between the highest quartiles.

Conclusion: The O3I is inversely related to adiposity, particularly in women. Thus it may serve as a marker of metabolic as well as cardiovascular health. It remains to be seen whether increasing LCn-3 PUFA consumption will improve body composition selectively in those overweight individuals with low O3I.

P099

In vivo ¹H-[¹³C]MRS measurements of postprandial lipid uptake and mobilization in intrahepatocellular lipid depots in Wistar rats

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Background: Insulin resistance and type 2 diabetes are associated with elevated intracellular lipid content in liver (IHCL). In this study, for the first time postprandial lipid storage in rat liver was measured in vivo using ^1H - ^{13}C magnetic resonance spectroscopy (MRS) and ^{13}C labeled lipids as tracers.

Objective: Assessment of ^{13}C enrichment of intrahepatocellular lipid content in healthy Wistar rats at baseline, 5 hours, and 24 hours after administration of ^{13}C labeled lipids.

Procedure: Six Wistar rats (16 weeks) were measured at baseline, 5 hours, and 24 hours after administration of 400 mg [U- ^{13}C] Algal lipid mixture. On a 6.3T horizontal Bruker MR system, localized 1H MRS was performed on a $4 \times 2 \times 4 \text{ mm}^3$ voxel placed in the liver using the LASER sequence, which was combined with a POCE element for ^{13}C editing. LASER-POCE spectra were acquired in an interleaved fashion with a ^{13}C editing pulse turned on every other experiment. ^{13}C enrichment of the IHCL-CH₂ signal at 1.3 ppm was calculated from the difference spectra.

Results: No significant differences were observed in total IHCL content throughout the study. At baseline, $0.9 \pm 0.7\%$ ^{13}C enrichment of IHCL was determined. At 5 h post ingestion of the ^{13}C labeled lipid, a significant increase in ^{13}C enrichment of IHCL to $4.8 \pm 0.9\%$ was observed ($p < 0.005$). ^{13}C enrichment of IHCL significantly decreased to $2.7 \pm 0.8\%$ 24 h after administration ($p < 0.005$).

Conclusion: The application of LASER-POCE in combination with ^{13}C enriched lipid administration allows for successful in vivo assessment of postprandial lipid uptake and mobilization in rat liver. In future research, this method will be used with an increased temporal sampling to detect abnormalities in lipid handling in the insulin resistant state and, as such, will contribute to a better understanding of the etiology of type 2 diabetes.

P100

Arachidonic and docosahexanoic acids deficits are associated with negative symptoms of schizophrenia

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Background: Membrane polyunsaturated fatty acids (PUFAs) are found to be reduced in patients with schizophrenia (SZ). However, there is uncertainty on the association of these deficits with psychopathology. Objectives: to examine erythrocyte membrane fatty acids in free-drug patients with SZ and the association between PUFAs and SZ symptoms.

Procedure: The study included 36 free-drug male patients with SZ and 36 healthy male subjects, matched

according to age and smoking habits. Patients were assessed by the following psychometrics instruments: Andreason's scale for assessment of positive symptoms (SAPS), Andreason's scale for assessment of negative symptoms (SANS), and the positive and negative syndrome scale (PANSS). Erythrocyte fatty acid profile was analyzed by capillary gas chromatography.

Results: Levels of arachidonic acid (AA) (9.07 ± 4.79 vs. 11.07 ± 2.90) and docosahexanoic acid (DHA) (1.88 ± 1.05 vs. 2.81 ± 0.83) were significantly lower ($p < 0.01$) in patients than controls. There was a negative correlation between AA and DHA levels and the SANS score. DHA levels were inversely related to "alogia", "anhedonia", "avolition", "blunted affect" and "disturbed attention" sub items of the SANS. Moreover, levels of AA, DHA, and total PUFAs tend to decline through the SANS tertiles. The decrease was significant for DHA (2.48 ± 1.20 ; 1.70 ± 0.92 and 1.23 ± 0.80 , for first, second and third tertile of the SANS, respectively). No correlation was observed between AA or DHA levels and the SAPS or its sub items.

Conclusion: Our data corroborate the hypothesis of altered PUFAs metabolism in SZ and clearly indicates that PUFAs deficits are associated with negative symptoms of SZ. While the underlining mechanisms remain unclear, the PUFAs deficit in the orbitofrontal regions of brain may reduce function of the cortical and prefrontal dopamine systems that contribute to negative symptoms of SZ.

P101

Plasma and membrane arachidonic and docosahexanoic acids are decreased in very low birth weight Tunisian infants

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Background: Long-chain polyunsaturated fatty acids (LCPUFA), most notably arachidonic (AA) and docosahexanoic (DHA) acids are critical nutrients for neurological development in infants.

Objectives: to examine plasma and erythrocyte membrane LCPUFA profile in neonates with very low birth weight (VLBW) and to test the association between these fatty acids and maternal-fetal factors.

Procedure: The study included 221 VLBW infants (26-36 weeks of gestation, birth weight ≤ 1500 g) and 172 full-term infants (37-42 weeks of gestation, birth weight ≥ 3000 g), born in the Center of Maternity and Neonatology (Tunis, Tunisia). Plasma and erythrocyte membrane fatty acid analysis were carried out by capillary gas chromatography.

Results: Plasma AA (8.13 ± 3.45 vs. 9.40 ± 2.92) and DHA (2.77 ± 1.13 vs. 3.42 ± 1.30), and erythrocyte membrane DHA (1.62 ± 0.62 vs. 1.85 ± 0.69) levels were significantly

lower ($p < 0.001$) in VLBW infants compared to full-term infants. Membrane DHA and total LCPUFA were inversely related to gestational age ($r = -0.24$; $p = 0.01$ and $r = -0.25$; $p = 0.008$, respectively) and birth weight ($r = -0.20$; $p = 0.04$ and $r = -0.22$; $p = 0.02$, respectively). No significant differences in plasma or membrane AA, DHA or total LCPUFA levels were observed according to twin pregnancy, gestational mellitus, gestational hypertension or fetal growth retardation.

Conclusion: Lower LCPUFA levels in VLBW infants may be explained by a reduction of their transplacental passage, which occurs mainly during the 3rd trimester of gestation. Thus, LCPUFA, especially DHA-rich milk is required in VLBW infants and may favor neurological and psychological development.

P102

Effect of oily fish in Chinese diet on serum fatty acids and cardiovascular disease risk markers in dyslipidemic male adults

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Background: Although the beneficial effects of n-3 fatty acids on several physiological functions have been widely reported, the information related to the effects of oily fish consumed within oriental diet on cardiovascular disease risk is scarce.

Objective: The aim of this 8-week randomised, paralleled, food-based intervention study was to investigate effect of oily fish in Chinese diet on serum fatty acids status and cardiovascular disease (CVD) risk markers in dyslipidemia male adults.

Procedure: Intervention lunch meals were prepared with 500 grams of pork/chicken/beef or habitual fish (carp & hairtail) or oily fish (salmon) per week respectively.

Subjects: were randomly assigned to pork/chicken/beef meal group (GP I, n=30) or habitual fish meal group (GP II, n=30) or oily fish meal group (GP III, n=32). Related circulating markers were analysed at baseline and the endpoint of intervention study. Results Inclusion of oily fish caused significant increase of dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) while decrease of n-6:n-3 fatty acid ratio ($P < .05$), and these changes were reflected by significantly elevated serum EPA and DHA concentration while lowered serum n-6:n-3 ratio ($P < .05$). Furthermore, oily fish intake resulted in significant reduction of serum triglyceride ($P = .036$), interleukin-6 ($p < .01$) and increase of high density lipoprotein cholesterol concentration ($P < .01$).

Conclusion: These results demonstrated that the inclusion of oily fish as part of Chinese diet can

significantly increase serum LCn-3PUFA level and lead to favourable changes of cardiovascular disease risk markers in dyslipidemic male adults.

P103

Metabolic syndrome is associated with decreased plasma $\omega 6$ and $\omega 3$ PUFA levels, and decreased $\omega 6/\omega 3$ ratio

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Background: It was suggested that saturated fatty acids (SFAs) favor the metabolic syndrome (MS) and that monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs); especially omega 3 PUFAs have potential protective effect against MS and its components.

Objective: to determine plasma and erythrocyte membrane fatty acid profiles in Tunisian's with MS.

Procedure: The study included 1975 individuals; 1225 females and 750 males, aged 35 to 70 years, randomly recruited from the Greater Tunis region between March 2004 and June 2005. MS was defined according to the NCEP ATP III criteria. Plasma and erythrocyte membrane fatty acid profiles were analyzed by capillary gas chromatography.

Results: Patients with MS showed significantly ($p < 0.001$) higher SFAs (29.1 ± 3.1 vs. 28.3 ± 2.9) and MUFAs (22.9 ± 4.3 vs. 21.3 ± 4.2) levels and a lower PUFAs (47.8 ± 5.7 vs. 50.5 ± 5.5). MS was associated with decreased levels of linoleic (LA), arachidonic (AA) and docosahexaenoic (DHA) acids and increased levels of alpha linolenic (ALA) and eicosapentaenoic (EPA) acids. The LA/ALA (46.9 ± 28.8 vs. 59.0 ± 37.7 ; $p < 0.001$), AA/EPA (8.98 ± 6.42 vs. 9.42 ± 6.61 ; $p < 0.01$) and omega 6/omega 3 PUFAs (9.47 ± 2.64 vs. 9.83 ± 2.64 ; $p = 0.03$) ratios were significantly lower in MS patients. No significant changes were observed in erythrocyte membrane fatty acid profile according to MS. **Conclusion:** In MS, changes in fatty acid profile were more obvious in plasma than in membranes. Our findings corroborate the adverse effect of SFAs and the favorable effect of both omega 6 and omega 3 PUFAs, but aren't in accordance with a protective role of MUFAs in MS.

P104

Enzymes involved in the conversion of arachidonic acid to eicosanoids in the skin of atopic dogs

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Background: Canine atopic dermatitis (AD) is a chronic inflammatory and pruritic skin disease which shares several characteristics with its human counterpart. Long-chain polyunsaturated fatty acids (PUFA) have the potential to modulate inflammatory responses by regulating the metabolism of lipids in the cyclooxygenase (COX) and lipoxygenase (LOX) pathways starting from membrane-derived arachidonic acid (AA). There is no insight in expression profiles of enzymes involved in these pathways resulting in inflammatory mediators.

Objective: Elucidation of the role of enzymes involved in prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) production and the expression of their receptors, would be helpful in developing new strategies to suppress inflammatory reactions in AD.

Procedure: In non-lesional and lesional skin from atopic dogs and control skin from healthy dogs mRNA expression of these enzymes and receptors were measured by quantitative real-time PCR.

Results: Significant differences in mRNA expression levels of several key enzymes (e.g. 5-lipoxygenase (5-LO), 5-LO activating protein (FLAP), leukotriene A4 hydrolase (LTA4H), prostaglandin E synthase 1 (mPGES-1)) and their receptors (prostaglandin E receptors 2 and 3) were found when non-lesional and lesional atopic skin were compared to healthy control skin. Also significant correlations were found between several of the enzymes, receptors and cytokines within the COX and the LOX pathway.

Conclusion: Based on significant higher mRNA expressions of important enzymes in the COX and LOX pathways, higher amounts of the inflammatory metabolites of the 3-series prostaglandins and 5-series leukotrienes are envisaged. Thus, the enzymes 5-LO, FLAP, LTA4H and mPGES-1 may be considered as interesting targets for therapy as their suppression may lead to lower amounts of inflammatory mediators and thereby to amelioration of clinical signs in canine atopic dermatitis.

P105

Dihomo-gamma-linolenic acid (DGLA) prevents scratching behavior in atopic NC/Nga mice through prostaglandin d1 production

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Background & Objective: Dihomo-gamma-linolenic acid (DGLA, 8,11,14-eicosatrienoic acid) is a biologically active n-6 fatty acid and an important source of 1-series prostaglandins (PGs). A new fermentation technique using a fungus *Mortierella alpina* was established to obtain triglycerides comprising 40% DGLA. Here, we examined the efficacy of oral administration of DGLA to prevent

atopic dermatitis (AD) in mice, and clarified the involvement of DGLA-derived 1-series PGs in the prevention of scratching behavior and AD development.

Procedure: NC/NgaTnd mice, which spontaneously developed AD-like symptoms, were fed a modified AIN-76A diet without (control group) or supplemented with DGLA (0.6% in diet, DGLA group) for 8 weeks. Clinical skin severity scores were recorded and scratching behavior was analyzed by using a scratching analyzer. Fatty acids and PGs in skin samples were quantified by GLC and LC-MS/MS methods, respectively.

Results: Clinical skin severity scores were markedly lower in the DGLA group. Scratching behavior was also reduced in the DGLA group, in association with histological improvement. DGLA content in phospholipids of skins, spleens, and plasma was increased in the DGLA group. At the end of the experiment, we quantified various PGs in skins and found that PGD1 levels were markedly increased in the DGLA group. Reduction in severity of dermatitis and scratching behavior was positively correlated with the increase of PGD1 levels. The other PGs were not found to be closely related with the AD parameters.

Conclusion: These findings indicate that oral supplementation of DGLA prevented AD development in NC/NgaTnd mice. In particular, PGD1 production was upregulated in DGLA-supplied mice, suggesting that PGD1 produced in the skin might suppress scratching behavior and result in reduced dermatitis severity. DGLA and/or PGD1 may be of key importance in the prevention of AD symptoms.

P106

Mass spectrometry-based analysis of eicosanoids and related compounds in an *in vivo ex vivo* human LPS challenge study

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Background: There is a growing interest in the diagnosis and classification of inflammatory processes. Acute inflammation is normally easy to diagnose, but a more subtle, often chronic, inflammatory response is more difficult to categorize and understand. However, increasing evidence suggests that subclinical inflammation plays an important role in diseases like diabetes type 2 and atherosclerosis and for that reason it is often called 'the secret killer'. Inflammation is a natural reaction of the body to pathogens. Its goal is to clear the body of pathogens and dead cell material, and to repair damaged tissue. White blood cells play an important role in these processes. It is still not completely understood how a chronic inflammatory process starts. However, it is clear that a disturbed

balance in the regulation of inflammatory cascades is involved.

Objective: Detection of subtle modulations in the inflammatory process, to compare in vivo and ex vivo experiments, using LC-MS techniques.

Procedure: Using lipopolysaccharide (LPS), a compound from Gram negative bacteria it is possible to induce an inflammatory response. LPS was administered under standardized conditions to healthy volunteers (in vivo) or to their blood cells using TruCulture tubes (ex vivo). Forty-one eicosanoids were analyzed simultaneously using a relative straightforward reversed phase UPLC-MS/MS method after sample clean-up and concentration using C18 solid phase extraction.

Results and Conclusion: An important issue is that eicosanoids are present at very low concentration in plasma and for that reason, are difficult to detect. Moreover the half life of many eicosanoids is short and they are susceptible to oxidation. This necessitates the use of special inhibitor cocktails. However a lot of similarities were detected between the in vivo and ex vivo experiments but also a lot of discrepancies. This may be caused by the different LPS doses used and stress factors.

P107

Integrated systems approach to study ω -3 long-chain polyunsaturated fatty acids in health and disease of the neural and vascular retina

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Background: Omega-3 long-chain polyunsaturated fatty acids (LCPUFAs) have the capacity to impact processes implicated in pathogenesis of diseases affecting the neural and vascular retina.

Objective: To apply an integrated analytic framework to examine the role of omega-3 LCPUFAs in health and disease of the retina.

Procedure: We first determined the impact of omega-3 LCPUFAs on gene expression by examining microarray-based profiles of primary retinal tissue from: 1) mice fed an omega-3 LCPUFA rich diet (2% of total fatty acids) to omega-3 LCPUFA-free controls; and 2) ex vivo human explants with defined omega-3 LCPUFA exposure (27 μ M

for 14 days) to LCPUFA-free controls. We used results from these 2 model systems to filter variants of genes dysregulated by 2-or-more fold from a 100K SNP array used in a 12-year prospective follow-up of 503 people from the Age-Related Eye Disease Study (AREDS). We tested these variants in age- and sex-adjusted models for association with advanced age-related macular degeneration (AMD). We used results to examine functional clustering of LCPUFA-regulated genes containing AMD-associated variants within ~500 gene sets, networks and pathways.

Results: 846 genes showed LCPUFA-altered expression in our model systems. 6044 SNPs on our 100K array existed within these genes. Of these variants, 68 showed associations with advanced AMD at p-values < 0.005; 3 SNPs from known risk loci on chromosome 10q26 attained p-values < 0.003. SNPs of the LCPUFA- and AMD-associated genes were most likely to exist in networks functioning in cellular growth, proliferation, cell death, hematological system development, and humoral immune response. The most enriched pathway was that driving leukocyte extravasation (p < 0.002).

Conclusions: Omega-3 LCPUFA exposure in model retinal systems alters expression of genes associated with advanced AMD. LCPUFAs may impact AMD risk by altering processes driving recruitment of non-specific leukocytes in innate immune response.

P108

The omega-3 index in heart failure: associations with clinical data and comorbidities

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Background and Objective: The omega-3 index assesses the quantity of the omega-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) in the erythrocyte membrane. Higher levels of this index have been shown to correlate with lower incidence of cardiovascular diseases and sudden cardiac death (SCD). Optimal “protective” levels are considered to range between 8 and 11%. Supplementation with EPA and DHA reduced total mortality and SCD in clinical intervention studies. Larger scale data on the distribution of the omega-3 index and its influencing factors are lacking in patients with heart failure (HF). We therefore measured the omega-3 index in a cohort of patients with systolic HF (left ventricular ejection fraction (LVEF) \leq 40%), and explored its correlates.

Procedure: EDTA blood samples collected as part of the

Interdisciplinary Network for Heart Failure (INH) Study were obtained at baseline and stored at -80°C . Fatty acid composition was determined using standardised and validated methodology, and the HS-omega-3 index calculated from these measurements.

Results: In 919 patients (68 ± 12 years, 72% male, 43% in NYHA class III/IV, LVEF $30\pm 8\%$) biomaterials were available. The median omega-3 index was 3.6% (IQR 3.0-4.4%). It showed weak associations with the following variables (all $p < 0.03$): age ($r = 0.09$), level of education ($r = 0.10$), uncured malignancy ($r = -0.09$), LDL:HDL cholesterol ratio ($r = -0.11$), triglycerides ($r = -0.12$), hemoglobin ($r = 0.07$), thrombocytes ($r = -0.07$) and atrial fibrillation ($r = 0.08$). In multivariable analysis, all variables except the last two retained their explanatory power. No association was found with LVEF, NYHA class, main cause of HF, and markers of renal function or inflammation.

Conclusion: In this large well-characterised cohort of patients with systolic HF, the omega-3 index was considerably lower than desirable. Furthermore, it was associated with a panel of established indicators of mortality risk in HF. Long-term follow up of the patients need to clarify whether the omega-3 index carries incremental prognostic information.

P109

Temporal profiles show differential contribution of lipid mediators in UV-induced inflammation in sun-reactive skin types I and IV

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Background: Exposure to sunlight is considered a major environmental factor contributing to skin photo-ageing and cancer development. Skin pigmentation has photo-protective properties as shown by studies reporting less incidence of skin cancer in individuals who tan readily without suffering sunburn.

Objective: To undertake a temporal examination of cutaneous lipid mediator production in sun-reactive skin type groups I (burn) and IV (tan), and link the outcome to the development UV-induced inflammation (sunburn).

Procedure: Healthy adult white Caucasian subjects (17 subjects skin phototype I and 17 phototype IV) were recruited. Suction blister fluid and skin punch biopsies were sampled from unirradiated and irradiated skin following a fixed dose of UVB ($120\text{mJ}/\text{cm}^2$), and up to 72h post-UV. Lipid mediators were assessed in blister fluid using LC/ESI-MS/MS. Cyclooxygenase (COX) and lipoxygenase (LOX) expression were assessed in biopsies by immunohistochemical staining.

Results: Individuals with skin phototype I developed erythema within 4h post-UV but this response was slower

for the phototype IV subjects. However, for both groups erythema reached the same level at 72h post-UV. Prostaglandins (PG) E_2 and E_1 were found to be up-regulated at 24h, in both groups. Although, in skin phototype I, PGE_1 and PGE_2 were still elevated at 72h post-UV, in skin phototype IV PG production had returned to baseline levels. UV upregulated COX-2 in the epidermis of phototype I, but had no such effect in phototype IV subjects. 12- and 15-HETE were upregulated post UVR: 12-HETE levels were similar in both groups but 15-HETE was produced at higher levels in skin phototype I at 72h.

Conclusion: Our results suggest that skin phototypes I and IV are different in their inflammatory response at the early time-points post-UVR. Lipid mediators contribute differently at the later stages of sunburn in phototypes I and IV, with possible implications for inflammation resolution and healing.

P110

Efficacy of essential fatty acid EPA in treatment of major depressive disorder: a large placebo control study

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Epidemiological and neurobiological data suggest that a deficit in essential fatty acids may play a role in major depressive disorder. Essential fatty acids may then represent an alternative in treatment of depression. The **goal** of our work was to study the efficacy of EPA (eicosapentaenoic acid) in the **treatment of depression**, as a unique treatment or combine with medication, **compare to placebo** (alone or with medication). Treatment duration: 8 weeks. After randomization, patients receive 1050 mg/day of EPA, or placebo made of sunflower oil and taste of fish.

As a primary efficacy outcome, IDS-SR₃₀, a self report measure sensitive for assessing change in large sample, was chosen. A total of 432 patients were randomized, 68,5% of them were women, mean age 46 years old, 40,3% were under antidepressant treatment, 72,7% had recurrent depressive episode and 52,5% of patients had a co-morbid anxious disorder. Diagnosis was based on MINI-International Neuropsychiatric Interview. **Principals analysis** did not demonstrate efficacy of EPA compare to placebo. Nevertheless, **analysis of sub-group**, planned in the study, did show a statistically significant effect of EPA compare to placebo, in depressive patients, but without co-morbid anxious disorder. **Size effect** was of 0,27, comparable to the effect seen in classic antidepressant.

In **conclusion**, 1 gr. of EPA might represent a valid treatment choice, alone or in combination with medication, for patients with depressive disorder, without co-morbid anxious disorder.

P111**Anticonvulsant effects of docosahexaenoic acid on pentylentetrazol induced seizures**

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Background: Epilepsy is a neurological disorder defined as spontaneous and recurrent seizures. It affects approximately 1% of the population. Anticonvulsant drugs are the first line therapy for epilepsy. However, these drugs come with side effects and are ineffective in 20-40% of patients. Therefore, there is a need for improved treatments. Growing evidence suggests n3-PUFAs have antiepileptic properties. Our laboratory has shown an increase in seizure threshold in rats following chronic fish oil supplementation and a reduction in seizure threshold following n-3 PUFA deprivation. A candidate fatty acid for these effects is DHA.

Objective: We explored the possibility that acute subcutaneous (s.c.) injection of DHA would increase seizure threshold in the maximal pentylentetrazol (PTZ) model, and correlated it to fatty acid levels in plasma.

Procedure: Two month old male Wistar rats were injected s.c. with varying doses (200, 300, 400, or 600 mg/kg) of DHA or control (400 mg/kg oleic acid). One hour post s.c. DHA injection, animals received an intraperitoneal (i.p.) injection of 105 mg/kg of PTZ. Latency to tonic-clonic seizure was measured. In another group of non-seizure tested animals, DHA was injected s.c. (400 or 600 mg/kg) and blood was collected 1hr post injection.

Results: DHA increased latency to tonic-clonic seizures, as compared to control (52.9 ± 3.9 sec). Maximal response was observed at 400 mg/kg (89.3 ± 12.8 sec). At 600 mg/kg, latency returned to control levels (50.7 ± 6.3 sec). Unesterified DHA concentrations were increased by up to 74%, as compared to control (400 mg/kg oleic acid) 1hr post s.c. DHA injection (400 or 600 mg/kg). Esterified DHA pools remained unchanged following DHA injection.

Conclusion: These results suggest that DHA may have antiepileptic properties, providing a potentially cheap therapy for epilepsy. More studies are needed to evaluate the involvement of the unesterified DHA pool in seizure protection.

P112**Age-related decrease of neural stem/progenitor cells in hippocampus is improved by ingestion of arachidonic and/or docosahexaenoic acids in rats**

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Background: Neurogenesis in hippocampus is related with learning and memory. Newborn neurons (NN) are differentiated from neural stem cells (NSC) or progenitor cells (NPC). Age-related decrease in NN and NSC/NPC was reported, but the detail was not revealed. It is unknown whether ingestion of long chain polyunsaturated fatty acids (LCPUFA) affects on the adult neurogenesis, whereas arachidonic acid drives NSC/NPC in postnatal stage.

Objective: To examine the effects of successive ingestion of arachidonic acid (ARA) and/or docosahexaenoic acid (DHA) on age-related decrease in the number of NSC/NPC.

Procedure: Male F344 rats were successively fed modified AIN-76A diets containing ARA and/or DHA (0.2% in diets, respectively) from 2 to 6, 12 and 18 months old. Rats were injected with 5-bromo-2-deoxyuridine (BrdU) at each time point and sacrificed after 1 day. BrdU-, SOX2-, and PSA-NCAM-positive cells in dentate gyrus of hippocampus were counted by the immunohistochemical assay to detect the NSC/NPC.

Results: The number of BrdU+ cells decreased to 63% (at 6 months), 30% (at 12 months) and 11% (at 18 months) of the cell number at 2 months. The numbers of BrdU+ cells in ARA-ingested rats at 6 and 18 months was significantly larger (118% and 165%, respectively) than those in the respective control rats. DHA ingestion tended to improve the number, but did not have a significant effect. The effect of ingestion of both ARA and DHA were similar to that of ARA. The ratio of SOX2+ cells (NSC/early-NPC) or PSA-NCAM+ cells (late-NPC) in BrdU+ cells was not changed significantly by LCPUFA ingestion at 6months.

Conclusion: Successive LCPUFA ingestion improved the age-related decrease in the number of NSC/NPC in rats. ARA is the most effective under these conditions. The effects of ARA ingestion may lead to improve age-related decrease in neurogenesis through maintaining NSC/NPC.

P113**Overexpression of sphingosine kinase in skeletal muscle protects against diet-induced insulin resistance**

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Background: Accumulation of lipids, such as ceramide, in skeletal muscle plays a crucial role in the aetiology of insulin resistance. Ceramide impairs insulin signalling either directly or indirectly via the activation of inflammatory molecules known to influence insulin resistance, such as c-Jun N-terminal kinase (JNK). Therefore, it has been hypothesised that inhibiting ceramide accumulation could combat many of the

underlying causes of insulin resistance. Enzymes involved in ceramide metabolism have, therefore, emerged as potential targets to treat insulin resistance. Sphingosine kinase (SphK) is one such enzyme that prevents ceramide accumulation by promoting its metabolism into sphingosine 1 phosphate.

Objective: We aimed to investigate whether mice that overexpressed SphK in skeletal muscle were protected from diet-induced insulin resistance and to examine possible mechanisms.

Procedure: Wild type (WT) and SphK transgenic (Tg) mice were maintained on a chow or high fat-diet (HFD) for 6 weeks. Glucose and insulin tolerance tests were performed and tissues were collected for biochemical and molecular analysis.

Results: Transgenic overexpression of SphK increased SphK enzyme activity by ~80 fold in muscle while no differences were found in liver or adipose tissue. Glucose and insulin tolerance was similar in WT and SphK Tg mice maintained on chow. However, HFD SphK Tg mice had improved insulin and glucose tolerance compared with fat-fed WT mice. Although the HFD markedly increased ($P < 0.05$) body weight and fat mass, no differences were observed between genotypes. The HFD-induced increase in JNK phosphorylation in muscle was reduced ($P < 0.05$) in SphK Tg mice compared with WT littermates.

Conclusion: These data indicate that overexpression of SphK can protect against diet-induced insulin resistance by blocking JNK activation in muscle.

P114

PPAR α -dependent and -independent mechanisms to maintain fatty acid composition in the mouse liver

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Background: Peroxisome proliferator-activated receptor (PPAR α) plays an important role in fatty acid (FA) metabolism in the liver. However, PPAR α -null mice show little abnormalities in the lipid metabolism on a standard diet even though expression levels of some lipid metabolic enzymes are significantly affected.

Objective: The aim of this study was to investigate the mechanism for maintaining FA composition in the liver of wild type and PPAR α -null mice on a standard and a fat free diet.

Procedures: FA compositions in the tissues of mice were determined by a time course study by using GC-MS. The changes in expression levels of the metabolic enzymes were measured by Western blots for proteins and by quantitative RT-PCR for mRNAs.

Results: The expression levels of several enzymes for FA synthesis and remodeling were markedly decreased in the liver of PPAR α -null mice, but the hepatic FA composition was almost normal except an increase in the level of

linoleic acid. The effect of the reduced expression of FA synthesis (FAS) and a desaturase (SCD1) on the FA composition, such as a significant decrease in the levels of monounsaturated (MU) FAs, became evident when FA compositions were compared using wild-type and PPAR α -null mouse primary hepatocytes in culture, suggesting the importance of extra-hepatic functions. Major source of MUFA in the liver was de novo synthesis in wild-type mice, whereas it was from a standard diet in PPAR α -null mice. Upon feeding a fat free diet, wild-type mice kept normal whereas the repressed de novo synthesis of MUFA in PPAR α -null mice was quickly recovered by transcriptional activation of the genes, leading to fatty liver. Thus PPAR α plays an essential role under these extreme conditions.

Conclusion: The liver has overlapping multiple mechanisms with or without PPAR α to maintain homeostasis of FA composition which is essential for cell function.

P115

PPAR α is essential in the dynamism of the hepatic lipid droplets

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Background: The liver is not a storage site of excess energy as triacylglycerides but a major site of carbohydrate storage, playing a vital role in glucose homeostasis. Little is known about the properties of the lipid droplets (LDs) in the liver responding to dietary excess, irregular fats, and potentially toxic compounds contained in a natural food diet.

Objective: The aim of this study was to clarify a distinct physiologic role of the hepatic LDs from those in lipid-storing tissues and the involvement of peroxisome proliferator-activated receptor α (PPAR α) in their dynamism.

Procedure, Results: We characterized the hepatic LDs in wild-type and PPAR α -null mice fed various natural plant diets with irregular fatty acid compositions by identifying the liver-enriched LD-associated proteins and the changes in lipid compositions. Natural diets induced several enzymes possibly involved in detoxification of plant-derived potentially toxic compounds and PPAR α plays an important role in the induction. The fatty acid compositions of neutral lipids in hepatic LDs were strongly influenced by those in the diet. The fatty acid compositions of the LD lipids quickly returned to normal after changing the diet to the usual one in wild-type mice. However, quick reversibility was not observed in PPAR α -null mice. These results suggest that PPAR α plays an important role in the induction of lipid-metabolizing/remodeling enzymes according to the contents of the diet.

Conclusion: Based on the currently available data, we

propose the hypothesis that hepatic LDs play vital protective roles against diet-derived excess fatty acids with irregular compositions and potentially toxic hydrophobic compounds by temporarily storing them as neutral lipids or compounds until completion of the remodeling of fatty acids and detoxification of the compounds in a PPARC-dependent manner. Thus PPAR α is critically involved in the induction of several remodeling and detoxification enzymes, thus enhancing the dynamism of the hepatic LDs.

P116

T10,c12-CLA-caused lipodystrophy is associated with profound changes of fatty acid profiles of livers and white adipose in mice

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Dietary supplementation with conjugated linoleic acid (CLA) has been shown to reduce body fat mass. To investigate the effects of individual CLA isomers on the fatty acid profiles of lipogenic (liver and white adipose) and lipid sensitive (erythrocytes) tissues, BALB/c were fed with one of two diets supplemented with either a c9,t11-CLA-enriched and t10,c12-CLA-free or a CLA-mixture containing both isomers in equal amounts [1% (w/w) of the diet] for 5 weeks. A control group was fed with a diet enriched in sunflower oil to energy balance the CLA. Compared to the t10,c12-CLA-free and the control diets, we observed significant adipose reduction accompanied by fatty livers in the CLA-mix fed group. These alterations in body fat distribution entailed a conspicuous shift of the fatty acid profiles of adipose and livers. Liver enlargement was mainly caused by accumulation of C18 monoenes that accounted for 67±1% of total fatty acid methyl esters. The significant reduction of the 18:0/18:1 desaturation index in the liver upon CLA-mix diet indicated high stearoyl-CoA desaturase activity. In contrast, reduction in white adipose was largely driven by percental reduction of monounsaturated fatty acids ($p \leq 0.001$). 16:0/16:1 and 18:0/18:1 desaturation indices for white adipose significantly increased, suggesting an inhibition of stearoyl-CoA desaturase upon CLA-mix diet. Fatty acid profile of the erythrocytes widely reflected that of livers, depending on the supplemented diet. These profound changes in fatty acid composition of lipogenic organs due to t10,c12-CLA intake may be the consequence of functional alterations of lipid metabolism.

P117

Circulating lysophosphatidylcholine species are reduced in human obesity and type 2 diabetes

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Background: The development of obesity and type 2 diabetes (T2DM) is associated with dyslipidemia characterized by increased levels of circulating free fatty acids and triglycerides. Despite this association, little is known about the specific molecular plasma lipid species in obesity and T2DM.

Objective: To determine circulating lipid species in human obesity and T2DM.

Procedure: Lipid profiles were characterized using LC-MS/MS lipidomics. Plasma samples from lean (BMI<25, n=11), obese (BMI >30, n=10) and obese T2DM individuals (n=9) were analyzed. Lipid concentrations were compared between groups and associations with indices of disease including BMI, glucose, insulin and cholesterol levels were assessed.

Results: Consistent with previous studies, several long chain saturated fatty acid diacylglycerol (DAG) species were significantly increased in obese and/or obese T2DM groups when compared with lean individuals, however, DAG 18:1 20:4 was the only lipid species that was higher when comparing obese T2DM people with obese individuals who had not progressed to T2DM. Ceramide 18:1 was also increased in obese T2DM individuals compared with lean controls. Importantly, concentrations of 11 of the 16 lysophosphatidylcholine (LPC) species were reduced in obese and/or obese T2DM groups when compared with lean individuals. Several of these LPC species were negatively correlated with BMI and glucose concentration while increases in many DAG species were strongly correlated with increases in BMI and indices of glucose metabolism. Increases in total, LDL and/or HDL cholesterol were also positively associated with increases in several triacylglycerol, DAG, ceramide and sphingosine concentrations.

Conclusions: Our results demonstrate that obesity is not only associated with increased circulating levels of deleterious lipid species, but that circulating LPC species are down-regulated in this patient population. Targeting LPC therapeutically may, therefore, be a novel approach for preventing the progression of insulin resistance and, ultimately T2DM in obese individuals.

P118

Erythrocyte polyunsaturated fatty acid status and memory, mood and cognition in older adults with mild cognitive impairment and healthy controls

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Background: Polyunsaturated fatty acid (PUFA) levels are reportedly altered in adults with cognitive decline and in those with depression compared to healthy controls. Depression is associated with the onset of cognitive decline and progression from mild cognitive impairment (MCI) to dementia.

Objective: To investigate associations between omega-3 (n-3) and omega-6 (n-6) PUFA and cognition, memory and mood in elderly adults with MCI and controls.

Procedure: Volunteers ≥ 65 years with MCI (N=44) and healthy controls (N=29) were tested for memory, cognition, and depressive symptoms. Erythrocyte PUFAs (% of total fatty acids) were measured in fasted blood samples.

Results: Compared with controls, eicosapentaenoic acid (EPA) was lower in those with MCI (0.98 % MCI; 1.2 % controls, $p < .05$) and docosapentaenoic acid (DPA) n-6 was higher (0.38 MCI %; 0.34% controls, $p < .01$). Those with MCI had higher total n-6 PUFA levels ($p < .05$) and a higher arachidonic acid (AA):EPA ratio than healthy controls ($p < .05$). Controlling for age, gender and education, higher n-6 docosapentaenoic acid (DPA) predicted poorer performance on Verbal Paired Associates ($R = -.27$, $p < .05$) and Excluded Letter Fluency ($R = -.28$, $p < .01$). Higher long chain n-6 PUFA predicted poorer performance on the Rey Auditory Verbal Learning Test (RAVLT) ($R = -.24$, $p < .05$) and higher arachidonic acid predicted poorer performance on Digits Backwards ($R = -.30$, $p < .05$). Lower EPA+DHA predicted higher self-reported frequency of forgetting ($R = -.33$, $p < .05$), and lower n-3 PUFA DPA predicted higher self-reported bodily pain ($R = .27$, $p < .05$) and poorer self-reported general health ($R = .269$, $p = .034$). Adults with MCI had higher scores on the Geriatric Depression Scale (MCI=3.13, controls=1.40, $p < .01$). After controlling for depressive symptoms in regressions, only n-6 PUFA associations remained significant.

Conclusion: Lower n-3 PUFA, higher n-6 PUFA and depressive symptoms may contribute to cognitive deficits and memory problems in older adults. Associations between n-3 PUFA and self-reported memory and health may be accounted for by depressive symptoms.

P119

Erythrocyte phospholipid molecular species and fatty acid status of Down Syndrome children compared to their non-affected siblings

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Background: A Down Syndrome (DS) child has a 20 fold higher risk of developing leukaemia, diabetes, heart disease and the majority develop 3 fattywAlzheimer's disease (AD) much earlier than usual. Although long chain 3) are cardio-protective and thought to be protective ofwacids (LC 3 status in DS.wneurodegeneration, little is known about the

Aim: To investigate if the extra copy of chromosome 21 alters the fatty acid (FA) composition of plasma and erythrocyte membrane phospholipids of DS children as compared to non-affected siblings.

Rationale: The erythrocyte membrane is a plasma-membrane with a similar composition to the vascular endothelium which has the highest plasma-membrane/cytosol ratio. An abnormality in the erythrocyte membrane is likely to reflect vascular abnormality. Because 3 in the plasma-membrane its study could also bewof the high concentration of LC relevant to the brain.

Method: Fifteen families of DS and non-affected siblings volunteered for this project. A 7-day food record was completed for each. Venous bloods from each child were analysed for the FA composition of plasma and erythrocyte phospholipids by thin layer and gas-liquid chromatography. Lipid molecular species (LMSp) were determined by MS-MS analysis.

Results: Dietary analysis showed no differences in nutrient intake. FA analysis showed an increased concentration of monounsaturated FA (MUFA) and decreased concentration of plasmalogens in major phospholipid fractions. The DS children had increased levels in the proportion of the following erythrocyte phospholipid molecular species: 16:0-16:0; 16:0-18:1 and 16:0-18:2 with reduced levels of 16:0-20:4 and 16:0-22:6 species.

Conclusions: Children in both groups met their nutritional requirements for FA, however children with DS present significantly higher levels of MUFA in both plasma and erythrocytes, as well as higher levels of saturated and monounsaturated molecular species. Further investigations are needed to test these findings with disease risk, and the onset of AD.

P120**A preliminary observation on the role of fatty acids in childhood disruptive behaviour disorders in Singapore**

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Background: The disruptive behaviour disorders - attention deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), and conduct disorder (CD) - have been linked to low levels of fatty acids.

Objectives: This ongoing study explores the link between fatty acids intake and clinical profiles of a sample of Asian children in Singapore diagnosed with ADHD, ODD and/or CD.

Procedure: Fourteen children aged between 9 to 16 were recruited from a child psychiatric outpatient clinic in Singapore. Participants received a diagnosis of ADHD (n=4), ODD / CD (n=3) or comorbidity (n=7), and were of Asian ethnicity (11 Chinese, 1 Malay and 2 of mixed origins). All participants completed a 3-day food diary; these were extracted and analysed using FoodWorks 2007 for fatty acid consumption as well as for dietary intake. Behavioural profiles were also measured, allowing participants' clinical symptoms to be compared with their fatty acid dietary profiles.

Results: In general, 64% (n=9) of the participants did not meet the Adequate Intake (AI) for all three essential fatty acids, as recommended by Australian dietary references. Specifically, 50% (n=7) of the participants had adequate intake of alpha-linolenic acid; the remaining 50% (n=7) had deficiencies. 64% (n=9) of the children met the AI for linoleic acid intake, whereas 36% (n=5) had inadequate levels. Finally, 86% (n=12) met the AI for total Long-chain Omega-3 (DHA+EPA+DPA) acids, while the remaining 14% (n=2) did not.

Conclusion: In our sample of Asian children with disruptive behaviour disorders, intake of essential fatty acids varied. Further empirical data is being collected to explore the role of Omega-3 supplementation in alleviating clinical behavioural symptoms.

P121**Phytanic acid enhances glucose uptake in primary porcine myotubes**

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Background: Phytanic acid (PA) is a bioactive compound, occurring predominantly in ruminant milk and tissue. As PA has been shown to regulate glucose homeostasis and fatty acid metabolism probably through the peroxisome

proliferator activated receptor (PPAR) signalling route, consumption of products rich in PA may improve health by reducing the risk of type-2 diabetes.

Objective: This study aimed to investigate if PA alone or in combination with insulin improves glucose uptake and incorporation into glycogen in primary porcine myotubes in culture.

Procedure: Satellite stem cells, isolated from three young pigs were cultured into differentiated myotubes, and then treated with different doses of insulin, with or without PA. Glucose uptake and glycogen synthesis was measured using 3 [H]-2-deoxy glucose and 14 C-glucose tracers, respectively.

QPCR analysis was conducted on Glucose transporter 1, Glycogen synthase and Glycogen branching enzyme 1 transcripts, using beta-actin expression for normalization and the $\Delta\Delta C_t$ method for quantification.

Results: PA alone increased glucose uptake by 60%. The effect of PA on insulin-stimulated glucose uptake was significant at lower concentrations of insulin. Glucose uptake was improved by about 100% in the presence of both 10nM insulin and PA.

Incorporation of glucose into glycogen was not significantly increased by PA, and none of the assayed genes were significantly affected by the PA treatment
Conclusion: Physiological concentration of PA enhances glucose uptake in primary porcine myotubes without concomitant increased incorporation of glucose into glycogen. The effect on glucose uptake is not explained by increased expression of Glucose transporter 1.

P122**Selected changes of 22:5 n-3 and of elongation products of MUFA in blood of Alzheimer's disease patients**

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Background: Interest in the role of dietary Long Chain Polyunsaturated Fatty Acids (LC-PUFA) of the n-3 series, namely eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) in Alzheimer's Disease (AD), is rapidly increasing. Several studies investigated possible alterations in the circulating levels of EPA and DHA in AD patients and whether dietary interventions with n-3 LC-PUFA may improve the clinical status, but results are not conclusive. Changes of the fatty acid (FA) status in AD may also derive from altered FA metabolism.

Objective: To assess the FA status, especially of the LC-PUFA, in a blood drop collected from fingertips in AD patients (31 males, age 73.9 ± 7.8 years and 22 females, age 78.1 ± 5.2 years), vs controls recruited in the CHECK (Cholesterol and Health Education and Control

Knowledge) study: (322 males, age 59.5 ± 10.0 years and 328 females age 57.2 ± 10.3 years).

Results: In the AD patients, females were older than males, with a prevalence of males below 70 years, and prevalence of females in the group above 80 years. As to blood FA, compared to controls, levels of total saturated FA, monounsaturated FA and PUFA were substantially identical. Compared to controls, the major differences in individual FA concerned the lower (-42 %) levels of docosapentaenoic acid (DPA 22:5 n-3), derived from EPA, and of 24:1 (-12 %) derived from 22:1, both generated from the precursor through an elongation step.

Conclusions: The selected reduction of DPA may be a marker of reduced activation of the metalloproteinase 9 (MMP-9), an enzyme involved in the degradation of β amyloid, the protein that accumulates in brain of AD. In addition the reduced levels of FA produced through an elongation reaction DPA and 24:1 suggests reduced activities of these enzymes, an event that has been associated with accelerated ageing.

P123

Mead acid inhibits angiogenesis - an *in vitro* study

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Background: Cartilage contains very high amounts of n-9 eicosatrienoic acid (Mead acid) compared with other tissues. We already found that Mead acid depressed osteoblastic activity using osteoblasts of scales of goldfish and an osteoblast cell line (Hamazaki et al, *Lipids* 2008). This is why the cartilage is free from ossification. Different from the other tissues, the cartilage does not have any blood vessels. So we asked whether Mead acid also inhibited angiogenesis.

Methods: A kit for angiogenesis (Angiogenesis Kit, Kurabo Industries, Ltd.) was used. This kit measures angiogenesis in the co-culture system of human umbilical vein endothelial cells and human diploid fibroblasts. Co-culture was performed in a medium containing vascular endothelial growth factor-A (10ng/mL) with various fatty acids (10^{-8} - 10^{-6} mole/L). Suramin was used as a positive (inhibitory) control substance. After 10 days of incubation with 3 medium changes, endothelial cells were immunostained for human CD31, and angiogenesis was calculated with image analyzer software.

Results: Compared with the control, addition of Mead acid depressed angiogenesis dose-dependently. The inhibition rate at 10^{-6} mole/L was about 25 %. This inhibitory activity of Mead acid was about one order of magnitude higher than suramin. Addition of oleic acid, dihomo-gamma-linolenic acid or arachidonic acid did not influence angiogenesis even at 10^{-6} mole/L.

Discussion: One of the reasons why the cartilage has no vascular system in it is probably the existence of high

amounts of Mead acid in the cartilage. We suggest that Mead acid might be applied to treatment when excess vasculature is a problem.

P124

Docosahexaenoic acid reduces fatty acid synthase in human breast cancer cells

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Background: Fatty acid synthase (FAS) is up regulated in breast cancer for the de novo biosynthesis of fatty acids mainly to be incorporated into phospholipids for the cell proliferation.

Objective: Docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) have opposites on carcinogenesis, with DHA suppressing and AA promoting breast cancer growth. However, the mechanism is not clear.

Procedure: We examined whether the effect is mediated through changes FAS expression. MCF-7 cells were cultured in normal medium containing 0, 10 or 60 μ M DHA, AA or oleic acid (OA, 18:1n-9) for 48 hrs, then were stimulated with insulin.

Results: 60 μ M DHA supplementation resulted in down regulation of FAS expression, a reduction on phosphorylated Akt, a decrease in the IC50 of FAS inhibitor cerulenin and enhancing cerulenin-induced cytotoxicity in MCF-7. In contrast, AA and OA had no such effects.

Conclusion: We propose that breast cancer cell viability is inhibited by DHA through down regulation of FAS expression and DHA has a synergistic effect on FAS inhibitor for the breast cancer therapy.

P125

Polyunsaturated fatty acids and blood circulation in the forebrain during mental arithmetic task

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Background: The effects of polyunsaturated fatty acids on blood circulation in the human brain have not been well investigated yet. The present horizontal study with healthy participants was based on the pre-intervention data set of a placebo-controlled, double-blind trial with Kampo medicine, keishibukuryogan-ka-yokuinin.

Methods: Healthy participants (14 men and 42 women) between 20 and 49 years of age were recruited. All female participants entered the trial at the start of their menstruation cycle. Before the measuring of blood

circulation in the forebrain, blood was sampled, and two questionnaires were administered (a questionnaire for participants' activeness using a 10-cm visual analog scale (VAS) and POMS (profile of mood states) for six mood states: tension-anxiety (T-A), depression (D), anger-hostility, vigor (V), fatigue, and confusion. Then forebrain blood circulation was continuously monitored during Uchida-Kraepelin Performance (UKP) Test as mental arithmetic task. Changes in oxyhemoglobin (ΔO_2Hb) and deoxyhemoglobin (ΔHHb) concentrations and the tissue oxygenation index (the ratio of O_2Hb to $O_2Hb + HHb$, TOI, a simplified index for cerebral blood circulation) were measured by near-infrared spectroscopy (NIRO-200, Hamamatsu Photonics KK). Multiple regression analysis was performed with sex, age, smoking and drinking as confounding factors.

Results: Linoleic acid (LA) had negative associations with activeness (VAS) and V. EPA and DHA were negatively associated with D; EPA was negatively associated with T-A. TOI during UKP Test was negatively associated with LA and positively with EPA. Activeness was positively associated with TOI during UKP test.

Conclusion: In general LA was adversely associated with mood and brain blood circulation during arithmetic task, and EPA was favorable for those parameters.

P126

Increased monounsaturated fatty acid levels in erythrocyte phospholipids and plasma of Sudanese woman with preeclampsia

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Background: Preeclampsia remains a major cause of maternal and foetal death. Plasma free fatty acid (pFFA) levels have been shown to be increased in preeclamptic patients, but little is known about the composition of pFFA during the clinical manifestation of the disease. Blood levels of monounsaturated fatty acids (MUFA) correlate with the onset of some metabolic disorders like altered glucose metabolism and hyperlipidemia.

Objectives: To investigate the fatty acid composition of pFFA, erythrocyte membrane ethanolamine (ePE) and choline (ePC) phosphoglycerides of normotensive and preeclamptic pregnant Sudanese women at the third trimester of gestation, whose diet is traditionally low in energy, fat and essential fatty acids.

Procedure: 65 preeclamptic pregnant women at the third trimester of gestation diagnosed with preeclampsia were recruited, matched for parity and gestational age with 60 normotensive pregnant women. Venous blood was

collected after overnight fasting during the first week of hospital admission prior to delivery. Total lipids were extracted from erythrocytes and plasma, and lipid fractions were analyzed by thin layer chromatography followed by gas chromatography.

Results: The concentration of C16:1n-7 and C18:1n-7 was higher in the ePE and ePC fractions of preeclampsia patients, as well as C18:1n-9 in ePE. C18:1n-9 is a major constituent of pFFA, and we found an expressive increase ($p=0.0002$) within this fraction. The activity of the stearoyl-CoA desaturase, as indirectly measured in this study by the ratio C16:0/C16:1n-7, was significantly increased in the ePE and ePC fractions.

Conclusion: The increased concentration of MUFA in pFFA, ePE and ePC of preeclamptic Sudanese women is consistent with previous findings in preeclamptic women, but of different ethnicities and dietary patterns. Thus, there is a correlation between blood non-essential fatty acids and the physiopathology of preeclampsia regardless of particularities on diet patterns, which in turn might correlate to other metabolic disorders like diabetes and hyperlipidemia.

P127

Characterization of stress resistance in rats receiving different n-3 polyunsaturated fatty acid (PUFA) supply

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Background: The growing unbalance between n-6 and n-3 PUFA in western diet is thought to negatively impact brain function. Several studies suggest that n-3 PUFA deficiency is associated to emotional disturbances that could lead to psychopathologies.

Objective: to compare the stress response of rats receiving different n-3 PUFA supply.

Procedure: Rats received either an n-3 PUFA deficient (deficient rats), n-3 PUFA balanced (providing the precursor α -linolenic acid : ALA-rats) or n-3 PUFA enriched diet (providing the precursor and the long-chain n-3 PUFA: DHA+EPA-rats). Half of the rats had a restraint stress experience at 6 months of age (6h/d for 21 days). Then brain lipid composition, monoamine contents and plasma corticosterone levels were determined. Behavioural stress responses were measured in the open-field test. Hippocampal synaptic efficacy was measured by electrophysiological recording.

Results: stress induced a weight loss, being the greatest in ALA-rats. The levels of dopamine, serotonin, noradrenalin and main metabolites were dramatically increased in the stressed DHA+EPA-rats as compared to all

5 other groups. Basal corticosterone levels were identical in the 3 dietary groups, but deficient rats exhibited a significant increase in the stress hormone 1 month after the end of the restraint procedure. Increased impulsivity was observed in deficient and in stressed rats as compared to non stressed ALA-rats or DHA+EPA-rats. Synaptic efficacy in the CA1 area of the non stressed DHA+EPA-rats was higher than in all 5 other groups. This was associated to decreased paired-pulse facilitation, suggesting an increased glutamate release. These effects were reversed by the stress procedure.

Conclusion: our results underline several parameters showing the influence of the n-3 PUFA supply on synaptic function and stress response. Additional investigation will be performed to explain the observed effects (expression of presynaptic markers and stress markers).

P128

Hepatocyte-specific inflammation directly enhances VLDL production in mice

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Objective: The low-grade inflammation that is associated with Type 2 Diabetes (DM2) and Cardiovascular Diseases (CVD) plays an important role in their pathophysiology. Shoelson et al have shown that activation of the inflammatory Nf- κ B pathway in the hepatocyte induces severe metabolic changes. We questioned whether hepatocyte-specific activation of Nf- κ B affects triglyceride and cholesterol metabolism directly.

Procedure: The LIKK-mouse has a transgenic hepatocyte specific expression of constitutive active I κ B kinase, IKK β . We crossbred this LIKK mouse with the APOE*3Leiden mouse for its human-like lipid metabolism and investigated the effects of this continuous activation of liver Nf- κ B on fasted plasma cholesterol, triglycerides and lipoproteins in 12 week old male LIKK.APOE*3Leiden and APOE*3Leiden mouse fed a chow diet (n=6).

Results: Basal triglyceride levels were increased in LIKK.APOE*3Leiden mice (p<0.05), which was confirmed by elevated levels of VLDL-triglycerides (0.576 vs 0.382 mM). Hepatic VLDL production was measured using Triton WR1339 to block peripheral VLDL lipolysis, in overnight fasted mice (n=8). VLDL-triglycerides were accumulating faster in LIKK.APOE*3Leiden mice than APOE*3Leiden mice (0.065±0.017 vs 0.044±0.013 mM/min, p<0.05), thereby showing an increased hepatic VLDL production, while clearance of VLDL-like emulsion particles was not different. Hepatocytes were isolated and a similar elevated triglyceride production in vitro was observed for

LIKK.APOE*3Leiden hepatocytes, indicating a direct relation between IKK β activation and triglyceride production within the hepatocyte.

Conclusion: These data show that hepatocyte-specific low grade inflammation directly increased VLDL-TG levels in plasma by up regulation of hepatic VLDL production. We can conclude that chronic low grade inflammation plays an important role in the pathophysiology of hypertriglyceridemia associated with Cardiometabolic risk.

P129

Long-chain omega-3 polyunsaturated fatty acids in relation to growth, gut integrity, and cognitive development of rural African infants

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Background: The growth faltering prevalent among Gambian infants is believed to be due to enteropathy and malabsorption caused by chronic exposure to pathogens. There is evidence that supplementary omega-3 long-chain polyunsaturated fatty acids (n-3 LCs) might ameliorate this damage by reducing gastrointestinal inflammation. Additionally, n-3 LCs have been shown to benefit cognitive development in infants, but this has not yet been tested in an African population.

Objective: To test whether early n-3 LCP supplementation results in improved infant growth, intestinal integrity, and cognitive development.

Procedure: A randomised, double-blind, controlled trial (2ml fish or olive oil per day for six months) was conducted in a population of rural African infants aged 3 - 9 months. Primary endpoints were anthropometric indicators and gut integrity (urinary lactulose-mannitol ratios). Plasma fatty acid status (fatty acid profiles), cognitive development (Infant Planning Test and an attention assessment at 12 months of age), intestinal mucosal inflammation (faecal calprotectin), and daily morbidities were the secondary endpoint measures.

Results: One-hundred and seventy-two Gambian infants completed the trial. Supplementation resulted in a significant increase in plasma n-3 LCP concentrations (p<0.001). Plasma n-6 fatty acid concentrations were little affected. Apart from an increase in mid-upper-arm circumference (MUAC) z-scores in the intervention group (average increase: 0.39; 95% CI: 0.06-0.56; p=0.017), no significant differences between treatment groups were

detected for growth or lactulose-mannitol ratios at 9 months. At 12 months MUAC remained greater in the intervention group, and significant increases in skinfold thicknesses were observed. No differences were detected for the other secondary outcomes.

Conclusion: Fish oil supplementation proved safe and successfully increased plasma n-3 fatty acid status. The results of this trial do not, however, support the use of supplementary n-3 LCPs in breast-fed, Gambian infants for improving overall growth, intestinal integrity, and cognitive development, or for reducing intestinal and systemic inflammation.

P130

Alterations in PUFA profile and oxidative status in liver and colon carcinogenesis

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Background: Deregulation of cell growth and survival in cancer development has been linked to an altered fatty acid (FA) metabolism, especially regarding the n-6 and n-3 PUFA. These alterations are linked to the modulation of membrane structure and cellular oxidative status which lead to changes in membrane function, activity of enzymes and signal transduction pathways. The incorporation and association of n-6 and n-3 FA with membrane dynamics involving cholesterol, phospholipids and membrane proteins, impact on cellular processes such as signal transduction pathways, cell to cell communication in the regulation of cell growth and alteration in cellular oxidative status.

Objective & Results: In rat liver, a characteristic lipid profile is associated with the growth and development of preneoplastic nodules. This profile entails decreases in the PC/PE phospholipid ratio, C20:4n-6 PC/PE ratio, n-3 PUFA content and oxidative status. Lipid analyses of liver biopsies from human patients with hepatocellular carcinoma indicated a similar altered lipid pattern observed in the rat preneoplastic nodules, i.e. a reduced level of n-6 PUFA in PC, C20:4n-6 PC/PE ratio, n-3 PUFA in PC and PE phospholipids with a resultant decrease in oxidative status. Utilising an animal colon cancer model, it is shown that a diet high in n-6 PUFA may play an underlying role in the development of colon polyps. An interactive role of SATS, MUFA and C20:4n-6 and the effect on membrane fluidity are highlighted as the main driving force to sustain the altered growth characteristics of the polyps. For comparison, colon cancer biopsies with matching surrounding tissue collected from human patients are currently undergoing analyses.

Conclusion: The modulation of the altered tumour lipid profile and oxidative status by dietary PUFA will be discussed in relation to their role in altering the growth characteristics of cancer cells.

P131

The antioxidant potential of oleic acid and effect on cell survival in carcinogenesis

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Background: In a cancer cell the redox balance is shifted towards a low oxidative status, mimicking proliferation in a normal cell. Control mechanisms in cancer cells appear to be disrupted, maintaining low levels of lipid peroxidation which contributes towards cell survival and promotion of an altered cell phenotype. The low lipid peroxidation level is due to decreased membrane long-chain PUFA content, resulting from impaired FA metabolism, and altered oxidative enzyme activity. Furthermore, cancer cells contain high levels of antioxidants such as vitamin E and oleic acid (OA, C18:1n-9), which has been reported to display antioxidant properties.

Objective: In the present study the antioxidant properties, of OA was compared to known antioxidants such as vitamin E, quercetin, reduced glutathione (GSH), ECGC and catechin in an in vitro microsomal assay. In addition, the antioxidant properties of OA was compared to vitamin E in rat primary hepatocyte cultures treated with the mycotoxin fumonisin B1 (FB1), known to induce lipid peroxidation in vitro and in vivo.

Results: In the microsomes, OA demonstrated a protective effect against iron induced lipid peroxidation exhibiting an IC50 of 760µM, half as effective as vitamin E (IC50 320µM), while GSH was less potent (IC50 10mM). The polyphenols showed strong inhibitory effects at low concentrations (EGCG, IC50 42µM; catechin, IC50 90µM; quercetin, IC50 17µM). In the primary hepatocytes, both OA (100µM) and vitamin E (10µM) counteracted the FB1 induced lipid peroxidation and tended to counteract the FB1-induced depletion of PUFA.

Conclusion: The present data suggest that OA plays a key role in the control of the oxidative status of a cell and contributes to the survival of a cancer cell.

P132

Erythrocyte membrane phospholipid molecular species in pregnant women with gestational and type 2 diabetes

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Background: Previously, pregnant women with gestational (GDM) and type 2 (DM2) diabetes were associated with a reduction in long-chain ω 3 and ω 6 fatty acids (FA) in erythrocyte phospholipids.

Objective: To investigate whether molecular species of phosphatidylethanolamine (PE) in pregnant women with GDM and DM2 were altered.

Procedure: Blood samples were obtained from 14 DM2 and 14 non-diabetic women during 1st trimester (7-16 weeks), and 13 GDM and 14 non-diabetic women during 3rd trimester (28-32 weeks). GDM samples were obtained before any intervention. Erythrocyte total lipids were extracted and PE molecular species were analyzed by electrospray ionization mass spectrometry (ESI-MS).

Results: More than 30 different molecular species of PE were identified in negative-ion ESI mass spectra. The main plasmenyl-phosphoethanolamine (pPE) molecular species identified contained long chain polyunsaturated FA (LC-PUFA), and the most dominant peak was 18:0-20:4/16:0-22:4. Interestingly, the contribution of the diacyl-phosphoethanolamine (diacyl-PE) subclass to the total PE was higher in all groups. Diacyl-PE molecular species also showed a high content of LC-PUFA, however it contained a higher proportion of 18:1 and 18:2 FA in the sn-2 position compared to pPE. There were no differences in the percent of PE molecular species between non-diabetic and diabetic women, regardless of the type of diabetes. However, when non-diabetic samples from the 1st and 3rd trimester were compared, those of the 3rd trimester contained higher proportion of molecular species with long-chain ω 3 FA.

Conclusion: Our preliminary results show that erythrocyte PE molecular species were not altered in pregnant women with GDM and DM2. However, a difference related to the gestational period has been observed, favouring the increase of molecular species containing ω 3 FA in erythrocytes in the 3rd trimester. This observation might indicate a physiological response to increased requirement for long-chain ω 3 FA by the foetus toward latter part of pregnancy.

P133

Low levels of ω -3 and ω -6 polyunsaturated fatty acids (PUFAs) in pregnancy: association with anxiety and depression

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Objective: Low levels of ω -3 PUFAs have been associated with adverse obstetric and neonatal outcome and are more often found in pregnant women with depressive

mood. This relation has not been reported for other mental health problems such as the prevalent anxiety disorders. The present study therefore aims at investigating the association of ω -3 and ω -6 polyunsaturated fatty acids (PUFAs) with anxiety and depression and their relevance for obstetric outcome.

Procedure: Prospective study following 93 women with depression, anxiety disorders and healthy controls from mid pregnancy to 1 week postpartum. At gestational week 29, blood samples were taken and washed to obtain red blood cells. Fatty acids were analyzed by means of gas chromatography (GC) after direct derivatization of the nonvolatile fatty acids into their corresponding volatile fatty acid methyl ester derivatives (FAME) with acetylchloride. Nutritional habits and psychometric data were assessed by questionnaire and interview. Information on course of pregnancy was extracted from patients' files.

Results: We found an association between high levels of anxiety and lower levels of ω -3 PUFAs (stand β = -0.34, p <0.01) and ω -6 PUFAs (stand β = -0.23, p <0.05), while depression showed no association. In addition, the association of anxiety with shorter gestations was partly mediated by lower levels of ω -6 PUFAs (stand β = -0.32, p <0.05). Higher levels of depression independent of levels of PUFAs predicted shorter gestations (stand β = -0.25, p <0.05).

Conclusion: Anxiety and depression are associated with adverse obstetric outcome. Especially for anxiety, lower levels of PUFAs could be a biomarker, and thus, target of intervention.

P134

Salmon in pregnancy study (SIPS): consumption of farmed salmon during pregnancy increases maternal and fetal EPA and DHA status

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Objective: To determine the effect of increased oily fish consumption during pregnancy on the EPA and DHA status in maternal and cord blood mononuclear cells (BMC) and plasma.

Procedure: At 20 weeks gestation, 123 women with high risk of having allergic offspring and low habitual intake of oily fish (\leq 2 times/month) were randomised to consume two portions of salmon per week (each portion provided 2 g of n-3 PUFA) (salmon group) or to continue their habitual diet of low oily fish consumption until delivery (control group). The women attended a clinic in the fasted state at weeks 20 (n=123) and 32-34 (n=111) of pregnancy, at which blood samples were collected, and a food frequency questionnaire administered. Umbilical

cord blood was collected at birth (n=101). Maternal and cord BMC and plasma phospholipid (PL) fatty acid composition was determined by gas chromatography. **Results:** In the control group EPA and DHA (% of total fatty acids) in maternal plasma PL decreased significantly from week 20 to 34 of pregnancy: EPA from 0.62% to 0.4% (p<0.001), DHA from 4.13% to 3.96% (p=0.013). In the salmon group EPA and DHA in maternal plasma PL increased significantly from week 20 to 34 of pregnancy: EPA from 0.71% to 0.88% (p=0.01), DHA from 4.77% to 5.10% (p=0.001). Cord plasma PL EPA and DHA contents were significantly higher in the salmon group compared with the control group: EPA 0.63% vs. 0.32% (p<0.001), DHA 7.40% vs. 6.44% (p=0.001). Similar trends were seen in maternal and cord BMC. **Conclusions:** Eating oily fish twice a week during pregnancy results in a higher maternal and cord status of long chain n-3 fatty acids (both EPA and DHA). This may result in improved growth, development and health in the offspring.

P135

PPAR δ agonist GW501516 strongly prevents development of atherosclerosis in APOE*3Leiden mice

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Introduction: PPAR δ is a nuclear receptor that regulates energy production and strongly modulates lipid metabolism mainly through fatty acid β -oxidation in skeletal muscle, and is consequently considered as a target for treatment of type II diabetes. In addition, PPAR δ agonists exert anti-atherogenic effects by enhancing cholesterol efflux via activation of ABCA1 in cultured cells. We investigated the effect of the PPAR δ -agonist GW501516 (GW) on plasma lipids, inflammation markers and atherosclerosis in a hyperlipidemic mouse model for atherosclerosis, the APOE*3Leiden transgenic mice.

Methods: APOE*3Leiden mice were fed an atherogenic diet alone or supplemented with GW (3 mg/kg bw/day). Effects on plasma lipids and inflammation markers and atherosclerosis were assessed after 20 weeks of treatment.

Results: In untreated control mice plasma cholesterol and triglyceride levels were on average 21.0 ± 6.1 mM and 2.0 ± 0.8 mM, respectively. GW lowered cholesterol levels by 41% (contained in the apoB-containing lipoproteins) and triglycerides by 36% (both p<0.001). Additionally, GW had favorable effects on plasma inflammation markers, as indicated by reduced plasma levels of SAA (-49%), MCP-1 (-36%) and VCAM-1 (-13%) (all p<0.05).

GW strongly improved various parameters of atherosclerosis, evidenced by a reduced lesion size (-82%, p<0.001), lesion number (-51%; p<0.001) and lesion

severity and by a 7-fold increase of undiseased segments (p<0.05).

Conclusions: The PPAR δ -agonist GW501516 exerts potent anti-atherogenic effects by reducing plasma lipid levels, inflammation markers and atherosclerosis in APOE*3Leiden mice.

P136

Beneficial effects of PUFA and fish oil on metabolic activity in neuronal cells

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Background: Two types of polyunsaturated fatty acids (PUFA) are essential: linoleic acid (n-6-PUFA) and alpha-linolenic acid (n-3-PUFA). Other omega-6 fatty acids, such as gamma-linolenic acid (GLA), can be synthesised in the body using linoleic acid as starting point. Similarly, omega-3 fatty acids, using alpha-linolenic acid as starting point of biosynthesis, include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Aging is a sensitive and critical period, where adequate PUFA bioavailability is crucial for proper brain function.

Objective: To investigate a protective impact of PUFA on neuronal cells, we studied metabolic activity and morphology in vitro using a human neuroblastoma cell line (SH-SY5Y).

Procedure: For this attempt, SH-SY5Y cells (control cells or cells over-expressing amyloid- β protein to represent endogenous Alzheimer-related stress) were used. After pre-treatment (1 day or 1 week) with n-3-PUFA, n-6-PUFA, or a fish oil mixture (EyeQTM standard mix) metabolic activity of mitochondria of the cells (MTT assay) was analysed to reveal improvement under basal condition as well as potential protection against endogenous and exogenous stress-induced oxidative cell damage.

Results: With regard to 1 day pre-treatment experiments, we did not see a protective effect with the n-3-PUFA EPA and the n-6-PUFA GLA, but with DHA there was a significantly protective effect on metabolic cell activity, since MTT reduction was increased for β -expressing cells and even for control cells after secondary insult. Fish oil as well protected cells from stress-induced damage in both cell types.

Currently, pre-treatment experiments with fish oil for 1 week as well as single and combination treatment of PUFA (EPA:DHA:GLA; 9:3:1) are under investigation.

Conclusion: Of note, our results emphasize findings from in vivo studies suggesting a beneficial impact of PUFA on cognition. It seems that a well-balanced diet of n-6 and n-3 PUFA is important for brain function, each family possessing unique properties in maintaining mental health.

P137**APOE*3Leiden.CETP transgenic mice as model for the metabolic syndrome**

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Background: The metabolic syndrome is characterized by the co-occurrence of several risk factors i.e. increased body weight (bw) and insulin resistance (IR) and at the same time adverse changes in plasma lipids as observed in diabetic dyslipidemia, with increased triglycerides and apoB-containing lipoproteins and decreased HDL. The aim of this study was to investigate whether the APOE*3Leiden.CETP (E3L.CETP) mouse is a useful translational animal model to investigate the metabolic syndrome.

Methods: Male E3L.CETP mice were put on a high fat diet and fructose in drinking water for 12 weeks to induce diet-induced obesity and IR. Then the mice were treated with either rosiglitazone (3 and 11 mg/kg bw/d), resveratrol (75 mg/kg bw/d), fenofibrate 12 mg/kg bw/d, atorvastatin 10 mg/kg bw/d) or niacin 720 mg/kg bw/d) for 4 weeks.

The effects on bw, plasma lipid and inflammation parameters and insulin sensitivity (via hyperinsulinemic euglycemic clamps) were assessed.

Results: Dietary treatment resulted in a human-like lipoprotein profile with a TC/HDL-C ratio of 3. Anti-diabetic compound rosiglitazone significantly increased insulin sensitivity and reduced plasma lipid levels. Established lipid lowering compounds atorvastatin, fenofibrate and niacin, and resveratrol improved the dyslipidemia.

Conclusion: The data indicate that the E3L.CETP mouse is a good translational animal model that combines all important features that underlie the metabolic syndrome and mimic the human response to clinically used treatments. We conclude that the E3L.CETP mouse is a promising model to investigate the effects of new drugs, alone or in combination, that affect insulin resistance and diabetic dyslipidemia.

P138**Pattern of phospholipid and of their fatty acids in lipid raft after supplementation with AA, EPA and DHA**

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Objective: We have studied the effects of PUFAs supplementation on composition and function of membrane lipid microdomains (rafts).

Procedure: Cells (MDA-MB.231 and MCF7) were treated with AA, EPA or DHA; lipid microdomains (rafts) were isolated. Phospholipids (PLs) were extracted and analyzed by HPLC/GC.

Results: After EPA treatment, the content of DPA and EPA increases, while the incubation with DHA determines an increment of DHA and EPA concentration, and a reduction of AA content. These data indicate that n-3 PUFAs are metabolized and incorporated in lipid rafts. Also AA is incorporated in microdomains but it induces a reduction of n-3 PUFAs content.

PUFAs are incorporated in lipid rafts with different specificity for the PLs moiety. In particular the treatment with EPA determines an increase of DPA, DHA and EPA content, especially in PI and SM, and a decrease of AA concentration in all phospholipids.

The DHA treatment determines an increase of its content, in particular in PI, in PC and PE, and a reduction of AA content only in PE.

The AA is incorporated especially in PS, PC and SM; it induces a reduction of EPA and DHA in PI, PS and SM.

Conclusion: Taken together, our results indicate that n-3 PUFAs 'feeding' might induce modifications of lipid rafts structure increasing the degree of fatty acid unsaturation. Moreover, we have demonstrated that DHA is the only fatty acid that induces a reduction of cholesterol concentration in lipid rafts.

We can suppose that these changes might modify signal transduction and cell-cell interactions. In fact in MDA-MB-231 cells, we have demonstrated that DHA slightly reduces the expression of EGFR, a receptor localized in lipid rafts. Moreover, DHA and EPA reduce the activation of EGFR, which mediate cell proliferation in breast cancer cells.

Biochemistry of Lipids**P139****PC12 pheochromocytoma cells possess the ability to synthesise omega-3 polyunsaturated fatty acids from alpha-linolenic acid**

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Background: Docosahexaenoic acid (DHA, 22:6 n-3) is the major n-3 fatty acid (FA) in brain tissue. It is either synthesized from alpha linolenic acid (ALA, 18:3 n-3) or directly incorporated via the diet. PC12 pheochromocytoma, a cell line widely used in molecular and cellular neurobiology contains little or no DHA.

Objectives: Investigate de novo synthesis of DHA from ALA versus direct enrichment by DHA supplements and characterize the polyunsaturated FA profile in proliferating and NGF-differentiated cells.

Procedure: Cells were incubated in DMEM with FA supplements such as ALA or DHA (10 μ M) in the absence or presence of NGF for designated time periods. At the end of incubation, phosphoglycerides (PG) were extracted and subjected to FA analysis using sequential thin layer and gas chromatography. Alternatively, PG molecular species were assessed by quadrupole/time-of-flight (Q-TOF) mass spectrometry combined with electrospray ionization (ESI).

Results: After incubation with 10 μ M ALA, eicosapentaenoic (20:5n3, EPA) level was exceeded by 14 fold, docosapentaenoic acid (22:5n-3, DPA) level by 3 fold and DHA level by 2 fold compared to controls in ethanolamine PG species. DHA supplements (10 μ M) on the other hand resulted in x1.5 increase in EPA level and x2 increase in DHA levels. A marked increase in the percent of EPA, DPA and DHA containing PG molecular species, undetected in non supplemented cells, including ethanolamine, serine and glycerol PGs were noticed after ALA supplements. Long term exposure of PC12 cells to NGF, inhibited the last step in DHA biosynthesis from ALA indicating an aberration in peroxisomal activity.

Conclusion: PC12 cells generate most of the n-3 intermediary metabolites including EPA, DPA and DHA. Lack of ALA in the regular culture medium may hamper the correct profile of PG and hinder many of the membrane properties of PC12 cells which ultimately may lead to erroneous biological interpretations.

P140

Characterization of monoacylglycerol lipase in *Saccharomyces cerevisiae*

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Background: Monoacylglycerols (MAG) are short-lived intermediates of lipid metabolism. Specific MAG species like 2-arachidonoylglycerol (2-AG) also act as signalling molecules. In mammals, the enzyme monoacylglycerol lipase (MGL) is thought to play a major role in MAG catabolism by facilitating the last step in triacylglycerol breakdown. In contrast, MAG metabolism in the yeast *S. cerevisiae* is barely described. Based on sequence similarity, Yju3p is thought to represent the yeast homologue to mammalian MGL, but its role in MAG metabolism has not been characterized yet.

Objective: The aim of this study was to investigate the role of Yju3p as a potential monoacylglycerol lipase in *S. cerevisiae*.

Procedure: Recombinant Yju3p was purified by affinity chromatography and catalytic activity was investigated using 1,3-(rac)-oleoylglycerol and 2-oleoylglycerol as substrates. Using a *yju3Δ* mutant strain, the contribution of Yju3p to monoacylglycerol metabolism in yeast was investigated.

Results: Purified recombinant Yju3p showed robust MAG hydrolase activity. *yju3Δ* cell lysates exhibited ~90% decreased MAG hydrolase activity compared to wild-type. Moreover, Yju3p-deficiency caused reduced fatty acid release and an accumulation of MAG during in vitro lipolysis experiments. Interestingly, we could not observe an accumulation of MAG in the *yju3Δ* mutant in vivo.

Conclusions: Yju3p accounts for 90% of MAG hydrolase activity in yeast cell lysates. Yet, *yju3Δ* mutants do not accumulate MAG in vivo. Thus, we conclude that additional enzymes exist, that contribute to the metabolism of MAG.

P141

Lipid droplet-like structure formation in stressed endothelial and adipose stromal vascular fraction cells in presence of TNF- α and FFA

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In 20-40% adipose tissue consists of the stromal vascular fraction (SVF) containing preadipocytes, and in 10-20% of preendothelial cells. The di-/dedifferentiation of SVF is poorly understood. Free fatty acids (FFA) and insulin resistance are responsible for lipotoxicity and lipid droplets (LD) formation. Nutritional overload induces ER-stress, changes in mitochondrial membrane potential, LD formation accompanied by autophagy important for survival and protection from apoptosis.

Study is aimed to find the sequence of the gene expression/protein biosynthesis and the lipid content changes characteristic for LD formation and disappearance in preadipocytes and HUVECs.

Methods: HUVEC and SFV were cultured with PA, AA, EPA (30 μ M), VEGF, or L-Arginine for (5ng/ml) for 1, 4 and 24hs (HUVEC). Changes at 24 hours (SVF), when with PA or TNF in gene expression was analyzed by microarray. Metabolic activity of mitochondria were analyzed by ATP production and oxygen requirement (Oxygraf 2-K, PAT protein and selected ER chaperones were followed by YDORoboros). The changes by the fluorescence microscopy imaging (Bioimager BD) and by freeze-fracture technique combined with replica

immunolabeling for high-resolution imaging.

Results: VEGF, as well EPA and AA inhibited, when PA promoted differentiation SVF to adipocytes. SVF metabolism (oxygen consumption and ATP) was higher than HUVEC. FFA did not significantly change oxygen consumption, but ATP generation was decreased by PA and OA. TNF induced expression of adipophyllin HUVEC. Microarray analysis revealed an induction of intracellular substrate transporters, metabolism and angiogenesis genes, as well as induction of ER-shock chaperones. The electron-microscopic technique of freeze-fracture replica immunolabeling illustrates the localization of the proteins p61ER, GRP78, and GRP94 on the E-face of the ER membrane, the E-face of the inner nuclear membrane and in the lumen of the ER.

Conclusion: The ER shock associates formation of the LD-like structures in lipid not accumulating HUVEC.

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P142

Activation of survival and apoptotic signaling pathways in lymphocytes exposed to palmitic acid

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Objective: There is increasing evidence that fatty acids can cause cell death through activation of apoptosis. In the present study, death signaling pathways were investigated in Palmitic Acid (PA)-treated Jurkat cell (human T-Lymphocyte lineage). Thus we investigated PA dependent activation of components of the insulin signaling pathway and glucose metabolism that impacted on cell survival or apoptosis.

Procedure: Jurkat cells were exposed to PA (50, 100 and 150µM) for 24h. Cell death was evaluated by DNA fragmentation assay, mitochondrial membrane depolarization and activation of proteins from apoptosis pathway. Insulin signaling, glycolytic pathway and lipid metabolism were also evaluated.

Results: PA induced DNA fragmentation and depolarization of the mitochondrial membrane mitochondrial in a dose dependent manner. PA treatment led to release of cytochrome c from the inner mitochondrial membrane to the cytosol, activated members of the MAPK protein family JNK, p38, ERK, and activated caspases-9 and 3. PA also increased insulin receptor and GLUT-4 levels in the plasma membrane. Insulin treatment (10mU/mL for 30 minutes) after exposure to PA promoted a significant increase in the phosphorylation state of the insulin receptor β-subunit, Akt and IRS-1. A correlation was found

between cell DNA fragmentation and expression levels of both the insulin receptor and GLUT-4. Glucose uptake by cells increased when incubated in the presence of PA. Glucose oxidation was diminished and lactate production augmented by PA treatment and there was an accumulation of triglyceride, phospholipids and cholesterol ester.

Conclusion: We suggest that, in an attempt to survive, lymphocytes activate insulin signaling pathways and glucose utilization in response to PA so stimulating the synthesis of lipid macromolecules therefore removing PA from mediating intracellular cytotoxic effects. However, in the presence of high concentrations of PA, apoptotic mechanisms prevail and the cells die.

P143

Exposure to commonly used surfactants induces alterations in the membrane fatty acid composition of Staphylococci

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Background: Human skin is colonised by a complex mixture of bacteria that are important for skin health and protection from pathogens. Species of staphylococci are a major component of this normal commensal microflora, but they can also be opportunistic pathogens. Bacterial growth can be inhibited by surfactants commonly used in wash products, e.g. sodium lauryl ethoxy sulphate (SLES) and laurylamidopropyl betaine (LAPB). Bacteria can adapt to environmental changes by altering their membrane fluidity and membrane fatty acids are useful biomarkers to assess this.

Objective: To determine minimum inhibitory concentrations (MICs) and monitor changes in membrane fatty acid composition of staphylococci when exposed to SLES and LAPB.

Procedure: Surfactant deposit levels on human skin were estimated by LC/ESI-MS. Findings determined concentrations used for in vitro challenge studies: 0.25-64 µg/ml SLES and 0.625-8 µg/ml LAPB. MIC determination used a panel of 34 strains of Staphylococcus spp. Reference strains *S. epidermidis* NCTC 11047 and *S. aureus* NCTC 10788 were exposed to different levels of surfactant, and membrane fatty acid composition was determined by GC.

Results: MIC assays indicated that SLES effected the growth of Staphylococcal strains, whilst LAPB did not. Thirteen fatty acids were identified in extracts from the two reference strains, including C19:0, a known marker of Staphylococcus. SLES exposure induced an increase in the

relative abundance of C17:0, C20:0 and trans-C18:1 and decreased that of iso-C15:0 and iso-C16:0. The effect of LAPB was less consistent but affected the amounts of C18:2, iso-C15:0, iso-C17:0, cis-C18:1, C15:0 in a concentration dependent manner.

Conclusion: When grown in the presence of SLES and LAPB at levels typically found on the skin, staphylococci can alter their membrane fatty acid composition. This provides insight into how the skin microflora is forced to adapt to the deposition of surfactants from wash products by reducing membrane fluidity.

P144

Deficient liver biosynthesis of docosahexaenoic acid correlates with cognitive impairment in Alzheimer's disease

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Background: Reduced brain levels of docosahexaenoic acid, a neurotrophic and neuroprotective fatty acid, might contribute to cognitive decline.

Objective: The present study was designed to investigate whether the liver enzyme system that provides docosahexaenoic acid to the brain is dysfunctional in Alzheimer's disease.

Procedure: Docosahexaenoic acid content was measured in post mortem brain samples from 17 control subjects and 37 Alzheimer's disease patients. Similar analyses were conducted in liver samples from an additional cohort of 9 control subjects and 14 Alzheimer's disease patients. Expression of genes involved in the biosynthesis of docosahexaenoic acid from dietary α -linolenic acid was also assessed.

Results: Docosahexaenoic acid levels were reduced in temporal cortex, mid-frontal cortex and cerebellum of subjects with Alzheimer's disease, compared to control subjects ($P=0.007$). Mini Mental State Examination (MMSE) scores positively correlated with docosahexaenoic/ α -linolenic ratios in temporal cortex ($P=0.005$) and mid-frontal cortex ($P=0.018$), but not cerebellum. Similarly, liver docosahexaenoic acid content was lower in Alzheimer's disease patients than control subjects ($P=0.011$). Liver docosahexaenoic/ α -linolenic ratios correlated positively with MMSE scores ($r=0.78$; $P<0.0001$), and negatively with global deterioration scale grades ($P=0.013$). Docosahexaenoic acid precursors, including tetracosahexaenoic acid, were elevated in liver

of Alzheimer's disease patients ($P=0.041$), whereas expression of peroxisomal D-bifunctional protein, which catalyzes the conversion of tetracosahexaenoic acid into docosahexaenoic acid, was selectively reduced ($P=0.048$).

Conclusion: A deficit in D-bifunctional protein activity may impair docosahexaenoic acid biosynthesis in liver of Alzheimer's disease patients, and possibly lessen the flux of this neuroprotective fatty acid to the brain.

P145

Toxicokinetic study of a highly purified DHA ethyl ester in rats

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Background: Two formulations of algal-DHA were used: MATK-90 and DHASCO[®]. MATK-90 contained 90% of DHA ethyl ester, while DHASCO[®] contained 40% of DHA as triglycerides.

Objectives: This study evaluated the systemic exposure of DHA after repeated administration in Sprague-Dawley rats for 91 days.

Procedures: Three doses of MATK-90 (1.3, 2.5, 5.0 g/kg/day) and one dose of DHASCO[®] (5g/kg/day=2g DHA) were administered once-daily by oral gavage at a volume of 10 mL/kg. The corn oil (vehicle) was administered to the control animals. Blood samples were obtained from 3 animals for each time point (1, 2, 4, 6, 8, 12, and 24 h post dose) on days 1, 28 and 91. C_{max} and area under the curve (AUC) for DHA plasma concentration vs. time were calculated using the linear trapezoidal method (linear interpolation).

Results: The half-life of DHA varied from 8 to 10.5 hours. On Day 1, DHA C_{max} after administration of 1.3 to 5 g/kg/day doses of MATK 90 increased, but markedly less than proportionally to the increase in dose. On Day 28, in males, DHA exposure (AUC) increased greater than proportionally to the increase in doses of MATK 90. In females, DHA exposure decreased. On Day 91, DHA exposure increased in both sexes, but less than proportionally. Exposure obtained with DHASCO[®] (2 g of DHA) was generally similar to that obtained with the same amount of MATK-90. In both sexes, exposure of DHA increased with repeated administration for 28 days vs. Day 1. However, in males, exposure tended to decrease from Day 28 to Day 91, whereas in females the general trend was for a continuing increase in exposure from Day 28 to Day 91.

Conclusions: Although there were sex differences in AUC values, the systemic exposure of DHA generally increased with repeated administration and increase in doses.

P146**Accumulation of bis(monoacylglycero)phosphate alters LDL-derived cholesterol homeostasis in cultured macrophages**

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Background: Bis(monoacylglycero)phosphate (BMP) is a unique phospholipid enriched in late endosomes. Using anti-BMP antibody, we previously showed that BMP regulates cellular distribution of LDL-derived cholesterol in macrophages. We also reported that BMP highly incorporates exogenous 22:6n-3 leading to the formation of 22:6/22:6-BMP. This molecular species was found to protect cholesterol against oxidation in vitro.

Objective: The present study evaluates the effect of both fatty acid composition and cellular content of BMP on cholesterol homeostasis in macrophages.

Procedure: RAW macrophages were supplemented with di-18:1n-9- or di-22:6n-3- containing phosphatidylglycerol (PG), a precursor of BMP. LDL-derived cholesterol metabolism and transport were then examined.

Results: BMP represents a minor phospholipid naturally enriched in 18:1n-9 in RAW macrophages. Incubation with 30 μ M of 18:1/18:1-PG liposomes induced a 4-fold increase of cellular BMP. BMP accumulated in late endosomes similar to endogenous BMP. The contents of other PL were not changed and PG did not accumulate. Fatty acid analysis of newly synthesized BMP showed that 18:1/18:1-PG was primarily converted to 18:1/18:1-BMP. Accumulation of 18:1/18:1-BMP resulted in a 30% decrease of LDL-stimulated cholesterol esterification and efflux to methylbetacyclodextrins or HDL whereas LDL uptake and hydrolysis were not modified. When incubated with 22:6/22:6-PG liposomes under vitamin E supplementation to prevent peroxidation, RAW macrophages accumulated 22:6/22:6-BMP. Cholesterol esterification and efflux were then also decreased by about 30% compared to control cells.

Conclusion: These results show that endosomal accumulation of BMP in RAW macrophages reduced both cholesterol esterification and efflux, confirming the role of BMP in cellular distribution of LDL-derived cholesterol. This regulation appears to be independent of DHA content in BMP. The specific role of 22:6/22:6-BMP towards cellular oxysterol formation remained to be ascertained.

P147**Structural basis for miscibility and immiscibility in lipid phase separations**

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Background: Lipids are critical components present in very diverse domains from biodiesel production to dietary polyunsaturated fats, to protein-lipid complexes in lipid bilayers, rafts and caveolae. Free miscibility of structurally different lipids could be evidence that lipid structure at the molecular level is amorphous.

Objective: to test the miscibility of structurally different lipids.

Procedures: Differential scanning calorimetry and computational chemistry.

Results: Our research demonstrates that immiscible lipid phases can readily be created. Lipids are critical components present in very diverse domains from biodiesel production to dietary polyunsaturated fats, to protein-lipid complexes in lipid bilayers and rafts. Our biodiesel research on methyl oleate (MeOA) determined that both a miscible and an immiscible phase formed from only 10% stearic acid (SA). Neither of the resulting MeOA phases contained 10% SA. Using differential scanning calorimetry, we confirm that, above a threshold level, addition of SA to MeOA, to various animal fats results in two miscible phases and an immiscible lipid phase. We propose the structural basis of this is that the same lipids at different mole ratios must be packing differently in the immiscible than the miscible phase. Cubic unit cells are the smallest volumes that explain the chemical composition of the two phases at both the molecular and the macroscopic level. Moreover, from the thermal data aided by computational chemistry, differences in the molecular structure in lipids can be correlated to changes in the cubic phase volume.

Conclusion: Immiscible phases invalidate any idea that lipids are amorphous liquids. Histo-pathological changes are known to occur following ingestion of large amounts of saturated type fats. A question raised is would a bolus of dietary fat in the circulation disrupt endothelial membrane lipids through this principle of immiscibility?

P148**Mitochondrial β -oxidation of unsaturated fatty acids in 3,2-trans-enoyl-CoA isomerase deficient mice**

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Background: Mitochondrial 3,2-trans-enoyl-CoA isomerase (mECI) is an auxiliary enzyme of the mitochondrial β -oxidation of unsaturated fatty acids. mECI catalyzes the isomerisation of the double bond in cis-3-enoyl-CoA or trans-3-enoyl-CoA to trans-2-enoyl-CoA. After isomerisation, trans-2-enoyl-CoA re-enters the β -oxidation cycle.

Objective: A genetic defect of mECI has not been found in humans yet. However, a mECI-KO mouse (dci-/- mouse) was recently described showing accumulation of lipids with unsaturated fatty acyl groups upon fasting. Additionally, the dci-/- mouse had dicarboxylic aciduria. This dci-/- mouse could be a good model to identify the presentation of human mECI deficiency. Here we define the impact of mECI deficiency on the oxidation of unsaturated fatty acids in the dci-/- mouse.

Procedure: Urine of WT and dci-/- mice was collected for three days. On the last day the mice were fasted for 24h, killed and dissected. Biochemical studies were performed in plasma, urine and tissues.

Results: We found that upon fasting, dci-/- mice had hypoglycaemia and slightly elevated levels of unsaturated acylcarnitines in plasma and blood. We found increased levels of adipic acid in urine. Remarkably, we did not observe accumulation of unsaturated acylcarnitines in tissues and urine. Furthermore, ECI enzyme measurements in purified mitochondria showed considerable residual activity, offering an explanation for the mild phenotype.

Conclusion: The phenotype of dci-/- is relatively mild suggesting compensatory mechanisms. Up to now, two enoyl-CoA isomerases in mice are known, one mitochondrial (mECI) and one peroxisomal (pECI). However, based on their targeting sequences as well as experimental evidence in rats, both mECI and pECI are localized to the mitochondria. We hypothesize that pECI can compensate for mECI deficiency. Furthermore, we identified a novel ECI gene that is responsible for the peroxisomal ECI activity.

P149

Analysis of LXR-dependent biological signatures after a dual extraction of both mRNAs and lipids from a single liver sample

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Background: Extractions of RNAs and lipids from large numbers of biological samples represent cumbersome and costly steps required for high-throughput transcriptomics and lipidomics approaches. To our knowledge most

protocols rely on independent extractions of nucleic acid and lipids respectively from a single sample, thereby increasing variability in data analysis.

Objectives: In this work we investigated whether it would be possible to use standard RNA extraction procedures in order to analyze not only mRNA levels but also lipids from a single liver sample.

Procedures: We bring evidence that the organic phase, obtained when using standard reagent for RNA extraction can be kept to analyze lipids such as neutral lipids and fatty acids by Gas Chromatography (GC). Once validated, we applied this protocol to explore in vivo the roles of Liver X Receptors (LXRs) in the regulation of are nuclear receptors that play ab and LXRahepatic gene expressions. LXR critical role in the control of cholesterol metabolism. However, LXR isoforms have also been shown to regulate fatty acid metabolism, inflammation and have distinct expression patterns both ofb and LXRaproliferation. While LXR them are expressed in mouse liver. Here, we investigated the respective contributions of each LXR isoform to liver homeostasis through a nutrigenomic -/- mice were fed with twoba-/- or LXRb-/-, LXRaapproach. Wild-type, LXR distinct diets for seven weeks. A standard diet was used as a control and a western diet (high-fat and high-cholesterol) was used to challenge LXR signaling.

Results: Changes in hepatic cholesterol and fatty acid composition were then measured through lipidomic analysis. Genome-wide microarray screenings of liver samples and complementary QPCR assays were performed on the same liver samples.

Conclusions: Using bioinformatic and statistical tools, including functional categories tests, we described biological pathways sensitive to the expression of individual LXR isoforms in the liver.

P150

Chronic hyperglycaemia impairs metabolic switching of human myotubes

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Objective: Skeletal muscle of insulin resistant individuals is characterized by lower fasting lipid oxidation and reduced ability to switch between lipid and glucose oxidation. The purpose of the present study was to examine if impaired metabolic switching could be induced by chronic hyperglycaemia.

Procedure: Human myotubes were treated with or without chronic hyperglycaemia (20 mmol/l glucose for 4 days), and the metabolism of [¹⁴C]oleic acid (OA) and [¹⁴C]glucose was studied.

Results: Acute glucose (5 mmol/l) suppressed OA

oxidation by 50% in normoglycaemic (5.5 mmol/l glucose) cells. Myotubes exposed to chronic hyperglycaemia showed a significantly reduced OA uptake and oxidation to CO₂, whereas acid-soluble metabolites were increased. Glucose suppressibility, the ability of acute glucose to suppress lipid oxidation, was significantly reduced to 21%, while adaptability, the capacity to increase lipid oxidation with increasing fatty acid availability, was unaffected. Glucose uptake and oxidation was significantly reduced by about 40%. Substrate oxidation in presence of mitochondrial uncouplers such as carbonylcyanide-4-trifluoromethoxyphenylhydrazone (FCCP) showed that net and maximal oxidative capacities were significantly reduced after hyperglycaemia, and the concentration of ATP was reduced by 25%. However, none of the measured mitochondrial genes were downregulated nor was mitochondrial content. Microarray showed that no genes were significantly regulated by chronic hyperglycaemia. Addition of chronic lactate reduced both glucose and OA oxidation to the same extent as hyperglycaemia, and this effect was specific for lactate.

Conclusion: Chronic hyperglycaemia reduces substrate oxidation and impairs metabolic switching of myotubes from healthy individuals. The effect is most likely due to an induced mitochondrial dysfunction. This shows that chronic hyperglycemia may induce a “diabetic phenotype” in former healthy cells.

P151

Effects of a n-3 fatty acid deficient diet on synaptic plasticity gene expression and spatial memory processes in rats

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Background: N-3 fatty acids (FA), particularly 22:6n-3 (DHA), are major components of brain membranes. Decreases in brain DHA are associated with functional alterations suggesting the involvement of brain DHA status in the regulation of cognitive functions. Vitamin A via its nuclear receptors (RAR and RXR) plays a central role in the maintenance of learning and memory processes by controlling the expression of numerous genes involved in synaptic plasticity. Vitamin A and n-3 FA can interact (at least in part) via their respective nuclear receptors, RXR and PPAR.

Objective: The present work deals with the hypothesis that a n-3 FA deficient intake may modulate spatial memory performances by altering the expression of synaptic plasticity genes.

Procedures: Male weaned Wistar rats received ad lib

either control diet (peanut and rapeseed oils, 50/50, w/w) or n-3 FA deficient diet (100% peanut oil) for 18 weeks. Experiment 1: quantitative RT-PCR for the quantification of the mRNA amount of PPAR, RAR, RXR, and synaptic plasticity genes (PSD-95, synaptophysin, CaMKII, NGF, BDNF) was performed from striatum and hippocampus of control and deficient animals. Experiment 2: after 17 weeks of feeding, the anxiety-like behavior of rats of both dietary groups was evaluated using the elevated plus maze test. Flexibility capacities were tested after 18 weeks of feeding using the Morris water maze.

Results: In deficient animals, the preliminary results report (i) a significantly ($p < 0.05$) decreased hippocampal mRNA expression of RXR γ , BDNF and CaMKII (-23, -15 and -10%, respectively); (ii) a higher level of anxiety-like behavior compared to controls (expressed as percentage of time spent in open arms (-52%, $p = 0.05$)); (iii) decreased capacities of flexible use of spatial representation.

Conclusion: These results show that a n-3 FA deficient intake may induce changes in memory performances by modulating the expression of synaptic plasticity genes and increasing the anxiety-like behavior.

P152

Interrelations between platelet activating factor and its metabolic enzymes in apparently healthy volunteers

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Background: Platelet activating factor (PAF), a potent inflammatory lipid mediator is synthesized via the remodeling and de novo route, key enzymes of which are Lyso-PAF acetyltransferase (Lyso-PAF-AT) and DTT-insensitive CDP-choline:1-alkyl-2-acetyl-sn-glycerol-cholinephosphotransferase (PAF-CPT), respectively. The remodeling route is thought to be activated under inflammatory conditions whereas the de novo route synthesizes PAF at basal levels. PAF-acetylhydrolase (PAF-AH) and lipoprotein-associated phospholipase-A₂ (Lp-PLA₂) are the main intracellular and extracellular catabolic enzymes of PAF, respectively.

Objective: The measurement of the aforementioned enzyme activities, along with PAF, in blood and leukocytes of healthy volunteers and the investigation of their interrelations.

Procedure: Apparently healthy volunteers (n=106) were recruited. Lyso-PAF-AT, PAF-CPT and PAF-AH were determined in homogenates of isolated leukocytes, Lp-PLA₂ in serum and PAF in blood according to published methods.

Results: PAF levels were 0.54 ± 1.01 pmol/mL of blood. Activities of Lyso-PAF-AT, PAF-CPT, PAF-AH and Lp-PLA₂ and were 8187 ± 5441 , 128 ± 83 , 334 ± 118 pmol/min/mg protein and 22.09 ± 5.37 nmol/min/mL serum, respectively. Principal Component Analysis revealed two PAF metabolic patterns: “increased biosynthetic activity” and “increased catabolic activity”. Indeed, Lyso-PAF-AT and PAF-CPT ($\rho=0.295$, $P=0.003$) as well as Lp-PLA₂ and PAF-AH ($\rho=0.197$, $P=0.057$) were positively correlated independently of age and BMI. In men, PAF-CPT together with age predicted PAF ($R^2=21.8\%$), whereas BMI was not a significant predictor. Moreover, in men PAF-CPT predicted Lyso-PAF-AT ($R^2=14.2\%$) irrespectively of PAF/BMI/age, which were not significant. PAF and Lyso-PAF-AT, either alone or together in the same model, predicted PAF-CPT in age/BMI adjusted linear regression models ($R^2=22.5\%$). No relation was documented in women.

Conclusion: The activities of PAF’s metabolic enzymes are interrelated, implying that (i) PAF’s biosynthetic routes are not independent and (ii) Lp-PLA₂ and PAF-AH may be influenced by similar stimuli. PAF-CPT is the strongest determinant of circulating PAF levels in men. These interrelations should be verified in other populations and pathophysiological conditions.

P153

Changes in liver fatty acid binding protein expression modifies fatty acid metabolism, growing and differentiation of Caco-2 cells

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Background: The intestinal epithelium assimilates and processes a large quantity of lipids incorporated with the diet. They are absorbed mainly as free fatty acids (FA) and sterols, and as monoacylglycerols; to be then reesterified inside the enterocytes, assembled and secreted as Quilomicrons into the lymph. This type of cells express large quantities of two Fatty Acid Binding Proteins (FABP): intestinal FABP (IFABP) and liver FABP (LFABP), which are suggested to be cytosolic buffers and/or transporters for hydrophobic ligands. Nevertheless, new functions have been recently proposed related to control of gene expression and cell differentiation.

Objective: To evaluate LFABP’s role in the lipid metabolism and cell biology of the enterocyte, employing a cellular model of Caco-2 cells with ablated expression of LFABP.

Procedure: We obtained an LFABP knock-down model in Caco-2 cells by anti-mRNA overexpression. We analyzed the effect of this change in expression on the assimilation, metabolism and secretion of FA, as well as on the cellular proliferation and differentiation processes.

Results: Several clones were isolated with less expression of LFABP compared to the control cells. No compensation by IFABP was observed in differentiated cells. Knock-down clones showed a marked decrease in oleate assimilation, while palmitate assimilation was increased. Differences in oleate distribution were observed at short times, but they disappeared after hours of incubation and no change in its distribution was observed in secreted lipids. On the other hand, cell proliferation and differentiation were slowed by the anti-sense clones compared to the control cells.

Conclusions: Our results suggest that intestinal FABP would facilitate the intracellular transport of FA and that they could also have a role in the regulation of lipid metabolism and other cellular processes. The results presented indicate that LFABP is an important factor for the proper function of the enterocytes.

P154

Changes in membrane fatty acid composition modulate insulin response in HT29 and HepG2 cells

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Background: HT29 and HepG2 cells are commonly used to assess the insulin response in human cells. Insulin operates by binding to a transmembrane receptor and initiating a cascade of phosphorylations, which lead to the activation/phosphorylation of the serine/threonine protein kinase B (PKB/AKT). Defects in insulin signalling pathway are known to be responsible for impaired glucose uptake, insulin resistance and type II diabetes. Although, the cause of these abnormalities has not yet been elucidated, a perturbation of membrane fatty acids composition has been implicated.

Objective: To investigate (1) the compositional changes in the phospholipids fatty acids (FA) in HT29 and HepG2 cells after incubation with alpha-linolenic (ALA), eicosapentaenoic (EPA), docosahexaenoic (DHA), linoleic (LA), and arachidonic (AA) acids and (2) the effect of FA on the activation/phosphorylation of the AKT protein.

Procedure: HT29 and HepG2 cells were incubated in growth medium with or without $40\mu\text{M}$ of LA, ALA, AA, EPA or DHA. After 48 hours incubation, cells were collected and analysed for fatty acid composition of choline- (CPG) and ethanolamine- (EPG) phosphoglycerides. In a parallel experiment, FA treated and untreated cells were stimulated with 50ng/ml of insulin for 30 minutes and the expression of phospho(Ser473)-AKT, and AKT were quantified by western blot.

Results: After ALA treatments, the incorporation of ALA was greater in HT29 (2-fold in CPG and 4-fold in EPG) than HepG2 cells. DHA was preferentially incorporated into the EPG of HepG2 cells, whereas it was AA that was predominantly incorporated in the EPG of HT29 cells. In HT29 cells, insulin incubation showed a 1.5-fold increase versus a 2-fold increase in cells pre-treated with LA or AA compared to control. The HepG2 cells showed a similar trend as HT29.

Conclusion: These data suggest that increased level of omega-6 fatty acids in the cell membrane enhance the activation of the AKT protein.

P155

The majority of polyunsaturated fatty acids correlate between blood samples taken from the fingertip compared with venous collection

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Background: Blood fatty acids are often used to assess fatty acids status of individuals. However, there is no consensus on which blood lipid analysis should be used to answer specific research objectives. Moreover, conventional fatty acid analysis is laborious and difficult for use in large epidemiological studies. The development of direct methylation of capillary blood seems to be useful in this aspect.

Aim: To compare the information gained from fingertip analysis of whole blood (direct methylation of capillary blood) with the separated lipid fractions from plasma and erythrocytes (extraction of total lipids, separation and analysis of individual lipid fractions) and venous whole blood.

Methods: Non-fasting venous blood was taken from ten healthy volunteers into EDTA tubes and a drop of capillary blood from the fingertip was collected on filter paper strips (Fluka 11312). The following fractions were analysed: (a) plasma total lipids, plasma phosphatidylethanolamine (pPE), phosphatidylcholine (pPC), triacylglycerols, cholesterol esters (pCE), (b) erythrocyte total lipids (eTL), phosphatidylethanolamine (ePE), phosphatidylcholine (ePC), and (c) total lipids of whole blood. Correlations between parameters were estimated by computing the Spearman correlation coefficient.

Results: There was a strong correlation between whole blood and fingertip analysis. Eicosapentaenoic, docosahexaenoic, linoleic and arachidonic acids were highly correlated with the fingertip, whole blood and all blood fractions analysed. However, alpha-linolenic acid showed no correlation between dried finger tip blood and the following lipid classes: pPC, pCE, eTL, ePE and ePC.

Conclusion: Fatty acids determined by direct methylation of finger tip blood reflected the fatty acid status of plasma and erythrocyte lipid fractions for some of the $\omega 3$ and $\omega 6$ fatty acids, but not all. Careful consideration must be taken before deciding which method to use in order to answer research objects where there may, for example, be specific questions regarding fatty acid and membrane function.

Lipids and Nutrition

P156

The analysis of the effect of fish oil capsules intake on endocannabinoid levels using liquid chromatography - tandem mass spectrometry

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Background: Consumption of docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) or fatty fish containing high levels of Omega-3 polyunsaturated fatty acids have been shown to reduce the inflammatory component and improve other characteristics of the metabolic syndrome. The exact mechanisms behind these effects are unknown, but evidence suggests that DHA and EPA can be converted to anti-inflammatory eicosanoids (e.g. resolvins). Another possibility is that their anti-inflammatory properties are due to an interaction with the endocannabinoid system. Fatty acids are precursors for N-acyl ethanolamines, a class including anandamide (AEA), with known anti-inflammatory properties. The local availability of a specific type of fatty acid precursor may determine product formation and hence bio-activity. This suggests a link with dietary intake, since it is well known that incorporation of fatty acids into membranes can be modulated by their proportional abundance in the diet.

Objective: Determine the effect of the daily intake of supplementary fish oil capsules on the level of endocannabinoids in human plasma using an endocannabinoid and related fatty acid acylamides platform.

Procedure: In a pilot study two non fish oil users consumed during one month 2 fish oil capsules per day and one frequent fish oil consumer stopped using fish oil capsules. The endocannabinoid levels were monitored in plasma during this time path. The endocannabinoids were analyzed using a reversed phase LC-MS/MS method after sample clean-up and concentration using C8 solid phase extraction.

Results and Conclusion: The results show that the short term daily fish oil intake has a minimal effect on the formation of docosahexaenoyl ethanolamine (DHEA) and no effect on the formation of eicosapentaenoyl ethanolamine (EPEA). However, when daily use of fish oil capsules by frequent users was terminated, a clear reduction in plasma DHEA levels was observed. Again no effect on EPEA was detected.

P157

Butterfat improves glucose tolerance compared to MUFA- and PUFA-based diets in obese mice

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Background: Despite high amounts of saturated fatty acids in milk, studies indicate that dairy fat might have beneficial effects on metabolic function. Such effects might be explained by the presence of bioactive fatty acids with agonist activity on nuclear-receptors. Phytanic acid (PA), is one such fatty acid. PA has reported agonist activity for retinoid-x-receptor (RXR) and peroxisome-proliferator-activated-receptor- α (PPAR α), and have been suggested to prevent development of diabetes. Since PA is formed from chlorophyll catabolism in the rumen, green plant material will cause PA accumulation in milk.

Objective: To determine if butter from cows fed green plant-material and butter enriched in PA, improves glucose-tolerance and fasting insulin levels in obese mice.

Procedure: Male C57bl/6J mice were fed 60 E% fat and 15% sucrose in drinking-water, to induce obesity for 10 weeks. Three groups were fed butterfat derived from cows being fed increasing amounts of green plant-material and control groups were given either high amount of PUFA or MUFA.

A dose-response study on PA was performed by adding PA ethyl ester to a butter low in PA, resulting in concentrations from 0.11 - 1.11 mass%.

Oral Glucose tolerance test was performed after the 10 weeks intervention and the animals were sacrificed two days after the OGTT.

Results: All groups fed butter had better glucose tolerance than control groups, although statistical significance was only reached between the MUFA-control and the group fed butter from 100% grazing cows. There was no effect of increasing PA from 0.11 to 0.61%, while 1.11% PA reduced glucose tolerance. Both groups given butter from green-fed cattle had lower weight gain than other obese groups. Results from other markers of metabolic dysfunctions will also be presented.

P158

Fat and fatty acids in colostrum, transitional, and mature milk from women delivering very preterm, preterm, and term infants

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Background: Fat and fatty acids proportions in human milk change according to gestational age and stage of lactation. Preterm infants fed banked human milk, however, often receive pooled mature milk obtained from women delivering at term. These practices may lead to an unpredictable and maybe not optimal composition of the milk provided to these newborns.

Objective: To study the changes in fat content and fatty acids proportions throughout lactation in very preterm (VPT), preterm (PT), and full term (FT) milk, and assess the differences among gestational age groups.

Procedure: Participants were women delivering before 30 (n=10), between 30 and 37 (n=10), and between 38 and 42 (n=23) weeks of gestation. They provided colostrum, transitional, and mature milk (between 2-4, 8-12, and 28-32 days post-partum, respectively). Fat content was estimated by the creatinocrit technique. Fatty acids, including the two main isomers of CLA, were analyzed by fast-gas chromatography. For each gestational age group, changes throughout lactation were assessed by a repeated-measures analysis of variance. Gestational age groups were compared using a one-way ANOVA with Bonferroni post-hoc test.

Results: Fat content of VPT colostrum and mature milk was significantly higher than in PT or FT samples. In the three gestational age groups, medium-chain saturated fatty acids, linolenic acid, and c9,t11-CLA proportions increased during lactation, while those of most longer-chain saturated fatty acids and long-chain polyunsaturated fatty acids decreased. Regarding differences with gestational age, medium-chain saturated fatty acid proportions were highest in VPT colostrum, while those of arachidonic and docosahexaenoic acids were lowest. Most of the differences found between colostrums at different gestational ages disappeared when comparing mature milks.

Conclusions: The particular composition of milk obtained at different gestational ages and stages of lactation should be taken into account when feeding newborns banked human milk.

P159

The effect of acylation stimulating protein antibody neutralization and supplementation on energy expenditure in wildtype mice

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Background: Acylation stimulating protein (ASP) is an adipogenic hormone that stimulates triglyceride (TG) synthesis and glucose transport in adipocytes. Previous studies have shown that ASP-deficient C3 knockout mice are hyperphagic yet lean, as they display increased oxygen consumption and fatty acid oxidation compared to wildtype mice.

Objectives: The present study aims to investigate the effects of ASP neutralization and supplementation in WT mice following initiation of a high-fat diet.

Procedures: Antibodies against ASP (Anti-ASP) and human recombinant ASP (rASP) were tested in vitro and in vivo. Continuous administration for 28 days via osmotic mini-pump of Anti-ASP or rASP was evaluated in wildtype mice on a high-fat diet (HFD) to examine their effects on body weight, food intake and energy expenditure.

Results: In mature murine adipocytes, rASP significantly stimulated fatty acid uptake (+243% vs PBS, $P < 0.05$) while Anti-ASP neutralized the rASP response. Mice treated with Anti-ASP showed elevated energy expenditure ($P < 0.0001$), increased skeletal muscle glucose oxidation (+141%, $P < 0.001$), reduced liver glycogen (-34%, $P < 0.05$) and glucose-6-phosphate content (-64%, $P = 0.08$) compared to control mice. There was no change in body weight, food intake, fasting insulin, adiponectin, CRP or TG levels compared to controls. Interestingly, HFD mice treated with rASP showed the opposite phenotype with reduced energy expenditure ($P < 0.0001$) and increased body weight ($P < 0.05$), cumulative food intake ($P < 0.0001$) and liver glycogen content (+59%, $P < 0.05$). Again, there was no change in circulating insulin, adiponectin, CRP or TG levels, however, plasma free fatty acids were reduced (-48%, $P < 0.05$).

Conclusion: In vitro, Anti-ASP effectively neutralized ASP stimulated fatty acid uptake. In vivo, Anti-ASP treatment increased whole body energy utilization while rASP increased energy storage. Therefore, ASP is a potent anabolic hormone that may also be a mediator of energy expenditure.

P160

Effect of maternal omega-3 LC-PUFA supplementation: A systematic review of clinical trials and animal studies

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Background: Omega-3 long-chain polyunsaturated fatty acids (LCPUFA) have been shown to inhibit fat cell differentiation and fat storage in adults, and this has led to the hypothesis that perinatal exposure to increased omega-3 LCPUFA concentrations will limit fat deposition in early life and reduce the subsequent risk of obesity.

Objective: To undertake a systematic review of the effect of omega-3 LCPUFA supplementation in pregnancy and lactation on postnatal body composition in randomised controlled trials and experimental animal studies.

Procedure: The Medline and Embase databases were searched for relevant articles. Outcome measures were percentage body fat or fat pad weight (animal studies) and BMI, BMI z-score or percentage body fat (human studies). Human trials had to be randomised in design. The quality of all included studies was assessed against set criteria and results of eligible trials compared.

Results: Only 4 human trials and 4 animal studies met our inclusion criteria, and considerable disparity existed in the nature and timing of the intervention, methods used for the assessment of body composition and trial quality. All human studies suffered from high attrition rates (<50%), and it was unclear whether there was adequate blinding of outcome assessments. Results were inconsistent between trials and difficult to interpret. The results from the animal studies all reported a decrease in body fat mass in omega-3 supplemented groups, however only one study restricted the intervention to the perinatal period.

Conclusion: This review highlights the need for high quality studies to definitively establish whether maternal omega-3 LCPUFA supplementation can reduce body fat mass in the offspring. There is a need for animal studies in which increased omega-3 LCPUFA exposure is confined to the perinatal period, and human clinical trials which are sufficiently powered, have appropriate placebo controls, adequate blinding of participants and investigators and high retention rates.

P161

IFOS: effects of high-dose fish oil supplementation on term infant neurodevelopment

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Background: The functional implications of omega-3 LCPUFA for term infants remains unclear, perhaps due to a limited number of neurodevelopmental measures undertaken, and an insufficient dose.

Objective: To assess whether high-dose omega-3 LCPUFA improves neurodevelopment according to a number of standardized clinical measurements.

Procedure: Participants were recruited at 36 weeks and randomized to receive fish oil (FO) (280mg DHA, 270mg EPA) (n=210) or placebo (olive oil - OO) (n=210). Supplementation occurred from birth to six months of age, with compliance measured via analysis of red blood cells (RBC) in both cord blood and at cessation of supplementation. Neurodevelopment assessment occurred at 18 months via the Bayley Scales of Infant Toddler Development (BSID) (n=248) and the Child Behaviour Checklist (CBCL) (n=239). Full data is expected by May 2010.

Results: Preliminary results revealed supplementation resulted in significantly higher RBC DHA for the FO group ($p < .05$). For the BSID measures, there were no significant differences between the supplementation groups. A sub-group analysis revealed that boys in the fish oil group had significant higher fine motor standard scores compared to the olive oil group ($p = .015$) (controlling for compliance, maternal education and SES status). For the CBCL, there were no significant differences between the FO and OO group, however higher cord blood DHA levels were predictive of lower levels of T scores for sleep problems (n=65, $p = .007$), and a further sub-group analysis showed that this trend was significant for girls only (n=30, $p = .015$).

Conclusion: The results indicate that there was a significant benefit of high-dose fish oil supplementation for the development of fine motor skills in boys only. Additionally, it appears that high levels of DHA in utero are predictive of superior sleeping patterns.

P162

Dietary supplement mix reduces cardiovascular risk factors and atherosclerosis in humanized mouse models

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Background: Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality in the Western world, despite successful cholesterol-lowering therapy by statins. The most effective way of reducing CVD is to prevent disease development by action on the primary risk factors for CVD. Among these risk factors is unhealthy diet through undesirable effects on blood lipids and inflammation. There are indications that specific nutritional components can counteract the adverse

effects of unhealthy diet and thereby dampen the development of atherosclerosis.

Research question: Can a well-defined dietary supplement mix, containing high amounts of antioxidant polyphenols, vitamins, long-chain unsaturated fatty acids and carotenoid reduce major atherosclerotic risk factors, viz. plasma lipid levels and inflammation markers in humanized mouse models?

Procedure and Results: The nutritional mix was tested in humanized mouse models of inflammation (huCRP mice) and of atherosclerosis (ApoE*3Leiden mice). First, we evaluated anti-inflammatory potency of the nutritional mix in transgenic mice expressing the inflammatory marker, human C-reactive protein. We found that treatment with the nutritional mix did not affect basal CRP expression but strongly quenched CRP levels after a challenge with IL-18. Next, we evaluated the potential lipid lowering and anti-atherogenic potency of the nutritional mix in ApoE*3Leiden mice fed a western-type diet containing 1% cholesterol for 16 weeks. Although food intake was similar between groups, supplementation of the nutritional mix resulted in a significant increase in body weight compared to control. Treatment with nutritional mix strongly decreased plasma cholesterol and triglyceride levels and improved liver health of ApoE*3Leiden mice. Analysis of atherosclerosis showed that supplementation of the nutritional mix strongly reduced plaque formation compared to control.

Conclusion: A well-defined dietary supplement mix containing antioxidant polyphenols, vitamins and polyunsaturated fatty acids reduces cardiovascular risk factors, improves liver health and strongly reduce atherosclerotic plaque formation in humanized mouse models under health-challenging conditions.

P163

Fish-oil supplementation of maternal rats prevents depletion of maternal brain regional docosahexaenoic acid levels and has a postpartum anxiolytic effect

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Background: Docosahexaenoic acid (DHA) and arachidonic acid (AA) are the major polyunsaturated fatty acids in the neuronal membrane. Most DHA and AA accumulation in brain occurs during the perinatal period and is supplied via the placenta and breast milk.

Objective: This study was to examine whether maternal brain and retinal levels of DHA and AA are depleted during pregnancy and lactation due to meeting the high demand of the developing nervous system in the offspring and to evaluate the effects of the reproductive cycle on serotonin metabolism and of fish oil on postpartum anxiety.

Procedure: Pregnant rats were fed a sunflower oil-based (n-3) fatty acid-deficient diet with or without fish oil supplementation during pregnancy and lactation and age-matched virgin rats were fed the same diets.

Results: The DHA depletion in the postpartum rats compared to the virgin females was seen in the hypothalamus, hippocampus, frontal cortex, cerebellum, olfactory bulb and retina. Maternal brain AA depletion was seen in the hypothalamus, hippocampus and frontal cortex but not in the other examined brain regions and retina. Serotonin levels were decreased and its turnover increased in the brainstem and frontal cortex in postpartum rats compared to virgin rats. Fish oil supplementation during pregnancy and lactation prevented the decrease in maternal brain regional DHA levels, inhibited monoamine oxidase-A activity in the brainstem, and decreased anxiety-like behavior.

Conclusion: We propose the reproductive cycle depletes maternal brain DHA levels and modulates maternal brain serotonin metabolism to cause postpartum anxiety and suggest that fish oil supplementation may be benefit for postpartum anxiety.

P164

Effect of myristic acid and other fatty acids on dihydroceramide $\Delta 4$ -desaturase in rat hepatocytes

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Background: Dihydroceramide D4-desaturase (DES) is the enzyme which catalyses the desaturation of dihydroceramide into ceramide (the common base of all sphingolipids). We have recently shown that recombinant DES is myristoylated and that myristic acid (C14:0) increases its activity via the myristoylation. Moreover, myristoylation of DES was responsible for its subcellular targeting to the mitochondria and for an induced apoptosis probably by increasing the quantity of intracellular ceramide.

Objective: We wanted to confirm the myristoylation of native DES and its activation by myristic acid in primary cultured rat hepatocytes. We also investigated the effect of other saturated (lauric and palmitic acids) and unsaturated (oleic, linoleic, α -linolenic, arachidonic and docosahexaenoic acids) fatty acids compared with myristic acid on DES activity and apoptosis.

Procedure: Cultured rat hepatocytes were incubated for 36h with saturated or unsaturated fatty acids and DES activities were measured. Apoptosis was determined by measuring the caspase activity and by counting cells with apoptotic nuclei. DES mRNA levels were measured by RT-PCRq.

Results: First, the results showed an increasing effect of myristic acid on DES activity in hepatocytes, compared with control cells. This effect was specific of myristic acid

because lauric and palmitic acids did not modify DES activity. Secondly, linoleic, α -linolenic and arachidonic acids inhibited DES activity and the degree of inhibition was correlated to the numbers of insaturation. DES mRNA level was not modified by these treatments. Further studies are in progress to determine the effect of these fatty acids on apoptosis.

Conclusion: This study suggests that myristic acid is the single fatty acid able to upregulate DES activity and that some unsaturated fatty acids inhibit its activity. Therefore, these results show the importance of fatty acids, and especially myristic acid, in the regulation of ceramide biosynthesis and their potential effect on its induction of apoptosis.

P165

Blood levels of n-3 long chain polyunsaturated fatty acids differ significantly in children from 8 European countries: the IDEFICS study

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Background: The European project IDEFICS (Identification and Prevention of Dietary and Lifestyle Induced Health Effects in Children and Infants) is based on a multicenter survey design of a population-based cohort of children (3-10 years) in different countries (Southern Italy, Estonia, Cyprus, Belgium, Sweden, Germany, Hungary, Spain), aimed at interventions in pre- and primary school settings. It explores biological markers, physiological regulation, selected genetic markers and early outcome measures.

Objective: To provide data on fatty acid (FA) profiles of blood lipids in children from the different countries, in order to evidentiare possible cross-country differences. The assessment of the FA status provides background information on the correlations between circulating FA and dietary habits.

Method: FA profiles in children from the above countries have been assessed by gas chromatography in a drop of

blood collected from a fingertip on a kit and shipped to our laboratory.

Results: Minimal differences were present for Saturated (SFA), Mono Unsaturated Fatty Acids (MUFA), and Poly Unsaturated FA of the n-6 series (linoleic and arachidonic acid), while major differences were observed for the n-3 fatty acids, 18:3 (α -linolenic acid, ALA), 20:5 (eicosapentaenoic acid, EPA) and 22:6 (docosahexaenoic acid, DHA). The highest DHA levels were found in children from Italy and Spain and the lowest in Hungary, while levels of EPA were highest in Estonia and Sweden, associated with higher levels of ALA, and the lowest in Italy and Spain.

Conclusions: The data indicate major selected differences in blood n-3 FA, supplied in very low amounts in most diets. This appears to reflect between countries differences, already described in the literature, in the intakes of preformed DHA or of precursor (ALA). The persistence of diversified blood n-3 PUFA levels, beyond childhood, in different countries may be partly responsible of different incidence of chronic diseases at later ages.

P166

N-3 fatty acids influence oxidative stress status in Atlantic salmon adipocytes

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Background: Regulation of oxidative stress (OS) in adipocytes is an important mediator of their development and dysfunction. n-3 highly unsaturated fatty acids (HUFAs) play essential roles in marine fish, where they have anti-lipogenic effects, but they are prone to peroxidation.

Objective: The aim of this study was to investigate how the effects of HUFAs in fish adipocytes are modulated by changes in their intracellular redox status.

Procedure: Adipocytes from Atlantic salmon were cultivated on HUFA-rich media and treated with buthionine sulfoximine (BSO) that depleted stores of the antioxidant glutathione (GSH) and exacerbated OS and α -tocopherol (α -TOCH) that protected cells from OS. Gene expression was assessed by qPCR. In addition, phospholipid composition, total fatty acid (FA) composition, TBARS and superoxide dismutase (SOD) activity were determined.

Results: Lipid accumulation and the profile of adipogenic markers were lower in GSH depleted cells than in α -TOCH supplemented cells. Large intracellular vesicles were prominent in the BSO and control groups. α -TOCH had anti-apoptotic and anti-inflammatory effects and induced the expression of activating transcription factor 6, a marker of ER-stress. Depletion of GSH was associated with the highest activity of SOD and the highest levels of

TBARS. α -TOCH mediated reduction of the OS in the GSH depleted cells, independent of glutathione peroxidases and the antioxidant SOD.

Conclusions: α -TOCH is strongly pro-adipogenic while OS induced by HUFAs and BSO have anti-adipogenic effects. Transcriptional modulation of the caspase 3-independent apoptosis in salmon adipocytes is sensitive to OS.

P167

Infant AA at delivery is independent of maternal AA status, while DHA biomagnification occurs only at low maternal DHA status

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Background: The long-chain polyunsaturated fatty acids (LCP) docosahexaenoic (DHA) and arachidonic (AA) acids are important for growth and development. The courses of maternal LCP in pregnancy and lactation in Western countries, and the higher LCP in infant serum lipids at birth compared with maternal lipids (biomagnification), suggest maternal depletion.

Objective: We studied maternal and infant DHA and AA status throughout pregnancy until 3 months postpartum in 3 populations in Tanzania with different fish intakes: Maasai (no fish), Pare (low freshwater fish) and inhabitants of Sengerema (daily freshwater fish).

Procedure: Erythrocyte (RBC) DHA and AA (in g%) were determined in mothers throughout pregnancy (n=209), mother-infant pairs at delivery (n=63), and mother-infant pairs after 3 months exclusive breastfeeding (n=104).

Results: Maternal RBC-AA was lowest in Sengerema and highest in Maasai, and showed a decreasing trend throughout pregnancy. RBC-AA of their infants were nevertheless comparable at delivery (Maasai 16.58, Pare 16.18, Sengerema 16.20). Infant RBC-AA at delivery was higher than maternal RBC-AA, but decreased thereafter to reach comparable values in all 3 groups. Maternal RBC-AA at 3 months postpartum was higher than at delivery. Maternal RBC-DHA at delivery (Maasai 3.6, Pare 4.7, Sengerema 7.2) related to fish intake and was lower than infant RBC-DHA, except for Sengerema mother-infant pairs. DHA biomagnification occurred at maternal RBC-DHA up to about 5.6 g%. Higher maternal RBC-DHA related to bioattenuation. Maternal RBC-DHA decreased during lactation. Infants exhibited increasing RBC-DHA during lactation if their RBC-DHA at delivery exceeded 5.9 g%, which corresponded with 6.3 g% maternal RBC-DHA at delivery.

Conclusion: Infant AA status at delivery and during lactation seems independent of maternal AA status. DHA biomagnification is confined to maternal RBC-DHA at delivery below 6 g%. A stable postpartum infant DHA status is reached when maternal and infant RBC-DHA at delivery amounts to 6 g%.

P168

Influence of droplet size and surfactants on the breakdown kinetics of emulsions in an *in vitro* digestion model

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Background: Obesity is rapidly developing into a threat of epidemic proportions. Therefore, the food industry is challenged to develop foods that do not only taste good but also regulate or limit food intake. However, little progress is achieved so far and fundamental knowledge on the gastro-intestinal behavior of emulsion systems is needed.

Objective: Lipolysis and lipid digestion products are examined in detail. The rate and extend of fatty acid released is studied in an *in vitro* digestive system.

Procedure Oil-in-water emulsions were prepared (varying droplet sizes, surfactant - whey protein isolate or gum Arabic) and triolein was used as the oilphase. The emulsion breakdown was characterized under constant pH-conditions in an artificial intestinal fluid. The initial production of fatty acids (FFA) was calculated. Lipid digestion products were studied using a GC method.

Results: The initial production of FFA (mol/min) increased with a higher available area but reached a plateau after certain area levels. To investigate if the amount of enzyme was the limiting factor at these high area levels we tested various amounts of enzyme but neither an increase nor a decrease of this plateau could be obtained. When comparing both emulsions under same assay conditions the initial slope was higher in gum Arabic stabilized emulsions compared to whey protein isolate (WPI) stabilized emulsions.

Conclusion: The rate of lipid digestion depends on the droplet size: it increases with higher available area but reaches a plateau after certain area levels. This plateau is not caused by limiting amounts of enzyme. Emulsions stabilized by gum Arabic are more easily digested than emulsions stabilized by WPI. WPI may form a more compact adsorbed layer which is less penetrable for lipase.

P169

Distribution of long-chain omega-3 fatty acids among cardiac lipid classes and sub-classes of individual phospholipids of Greenland Inuit

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Background: Higher levels of the long-chain omega-3 fatty acids in heart tissue lipid as a result of seafood consumption have been implicated in anti-arrhythmic effects and reduced risks of sudden cardiac death.

Objective: To date, no detailed reports have been published on the distributions of the individual long-chain omega-3 fatty acids among the various lipid types and sub-classes of individual phospholipids. Since the hearts of the Greenland Inuit are highly enriched in EPA (eicosapentaenoic acid, 20:5 n-3), DPA (docosapentaenoic acid, 22:5 n-3), and DHA (docosahexaenoic acid, 22:6 n-3), the absolute amounts of these omega-3 in the various lipid classes (total, TG, PL, FFA, PC, PE, PS, PI, SPH, DPG, LPC) and sub-classes (diacyl, alkenylacyl, alkylacyl) of PC and PE were measured.

Procedure: Total lipid extracts were prepared from the hearts of 9 Greenland Inuit followed by quantification of the EPA/DPA/DHA levels in the lipid classes by appropriate chemical and analytical procedures (TLC, GLC) using derivatives as needed.

Results: For all three omega-3 fatty acids, the absolute amounts (mg per 500 gm heart) were highest in the total PL with PE contributing more than PC and the diacyl species contributing moderately more than the alkenylacyl species. The relative distributions for each of the omega-3 fatty acids differed markedly.

Conclusion: These heterogeneous distributions of the omega-3 fatty acids among the individual lipid types could underlie their respective contributions to cardioprotection.

P170

Specific interest of human milk phospholipids compared to other animal species through ³¹P NMR analysis

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Background: Dietary phospholipids (PL), mainly provided by human milk, are nutrients of potential high interest for newborn development, especially for brain (plasmalogen, PL-enriched in DHA) but also intestine (sphingomyelin).

Objectives: Current analytical methods fail to give a complete quantitative and qualitative profile of PL species in milk. Considering the relative low PL content in milk, we aimed to set-up a quantitative and sensitive

method allowing to differentiate PL species using a procedure avoiding the loss of any PL.

Procedure: Total lipids were extracted from different samples of milk from human (n = 23), cow (n = 15), camel (n = 8), and mare (n = 8) by Folch's modified method, and mixed with a deuterated solvent before ³¹P NMR acquisitions. Analyses were conducted on an Avance 400 Bruker spectrometer (9.4 T) using 5mm tubes and a multicore probe.

Results: Procedure was optimized for solvent proportion, pH and temperature to allow separation of a maximum of PL classes. Each kind of milk exhibited specific PL fingerprint. Median total PL content is variable according to animal species, being higher in camel milk (0.517 mM), and lower in mare milk (0.101 mM) than in human and cow milk (0.321 mM and 0.267 mM, respectively). Human and mare milk samples provide a broader variety of PL species, and human milk is specifically richer in sphingomyelin and plasmalogens compared to other milk species.

Conclusion: The proposed method of quantification exhibits a good sensitivity, associated with high resolution, easy sample preparation and minimal loss of target molecules. This optimized procedure is suitable to identify a large number of species, especially for plasmalogens. A better knowledge of milk phospholipid bioactive molecules composition could be useful to improve infant milk substitutes or fortifier formulation.

P171

Investigation of association of microsomal triglyceride transfer protein gene MTTP -164 T>C promoter polymorphism with metabolic syndrome variables

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Background and objective: the microsomal triglyceride transfer protein (MTTP) is required for the assembly and secretion of apolipoprotein B (apoB)-containing lipoproteins from the intestine and liver¹. Single nucleotide polymorphisms (SNPs) in the MTTP gene have shown associations with LDL-cholesterol and related traits of the metabolic syndrome. MTTP gene promoter SNP -164T>C (rs1800804) C-allele has been shown to associate with higher transcriptional activity in gene-reporter assays².

Procedure: we genotyped -164T>C in 466 healthy males and females in the RISCK Study, a randomised parallel controlled trial, which aims to test the impact of specific dietary changes on cardiovascular risk factors associated with the metabolic syndrome³. We tested association of genotype with mean quantitative metabolic phenotypes, LDL-C, HDL-C, TG, TC, apoB, NEFA and insulin sensitivity. Baseline measurements were taken after following a reference diet containing (38% energy (%E) fat, 18%E SFA,

10%E MUFA, 6%E PUFA) for one month.

Results: the numbers of each genotype were in Hardy-Weinberg proportions as follows: T/T 226, T/C 179, C/C 37; C-allele frequency = 0.29. No associations with mean quantitative metabolic phenotypes were found after adjustment for age, gender and BMI. A significant association was found with HDL-cholesterol in males, in which carriage of the variant C-allele was associated with lower fasting serum HDL-C (P = 0.013). We found no significant variation in fasting serum LDL-C with respect to genotype.

Conclusion: These findings suggest that the genetic variability at the MTTP -164 T>C locus may account for small inter-individual variation in fasting HDL-cholesterol concentration in obese/ overweight males. Males homozygous for the C-allele had significantly lower serum HDL-cholesterol compared to other genotypes, which may modulate risk of the metabolic syndrome

P172

Possible mechanisms of dietary fatty acid efflux in the human placenta: a mathematical-computational approach

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Background: DHA and AA are essential for neurodevelopment. Whilst AA is involved in cell division, cell signalling and inflammation, DHA is crucial for retinal development and accumulates in the fetal brain at 50-70mg/day in the last trimester of pregnancy. DHA and AA cannot be synthesised by the fetus and are obtained from the maternal blood supply through the syncytiotrophoblast layer of the placenta. The mechanism of this transfer is yet to be fully understood though it is known that these essential fatty acids are insoluble and therefore always associated with proteins such as integral membrane fatty acid transport proteins (FATP) and intracellular fatty acid binding proteins (FABP).

Objective: To explore the function of FABP1, 3, 4 and 5 and members of the FATP family in the placental syncytiotrophoblast.

Procedure: Partners of FATP5 and 6 and FABP were identified using the protein-protein interactions identified in the Human Protein Reference Database. A 'distance' matrix was generated with these partners, incorporating weighted multiplicities of paths (with a discounting factor gamma for each path length unit) between interacting protein partners. Hierarchical clustering was performed and results were displayed as dendrograms

Results: Initial results (using a gamma value of 1×10^{-4}) reveal four distinct FABP clusters. FABP3 forms a cluster with cardiac ankyrin repeat kinase and vacuolar sorting protein 28 homolog, both of which are expressed in the placenta. FABP5 interacts directly with calcium binding

protein A7. Interestingly FABP4 clusters with FATP6 and FABP1 with FATP5.

Conclusion: Results suggest distinct functions for FABP1, 3, 4 and 5 in the placental syncytiotrophoblast. FABP4 appears to be involved in efflux with FATP6 and FABP1 with FATP5. Overall this would suggest there are two pathways for fatty acid entry and exit in this particular transporting epithelium.

P173

On the evolutionary roots of fatty acid desaturase 1 and 2 polymorphisms

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Background: Delta-5 (FADS1) and delta-6 (FADS2) desaturases are key regulatory enzymes in LCP synthesis. They exhibit many SNPs, notably in non-coding regions, which are likely to affect transcription and translation. Carriers of FADS1 minor alleles have lower arachidonic acid status in Western countries. The high prevalence of SNPs in FADS1 and FADS2 suggest that their occurrence in evolution did not have profound consequences. This concurs with the much higher intakes of long chain polyunsaturated fatty acids from a Paleolithic diet, suggesting that the current influence of these SNPs is caused by the Westernization of our diet.

Objective: We explored the origin of the FADS1 and FADS2 minor alleles by comparing their abundances in Dutch women (n=112) with the abundances in Tanzanian women (n=175).

Procedure: SNPs rs 174545 (FADS1) and SNPs rs 174575 (FADS2) were genotyped using real-time PCR with commercially available kits for allelic discrimination.

Results. For the Dutch group we found: 48.2%, FADS1 homozygosity for the major allele (CC), 39.3%, heterozygosity (CG) and 12.5% homozygosity for the minor allele (GG). For FADS2 we found: CC 54.5%; CG 38.4%; GG 7.1%. For FADS1 in Tanzania we found: CC 96.6%, CG 3.4 % and GG 0%, and for FADS2: CC 59.4 %, CG 33.7 % and GG 6.9 %. All genotype distributions proved in Hardy-Weinberg equilibrium. The FADS1 genotype distribution in Tanzania was significantly different from The Netherlands ($p < 0.0001$).

Conclusion: The FADS1 minor allele frequency in Tanzania is remarkably low. It suggests origination and conservation by positive selection after our 'Out-of-Africa movement', beginning some 100,000 years ago, or its disappearance in Africa by negative selection. Data from more indigenous populations in the world are needed to comprehend the evolutionary roots of FADS polymorphism.

P174

Docosahexaenoic acid in plasma of very preterm infants: impact of an early supplementation of the mother during lactation

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Background: Very preterm infants are vulnerable to deficiency in docosahexaenoic acid (DHA), with potential negative consequences on their neurodevelopment.

Objective: In DHA supplemented mothers, evaluate extend of the increase in DHA intake and DHA status in very preterm infants receiving their breast milk.

Procedure: Ten mothers who delivered prematurely (≤ 29 week gestation) and planning to breastfeed received a DHA supplement (1.2g/d) until 36 weeks after conception. DHA intakes were assessed in their 12 preterm infants from birth to Day 49. Fatty acid profiles were measured weekly in breast-milk and in the plasma of mothers and premature infants at baseline, Day 14, and Day 49. Fatty acid profiles of the milk and plasma at Day 49 of a reference group of 22 mothers and their 24 babies, whose mother did not receive DHA supplements during lactation was use for comparison.

Results: Plasma DHA concentrations in babies receiving the DHA-enriched was significantly and positively increased over the study period ($p=0.0143$). At Day 49, DHA in mothers' milk was 12 times higher in the DHA group compared to the reference group. There was also a significant linear increase ($p < 0.0001$) in the amount of DHA provided to the very preterm infants in the supplemented DHA group over time. Although the infants from both groups had similar enteral intake at Day 49, the supplemented DHA group received 55.2 ± 37.6 mg/kg/day of DHA compared with 7.2 ± 11.1 mg/kg/day in the reference group ($p=0.0017$).

Conclusion: Early supplementation with DHA to lactating mothers with low dietary DHA was successful in increasing DHA status in very preterm infants.

P175

N-3 fatty acids affected glial morphometry during aging in Fat-1 mice

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Background: Glial cells are the cellular components of the brain immune system. During aging, both microglia and astrocytes are activated, display morphometric changes and produce proinflammatory cytokines. In addition, in response to proinflammatory stimuli, primed

microglia overproduce proinflammatory cytokines which trigger neuronal death and cognitive disorders. In this context, limiting the activation of glial cells and the production of proinflammatory cytokines seems very relevant. N-3 polyunsaturated fatty acids (n-3 PUFA) are promising micronutrients due to their anti-inflammatory properties. However two parameters are influent on brain n-3 PUFA content, especially on long chain n-3 PUFA such as docosahexaenoic acid (DHA): firstly our western diet is characterized by a growing unbalance between n-6 and n-3 PUFA that restrains n-3 PUFA and secondly during aging brain n-3 PUFA decreased. This decrease can be restored by food supplementation.

Objective: The aim of this study was to determine the effects of brain n-3 PUFA content on glial morphometry during aging in mice.

Procedure: We used Fat-1 model mice that convert n-6 to n-3 PUFA. We determined glial activation in hippocampus of Fat-1 and wild type (wt) mice aged 4.5 and 23 months by immunostaining of astrocytes and microglial cells. Images were captured with a confocal microscope in the Dentate Gyrus (DG), CA1 and CA3 of the median hippocampus. Morphometry of astrocytes and microglial cells was analysed using a three-dimensional reconstructing Imaris software and process length and number were measured. The expression of hippocampus pro-inflammatory cytokines was measured by quantitative RT-PCR.

Results: Morphometry of glial cells of Fat-1 mice was less affected by aging than that of wt mice. Expression of IL-6 was increased in aged wt mice while it was not the case in aged Fat-1 mice.

Conclusion: These data suggested that preservation of the morphology of glial cells may limit the expression of IL-6.

P176

Omega-3 fatty acids and brain glucose utilization: an ¹⁸F-fluorodeoxyglucose positron emission tomography (FDG-PET) study in the rat

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Background: Several in vivo and in vitro studies suggest that docosahexaenoic acid (DHA) may be a regulator of brain energy metabolism by affecting glucose metabolism and its transporter GLUT1.

Objective: The purpose of this study was to evaluate

whether cerebral glucose metabolism measured by FDG-PET would reflect decreased GLUT1 expression in the brain of omega-3 deficient rats.

Methods: We measured the cerebral metabolic rate for glucose with FDG-PET for small animal (microPET) in adult rats (10 wk-old) receiving an omega-3-adequate (control, n = 6) or an omega-3-deficient (omega-3 def, n = 6) diet. Dynamic PET scans were performed during 45 min after injection of ¹⁸F-FDG (60 MBq). Data were reconstructed and superposed with MRI slices of the rat brain to calculate radioactivity in regions of interest (ROIs). Fatty acid content in brain phospholipid classes was determined by gas chromatography and the mRNA and protein expression of GLUT1 using real-time PCR (TaqMan low-density array, TLDA) and western blotting, respectively.

Results: Omega-3 PUFA-deficient rats had 60-70% lower DHA in their brain membrane phospholipids. Significantly decreased ¹⁸F-FDG uptake was observed in the brain of omega-3 deficient rats, corresponding to both a lower rate of FDG uptake during the early phase (0-15 min) and a lower plateau level of ¹⁸F-FDG incorporation during the later plateau phase (15-45 min). The gene and protein expression of GLUT1 was also lower in the omega-3 def group.

Conclusion: In rats deficient in omega-3 PUFA, lower expression of GLUT1 in the brain is consistent with in vivo results using FDG-PET that show glucose hypometabolism in the brain. Dietary intake of omega-3 fatty acids therefore clearly appears to modulate brain glucose uptake, a mechanism by which omega-3 fatty acid status may influence cognitive function known to be at risk during aging.

P177

Effect of diets enriched in linoleic or α -linolenic acids: a focus on Δ 5- and Δ 6 desaturases

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Background: Ensuring the n-6 and n-3 long chain polyunsaturated fatty acid (LCPUFA) bioavailability is one of the topical issues for nutrition research. To this end, it is important to clearly define the dietary precursor's contribution in the biosynthesis of LCPUFA. Two parameters have to be considered: the level of linoleic (LA) and α -linolenic acid (ALA) intakes, and the ratio between these two essential precursors.

Objective: The aim of this study was to analyse the effect of diets enriched either in LA or ALA on LCPUFA biosynthesis.

Procedure: Sprague Dawley rats were fed control diet or precursor enriched diets for two months. The enrichments compared to control diet were physiologically relevant (in % of energy: from 2.5% to 5% for LA and from 0.5% to 2.2%

for ALA) and balanced by a decrease in palmitic acid level. Diets were isoenergetic and isolipidic.

Results: LA diet did not affect the n-6 LCPUFA profile, whereas ALA diet significantly enhanced eicosapentaenoic and docosapentaenoic acids in the liver and brain, and docosahexaenoic acid in the brain (+1.6% of total FA). Hepatic $\Delta 5$ - and $\Delta 6$ -desaturase activities were significantly enhanced in rats fed with LA and ALA diets. ALA diet led to a stronger activation (+56.3% for the $\Delta 5$ -desaturase and +51.7% for the $\Delta 6$ -desaturase as compared to the control diet). This result could be part explained by the RNA level: animals fed LA and ALA concordantly displayed a higher expression of both desaturases.

Conclusion: This study underlined that an equivalent increase of dietary LA and ALA did not have the same effects on LCPUFA biosynthesis. Both diets up-regulated desaturase activities in the liver, but only ALA diet led to an increase in n-3 derivatives, showing the necessity to ensure a sufficient ALA intake in human diets.

P178

Gastric stable emulsions provide increased bioavailability of long chain ω -3 fatty acids

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Objective: The present study comprises the design of as well as the effect of pre-emulsification of ω -3 fatty acids on the bioavailability of docosahexaenoic acid and eicosapentaenoic acid. In vitro studies have shown that long-term steric stabilization of an o/w-emulsion is obtained by arresting the oil droplets in a gelatin continuous gel matrix. The emulsion was also stable upon dissolution of the gel matrix at physiological conditions in vitro and is hence referred to as a gastric stable emulsion (GSE).

Procedure: In the bioavailability study, healthy young students were recruited and presented two different single-dose treatments of fish oil containing 5 grams of ω -3 fatty acids; one group receiving the fatty acids in traditional soft gel capsules, whereas the other group received the fatty acids using the GSE technology. Time resolved (2 - 26 hours) blood plasma analysis after intake of this single dose ω -3 fatty acids revealed significantly increased AUC_{0-26h} and C_{max} of EPA (45 and 100%, respectively) and EPA + DHA (43 and 106%, respectively) when administered as GSE compared to traditional soft gel capsules.

Results: In vitro results indicated that the fish oil

presented in soft gel capsules may not have been as effectively emulsified in the stomach compared to fish oil in a GSE formulation, which in turn would have led to a reduced conversion rate (per unit time) of the pancreatic lipases.

Conclusion: The overall conclusion hence becomes that ω -3 fatty acids, such as docosahexaenoic acid and eicosapentaenoic acid, will exhibit improved bioavailability and absorption in blood plasma by being pre-emulsified into an acid stable emulsion prior to oral ingestion.

P179

Effects of conjugated linoleic acid (CLA) upon cytokine profile in epididymal adipose tissue of cachectic tumour bearing rats

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Background: Cancer Cachexia is a syndrome characterized, among many symptoms, by the disruption of lipid metabolism, both in animal models and cancer patients. Conjugated Linoleic Acid supplementation has been related with the improvement of lipid metabolism. **Objective:** We investigated the effects of CLA supplementation upon adipose tissue lipid metabolism in cachectic rats.

Procedure: Control (C) and cachectic (TB) rats were divided into three groups and supplemented with CLA (CLA), sunflower oil (SF) or water (C) for 14 days. Epididymal adipose tissue (EAT) fat (Stansbie et al., data) and protein (Lowry et al, DATA) content were measured, and IL-1 β , IL-6, TNF- α tissue concentration determined by ELISA.

Results: Fat content in EAT decreased in TBCLA when compared with control (C), with TBSF, and with CCLA (P<0.01). TB and TBCLA showed increased protein content in EAT when compared with C, CCLA, CSF and TBSF (P<0.05). Decreased IL-1 β concentration in CCLA was found, when compared with C, but higher TBCLA IL-1 β concentration was observed in relation to C and TB and TBSF (P<0.01). No differences were observed in EAT IL-6 concentration in CCLA when compared with C. TBCLA, however, showed increased IL-6 concentration when compared with TB and CCLA. TNF- α concentration was not different in CCLA in relation to C. TBCLA showed higher TNF- α concentration when compared with TB and CCLA (P<0.01).

Conclusion: CLA supplementation increases inflammatory cytokine content in the epididymal adipose tissue, possibly contributing to enhanced fat delipidation in cachectic rats.

P180**Oleic acid attenuates growth inhibitory and pro-inflammatory responses of stearic acid in human aortic endothelial cells**

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Background: Long-chain saturated fatty acids (SFAs) have been linked to an increase in cardiovascular disease complications. SFAs represent significant components of both enteral and parenteral formulations. However, the effect of SFAs upon acute inflammation is less clear. Recently, it has been realized that oleic acid, a monounsaturated fatty acid (MUFA) present in olive oil, can reduce the detrimental effects of SFAs.

Objective: The objective of the present study was to investigate if oleic acid (C18:1), a MUFA, reduces the growth inhibitory and pro-inflammatory effects of stearic acid (C18:0), a SFA, in human aortic endothelial cells (HAECs).

Procedure: Growth inhibition and cytotoxicity assays were quantified by a WST-1 assay. ICAM-1 expression and apoptosis was quantified by flow cytometry. Caspase-3 and NF- κ B activation was assayed by western blotting. Incorporation of stearic and oleic acids was determined using capillary gas chromatography.

Results: Stearic acid- induced growth inhibition at concentrations less than 50 μ M, whereas higher concentrations invoked cytotoxicity. Stearic acid-induced growth inhibition and cytotoxicity were attenuated upon addition of oleic acid. Oleic acid, as low as 5 μ M, inhibited the stearic acid-induced increase in ICAM-1 expression. Stearic acid- induced NF- κ B phosphorylation was also reduced by oleic acid. HAECs, treated with either stearic or oleic acid, resulted in significant increases in C18:0 and C18:1n-9, respectively. Importantly, when HAECs were co-incubated with stearic acid (50 μ M) and oleic acid (5- 50 μ M), the amount of C18:0 incorporation was significantly reduced by the addition of even the lowest amount of oleic acid. Stearic acid addition did not result in an increase in triglycerides, but oleic acid (50 μ M) addition, with or without stearic acid, induced an accumulation of triglycerides in HAECs. **Conclusion:** Our data suggests that oleic acid has the ability to reduce the growth inhibitory and pro-inflammatory effects of SFA, specifically stearic acid, on HAECs.

P181**Trans-11 vaccenic acid is bioavailable from different food sources and reduces intestinal chylomicron secretion in the JCR:LA-cp rat model**

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Background: *Trans*-11 vaccenic acid (VA) is the predominant trans isomer in ruminant fat and is the major precursor to endogenous synthesis of *cis*9,*trans*11-conjugated linoleic acid (CLA) in humans and animals. Our group have previously shown hypolipidemic benefits of dietary VA supplementation in a rat model of dyslipidemia and metabolic syndrome (JCR:LA-*cp*).

Objectives: 1) to measure the intestinal bioavailability of VA from different food sources, and 2) to study the acute effect of VA on intestinal lymph chylomicron production.

Procedure: The superior mesenteric lymph duct of obese JCR:LA-*cp* rats (n=24) was cannulated and rats were randomized to one of four lipid/meal emulsions infused via a gastric cannula: 1) a purified VA mixed meal, 2) a VA-enriched beef fat mixed meal, 3) purified VA in triolein or 4) triolein alone. Lymph was collected for 10 hours following infusion of an emulsion. The fatty acid composition of lipid/meal emulsions and mesenteric lymph was determined by GC and the bioavailability of VA calculated. Lymph triglyceride (TG), total cholesterol and chylomicron particle number (measured by apoB48) were quantified.

Results: There was no significant difference in the bioavailability of VA between natural beef-fat meal (1.42 \pm 0.34%), synthetic VA-triolein emulsion (1.11 \pm 0.43%) or the VA-mixed meal (0.71 \pm 0.18%). The conversion of VA to CLA was 2-fold higher in the VA-triolein emulsion (24.24 \pm 3.81%) compared to the VA-mixed meal (8.26 \pm 1.56%). There was a 40% reduction in lymph-TG (p<0.05) and a 30% reduction in chylomicron particle (apoB48) secretion (p<0.01) in obese rats infused with the VA-triolein emulsion, relative to triolein alone.

Conclusion: Dietary VA has similar bioavailability from natural beef and synthetic sources. VA reduces chylomicron TG and production rates, which might be contributing to the hypolipidemic effects observed in the JCR:LA-*cp* rat model.

P182**Maternal glucose control and arachidonic acid status have long-term consequences on bone health in the adult offspring**

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Background: Newborn infants of diabetic mothers may have low arachidonic acid (AA) and compromised bone growth and mineralization. Maternal diabetes suppresses Δ 9, Δ 6 and Δ 5 desaturase activity suggesting that AA supplementation may be required.

Objective: To define the effect of maternal diabetes and

AA supplementation on offspring body composition and bone health up to 12 weeks of age.

Procedure: Dam were randomized into 6 groups (n=5/group) using a 3 × 2 design and the treatments were Saline-Placebo, streptozotocin (60 mg/kg) induced-diabetes with glucose controlled at <13 mmol/L (STZ/GC), or poorly-controlled at 13-20 mmol/L (STZ/PC) using insulin; and fed either a Control or AA diet (0.5 % of fat w/w). After 1 wk adaptation to diet and treatment, rats were mated. Offspring were nourished by their biological mother and weaned offspring were fed a regular chow to 12 weeks of age. Measurements included offspring body composition and liver fatty acids of dam and offspring. The liver $\Delta 9$ (16:1n7/16:0), $\Delta 6$ (18:3n6/18:2n6) and $\Delta 5$ (20:4n6/20:3n6) desaturase indices of day 29 offspring were calculated. The Main and interaction effects of treatment and diet were assessed using factorial ANOVA.

Results: Comparable to offspring of Saline-Placebo, the offspring of STZ/GC gained more (P=0.009) weight and had greater whole-body and regional bone area than offspring of STZ/PC (all P<0.05). Maternal glucose was negatively correlated with offspring bone area at 4 and 12 weeks of age (all P≤0.04). Dam liver AA was positively correlated with female offspring regional bone mineral content and density at 8 and 12 weeks of age (all P<0.05). The liver $\Delta 6$ desaturase activity of STZ/PC offspring was significantly lower (all P≤0.04) than STZ/GC and Saline-Placebo offspring. Maternal AA supplementation increased (P=0.0019) offspring liver $\Delta 9$ desaturase activity.

Conclusion: Maternal glucose control and AA status have long-term consequences on bone health in the adult offspring.

P183

Plasma endocannabinoids: is there a difference in regulation among pregnant versus non-pregnant women?

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Background: Endocannabinoids, endogenous cannabinoids, are lipid messengers and analogs of polyunsaturated fatty acids. This class of lipids is emerging as important regulators of physiological processes, including functions in reproduction and development. We are interested in the regulation of these compounds during pregnancy compared to the non-pregnant state and have established methodology for the simultaneous assessment of 12 plasma analytes of the endocannabinoid metabolome. Here we report on 6 of the 12 measured analytes.

Objective: Compare plasma analytes of the endocannabinoid metabolome in pregnant versus non-pregnant women.

Procedure: We quantified 12 plasma analytes of the endocannabinoid metabolome in pregnant women (n=31, 20-22 weeks gestation) versus non-pregnant women (n=6) using liquid chromatography-mass spectrometry.

Results: As a class of endocannabinoids, the ethanolamides were higher in pregnant versus non-pregnant women (mean ± SD): arachidonylethanolamine (0.68 ± 0.18 pg/μl versus 0.44 ± 0.06 pg/μl, p < 0.01) and palmitoylethanolamine, (4.20 ± 1.38 pg/μl versus 2.84 ± 1.00 pg/μl, p=0.03). There was no difference in oleoylethanolamine between groups (3.78 ± 1.31pg/μl versus 2.87± 0.63 pg/μl, p<0.11). The glycerols were lower in pregnant versus non-pregnant women: 2-arachidonolglycerol (180.98 ± 91.82 pg/μl versus 506.17± 242.53 pg/μl, p<0.01), 2-palmitoylglycerol (3522.75 ± 1363.20 pg/μl versus 5885.67 ± 1396.20 pg/μl, p<0.01) and 2-oleoylglycerol (690.03 ± 334.48 pg/μl versus 1386.83 ± 457.57 pg/μl, p<0.01).

Conclusions: These preliminary data demonstrate that pregnancy alters levels of both ethanolamides and glycerols in the endocannabinoid system. Funded in part by: LSU AgCenter, Nestle, Ltd (Switzerland) and Agriculture and Food Research Initiative Grant 2009-65200-05991 from the USDA National Institute for Food and Agriculture.

P184

Acute lipid induced insulin resistance in women and men is not accompanied by impaired proximal insulin signaling

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Background: We have previously shown that women have a higher insulin sensitivity despite a higher IMTG concentration and a higher muscle mRNA level of several proteins related to muscle lipid metabolism compared with matched men.

Objective: We therefore hypothesized that women would be able to handle a lipid load better than men and therefore be less prone to intralipid-induced insulin resistance than men.

Procedure: Whole body insulin sensitivity and leg glucose uptake were studied in 16 young healthy matched men and women infused with intralipid or saline for 7 h. Muscle biopsies were obtained before, during and after an euglycemic hyperinsulinemic (≈70 uU/ml) clamp. Glucose transport in giant sarcolemmal vesicles from hindlimb rat muscles were measured at different concentrations of linoic acids.

Results: Intralipid infusion reduced whole body insulin sensitivity to a lesser extent in women than in men. In contrast, insulin stimulated leg glucose uptake was reduced to the same extent in women and men after intralipid infusion. The lipid induced reduction could not be explained by impaired insulin signalling, an accumulation of lipid intermediates or increased IMTG content. Glucose transport in giant sarcolemma vesicles was not decreased by physiological levels of fatty acids. **Conclusion:** Based on the present findings we suggest that the lipid induced effect on insulin sensitivity might be located at hitherto unexplored parts of the insulin signalling pathway or perhaps directly interfering with GLUT4 translocation and docking to the plasma membrane.

P185

Post weaning restoration of brain DHA levels in deficient omega3-rats is better with butter diets independently of ALA levels

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DHA is the major brain FA and omega-3 deficiencies during gestation and/or lactation could have dramatic impacts on health: obesity and also cognitive and mental diseases. Most of infant formulas contained blends of vegetable oils to follow the recommended values of LA and LA/ALA ratio.

Objective: To evaluate the butter fat impact on brain composition of young deficient omega3-rats compared to the usual vegetable blends used in infant formula

Procedure: Dams were fed a 5%fat (w/w) diet for 6weeks before mating, during gestation and lactation, with a minimal-omega3 level (ALA0.4% of FA), obtained with a palm/soya (97/3) oils blend. After weaning, young pups (females and males n=10each) received a 10%fat (w/w) diet for 6 weeks. Group1 (G1) received the palm blend diet with a minimal-ALA level (0.4% of FA); 2 groups received diets including rapeseed to provide the same ALA level (1.5% of FA) respectively blended with palm oil (G2) and butter (G3). Two control-groups were tested: (G4) a pure milk fat diet (0.8%ALA) and (G5) a pure rapeseed fat diet (8%ALA).

Brain FA profiles were determined in weaning pups, and after the post-weaning 6week-diets.

Results: Six-week post-weaning restoration of brain DHA levels in young omega3-deficient rats is better with butter compared to palm/rapeseed blends. The 1.5%ALA blends from butter and palm increase the brain DHA levels respectively by 80% and 60%. The brain DHA

increase with pure butter diet (0.8%ALA) is more than 3times the increase obtained with the 0.4%ALA palm-blend. Furthermore the pure butter (0.8%ALA) diet is as efficient as the pure rapeseed oil (8%ALA) diet to restore completely the normal levels of brain DHA

Conclusion: Butter fat diets seem to be the best to restore within 6weeks the levels of DHA in the brain of deficient-omega3 post-weaning rats.

Abstracts - Tuesday June 1

Plenary: Biochemistry of Lipids 2 : Lipid droplets and transport

08.30 - 09.15

Fatty acid chaperones and transporters

JFC Glatz

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While long-chain fatty acids and lipids serve a wide variety of functions in mammalian homeostasis, particularly in the formation and dynamic properties of biological membranes and as fuels for energy production in tissues such as heart and skeletal muscle, it is well documented that fatty acid metabolites may exert toxic effects on cellular functions and cause cell injury. Therefore, fatty acid uptake into the cell and intracellular handling need to be carefully controlled. In the last few years our knowledge of the regulation of cellular fatty acid uptake and transport has dramatically increased. Notably, fatty acid uptake was found to occur by a mechanism that resembles that of cellular glucose uptake. Thus, following an acute stimulus - particularly insulin or muscle contraction - specific fatty acid transporters translocate from intracellular stores to the plasma membrane to facilitate fatty acid uptake, just as these same stimuli recruit glucose transporters to increase glucose uptake. This regulatory mechanism is important to clear lipids from the circulation post-prandially and to rapidly facilitate substrate provision when the metabolic demands of heart and muscle are increased by contractile activity. Intracellularly, cytoplasmic fatty acid-binding proteins (FABP) act as chaperones for incoming fatty acids. Studies in both humans and animal models have implicated fatty acid transporters in the pathogenesis of diseases, particularly the progression of obesity to insulin resistance and type-2 diabetes. As a result, membrane fatty acid transporters are now being regarded as a promising therapeutic target to re-direct lipid fluxes in the body in an organ-specific fashion.

In this lecture a brief overview will be presented of our current understanding of membrane and cytoplasmic fatty acid transporters as regulators of lipid metabolism. Particular emphasis will be put on the molecular functioning and the (pathio)physiological significance of the membrane protein CD36.

Reference: Glatz JFC, Luiken JJFP, Bonen A. *Physiol Rev* 90: 367-417, 2010.

09.15 - 10.00

Metabolic Lipases and Disease: Cachexia

Suman Das, Paul Vesely, R. Zimmermann, G. Haemmerle, G. Hoefler, Rudolf Zechner
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Lipolysis is a key pathway to provide energy substrate, lipid mediators for cell signaling, and lipid precursors for the synthesis of membrane lipids. Accordingly, lipolysis is essential for lipid and energy homeostasis. Defective lipolysis leads to either lipid storage disorders or the opposite, severe loss of body fat. The uncontrolled loss of body fat that cannot be prevented by nutritional measures is called cachexia and most commonly observed in cancer. To investigate the role of lipases in the development of cancer induced cachexia, we studied tumor growth, muscle and adipose tissue lipid metabolism, and energy homeostasis in mice that lacked the major enzymes for triacylglycerol hydrolysis. Cachexia was induced in wild-type C57BL6 mice, ATGL-deficient mice, and HSL-deficient mice by subcutaneous injection of Lewis lung carcinoma cells. We show that lipases play an important role in the pathogenesis of cancer-induced cachexia.

Concurrent Session - 13

Lipids and Health: Lipids and inflammation

10.30 - 11.00

Invited contribution**Pro-resolving actions of resolvin E1 in airway inflammation, injury and infection**B Levy*Harvard Medical School, Harvard, United States of America*

Acute inflammation in the lung is fundamentally important to host defense, but excessive inflammation can lead to acute lung injury and the acute respiratory distress syndrome. Inflammation resolution is an active process with specific mediators. Resolvin E1 (RvE1) is an endogenous mediator that displays pro-resolving actions. In a model of aspiration pneumonia, intravenous administration of RvE1 (-0.005 mg/kg) prior to acid injury of the lung selectively decreased neutrophil accumulation by 55% and enhanced bacterial clearance. RvE1 significantly decreased lung tissue levels of several pro-inflammatory chemokines and cytokines, including IL-1 α , IL-6, HMGB-1, MIP-1 α , MIP-1 β , KC and MCP-1, in a manner independent of the anti-inflammatory mediators IL-10 and lipoxin A₄. In addition, RvE1 treated animals had a marked improvement in survival. Together, these findings in experimental aspiration pneumonia have uncovered protective roles for RvE1 in pathogen-mediated inflammation that are both anti-inflammatory for neutrophils and protective for host defense. Understanding the biosynthesis and actions of endogenous pro-resolution molecules such as RvE1 is providing insights into the molecular pathophysiology of disease and new opportunities for therapeutic intervention.

11.00 - 11.15

Novel 18S-E-series resolvins: stereoselective biosynthesis and pro-resolving actionsS Oh, PS Pillai, A Recchiuti, R Yang, CN Serhan*Harvard Medical School, Boston, United States of America*

Background: The protective actions of low-dose aspirin are well recognized in many clinical studies. In addition to anti-thrombotic actions, aspirin triggers specialized pro-resolving mediators (SPM), including lipoxins and resolvins, which may explain some of the benefits of aspirin and essential polyunsaturated fatty acids.

Objective: 1) To identify novel aspirin-triggered eicosapentaenoic acid-derived epimeric lipid mediators, namely the 18S-E-series resolvins and 2) to investigate their anti-inflammatory, pro-resolving actions.

Procedure: In order to elucidate stereoselective biosynthetic pathways of E series resolvins, we carried out chiral HPLC-tandem mass spectrometry-based lipid profiling and subsequent enzymatic experiments. We also assessed bioactions of novel mediators with in vitro overexpression system, primary cell cultures and experimental diseases.

Results: Aspirin administration increased endogenous formation of 18S-hydroxyeicosapentaenoate (HEPE), a precursor of new mediators, in healthy human subjects. This was confirmed with recombinant human cyclooxygenase-2 that generates 18S-HEPE > 18R-HEPE (S:R ratio ~4:1) when treated with aspirin. The human 5-lipoxygenase (LOX), a pivotal enzyme in resolvin E1 (RvE1) and E2 (RvE2) biosynthesis, also utilized 18S-HEPE as a substrate to form 5S-hydroperoxy-18R/S-HEPEs and 5S(6)-epoxy-18R/S-HEPEs. In addition, LTA4 hydrolase (LTA4H) converted these 5S(6)-epoxide containing intermediates to both RvE1 and 18S-RvE1. 18S-RvE1 was essentially equipotent to the original E-series resolvins, although 18S-RvE1 was more rapidly dehydrogenated and inactivated. 18S-RvE1 was more potent than RvE1 for activating an RvE1 receptor overexpressed in CHO cells in vitro, suggesting its high-affinity interaction with the target GPCR.

Conclusion: Together these results demonstrate two parallel stereospecific aspirin-triggered pathways to form EPA-derived E-series resolvins.

11.15 - 11.30**Protective effects of brain DHA in a mouse model of neuroinflammation**

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Background: DHA is the major brain n-3 PUFA, and it is possible that it is anti-inflammatory within the brain as it is known to be in the periphery.

Objective: To test the effects of brain DHA levels on neuroinflammation.

Procedure: Brain DHA levels were altered using a combination of transgenic and dietary mouse models, and the neuroinflammatory response in the hippocampus was tested upon intracerebroventricular infusion of either 5µg of lipopolysaccharide (LPS; neuroinflammatory agent) or vehicle (control).

Results: Our first approach used the fat-1 transgenic mouse which endogenously converts n-6 to n-3 PUFA. Fat-1 mice and their wildtype (WT) littermates consumed an n-6 PUFA containing, n-3 PUFA deprived chow (10% safflower oil; SO). Fat-1 mice had higher hippocampal DHA concentrations in phospholipid (50%) and unesterified (65%) pools compared to WT mice. LPS-treated fat-1 mice, compared to LPS-treated WT mice, had lower mRNA levels of several neuroinflammatory markers (including cytokines, chemokines, and glial markers). Fat-1 mice also experienced less neuronal degeneration in response to LPS.

In a second study, we fed fat-1 WT littermates an n-3 PUFA containing diet (2% fish oil, 8% safflower oil; FO) and compared their neuroinflammatory response to fat-1 mice fed SO chow. There was no difference in neuroinflammation between the two groups, suggesting that DHA feeding attenuates the neuroinflammatory response similar to the fat-1 mouse. Fatty acids will be measured and presented.

Conclusion: Fat-1 mice have higher brain DHA levels in phospholipid and unesterified fractions compared to their WT littermates. Fat-1 mice also experienced an attenuated neuroinflammatory responses and less neuronal degeneration. This attenuation can be mimicked by feeding wildtype littermates an n-3 PUFA containing diet. Taken together, DHA appears to have anti-inflammatory properties within the brain.

11.30 - 11.45**Impairment of NFκB activity by unsaturated fatty acids**

J Schumann, H Fuhrmann

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Objective: It is evident that polyunsaturated fatty acids (PUFA) modulate lymphocyte proliferation, antigen presentation, cytokine synthesis, oxidative burst as well as expression of adhesion molecules. In this regard, PUFA are speculated in part to exert their effects on inflammatory gene expression through direct actions on intracellular signaling pathways. However, concerning the action of unsaturated fatty acids on NFκB conflicting data do exist. In fact, it is not known if the interrelation between fatty acids and NFκB is restricted to special fatty acid families. In addition, the impact of the degree of saturation of a fatty acid is unidentified so far.

Procedure: Given the central role of NFκB in cellular processes and concerning the importance of fatty acids for human health we conducted the first systematic study investigating acute as well as long-term effects of PUFA from the n-3, the n-6 as well as the n-9 family on NFκB activity by mean of a luciferase reporter cell line.

Results: We identified PUFA to impair NFκB signaling. Furthermore, we could demonstrate the PUFA ability to derogate NFκB activity to be independent from the family the fatty acid belongs to. Instead, we found a correlation between the number of bis-allyl-methylene positions of the PUFA added and the NFκB activity of stimulated, long term supplemented cells.

Conclusions: The data presented provides new insights into the biological mechanisms PUFA exert their anti-inflammatory effects. Since suppression of NFκB activity could be of benefit in a number of inflammatory diseases as well as cancer, our findings are of clinical implication. According to our data dietary supplementation with PUFA-containing oils is likely to provide an at least palliative therapy for disorders linked to inappropriate NFκB signaling.

11.45 - 12.00

Polyunsaturated fatty acids alter the phenotype of human mast cells in vitro

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Background: The increased *n*-6:*n*-3 fatty acid ratio in Western diets may contribute to the rapid increase in prevalence of allergic diseases. Key effector cells in allergy are mast cells (MC).

Objective: The effect of different fatty acids on MC activation was studied.

Procedure: Separate long chain *n*-6 (arachidonic acid, AA) and *n*-3 (eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA) polyunsaturated fatty acid (PUFA) incorporation was investigated in human MC lines (LAD2, HMC-1). Next to degranulation and mediator secretion (release of PGD₂ and cytokines such as TNF- α , IL-4 and IL-13), generation of reactive oxygen species (ROS) and phosphorylation of mitogen-activated protein kinases (MAPK) was examined.

Results: Incubation of MC with AA, EPA or DHA for 24 hours increased PUFA content of the cellular membrane. Incubation with PUFA did not reduce IgE-mediated degranulation by LAD2 cells. However, mediator release of ionomycin/PMA stimulated HMC-1 cells was differentially regulated. IL-13 ($p < 0.01$ for all PUFA) and IL-4 ($p < 0.05$ for EPA and DHA) secretion were inhibited, whereas AA enhanced TNF- α release ($p < 0.05$). The effect of DHA on IL-13 release was most pronounced and associated with a reduction in ROS generation ($p < 0.01$). AA incubation increased PGD₂ secretion, whereas *n*-3 PUFA reduced PGD₂. Cyclooxygenase (COX) inhibitors showed that the reduction in IL-13 secretion by PUFA was independent of COX.

Preliminary results demonstrated that the ionomycin/PMA-induced phosphorylation of MAPK was inhibited by *n*-3 PUFA.

Conclusion: Long chain PUFA differentially alter mast cell activation which may affect the development of allergic diseases.

Concurrent Session - 14

Biochemistry of Lipids: Diabetes, lipid droplets, mitochondria

10.30 - 11.00

Invited contribution

The formation of lipid droplets: possible role in the development of insulin resistance/type 2 diabetes

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Neutral lipids are stored in the cell in so called lipid droplets, now recognized as dynamic organelles. Accumulation of triglycerides in such droplets in skeletal muscle is highly related to the development of insulin resistance. Lipid droplets are formed from microsomal membranes as 0.1-0.4 μ m large structures. The assembly process requires triglyceride biosynthesis. We also identified phospholipase D1 as important for the process. Most likely this can explain why the assembly is highly dependent on phosphatidylinositol(4,5) bisphosphate. Moreover ERK2 was needed for the assembly process. ERK2 sorted the motor protein dynein onto the droplets allowing them to transfer on microtubules. This transfer is a prerequisite for the increase of the size of the droplets by fusion, a process that is catalyzed by the SNARE proteins SNAP23, syntaxin-5 and vamp 4. SNAP23 is also essential for the insulin dependent translocation of GLUT4 to the plasma membrane i.e. for the insulin dependent uptake of glucose. In cultured cardiomyocytes, fatty acids diverted SNAP23 from the plasma membrane to the interior of the cell, a process that was paralleled by the development of cellular insulin resistance, which could be overcome by increasing the levels of SNAP23. A redistribution of SNAP23 to the interior of the cell is also seen in skeletal muscle biopsies from patients with insulin resistance/type 2 diabetes, when compared to non-diabetic controls. Time laps studies of microinjected SNAP23-CFP as well as chase studies of transfected SNAP23-FLAG indicated that the SNARE is formed in the cytosol and from this compartment sorted to lipid droplets and to the plasma membrane. SNAP23 appeared in the plasma membrane later than in the lipid droplets. Based on these results we propose

that changes in the sorting of SNAP23 from the site of synthesis to the plasma membrane is of importance for the development of insulin resistance/type 2 diabetes.

11.00 - 11.15

Dysbalance in skeletal muscle lipase content and activity in obesity and type 2 diabetes

JWE Jocken

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Background: The obese insulin resistant state is characterized with elevated lipid storage in skeletal muscle, in the form of triacylglycerol (TAG), and lipotoxic diacylglycerol (DAG) and ceramides.

Objective: We tested the hypothesis that a dysbalance in muscle lipase content and activity might result in an incomplete in vivo lipolysis and the accumulation of lipotoxic lipid metabolites, in non-obese and obese type 2 diabetic (T2D) men.

Procedure: Skeletal muscle biopsies were taken to measure lipase protein content (ATGL, HSL and CGI-58), activity and lipid content (TAG and DAG).

Results: We show that muscle ATGL protein content is elevated (2.17 ± 0.40 vs. 0.42 ± 0.23 ; $P=0.008$) and HSL protein content is reduced (0.39 ± 0.07 vs. 1.00 ± 0.19 ; $P=0.004$) in obese men. This dysbalance in lipase content is accompanied by a 60% reduction in the ratio of DAG to TAG hydrolase activity (11.4 ± 2.3 vs. 26.5 ± 7.3 ; $P=0.045$), suggesting incomplete lipolysis. The dysbalance in lipase content was only apparent in obese T2D and not in non-obese T2D men. Despite incomplete lipolysis, muscle total DAG content was not elevated in obese men, and was even decreased (9.4 ± 0.9 vs. 6.2 ± 0.7 ; $P=0.017$), TAG content was not significantly different between groups (70.4 ± 12.4 vs. 84.7 ± 18.9 ; $P=0.543$).

Conclusion: Together, these data suggest that obese subjects have a dysbalance in lipase content, resulting in incomplete lipolysis but not elevated DAG content. This dysbalance is confined to obesity per se. We propose that total DAG content might not reflect the DAG stereoisomers that are preferred HSL substrates.

11.15 - 11.30

In vivo dynamics of zebrafish adipocyte lipid droplets in response to nutritional status and pharmaceuticals regulating lipid metabolism

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Background: Knowledge of adipocyte development and physiology is largely derived from research using mammalian model systems and cell cultures. In addition, it is very difficult to visualize the adipocyte cell line during the in utero development of mammals.

Objective: The aim of the present study was to use the zebrafish model to monitor white adipocyte lipid droplet dynamics in response to starvation and refeeding and after pharmaceutical treatments.

Procedure: In vivo labelling of zebrafish adipocytes was performed with vital Nile Red staining on live unanaesthetized animals and mature white adipocytes, containing usually a single large droplet, were monitored by in vivo microscopy under a fluorescence microscope. Quantitative analysis of neutral lipid deposit fluorescence signals was performed using free-processing ImageJ software (NIH) and correlated to triacylglycerol contents extracted from individual animals.

Results: The chronology and topography of adipocyte appearance in several anatomic sites during zebrafish development were defined and a distinctive pattern of gene expression among neutral lipid deposition sites was demonstrated by RT-PCR and in situ hybridization. Fat depots in unilocular adipocytes which are mobilized in response to starvation and deposited in response to refeeding were correlated to triacylglycerol content. Refeeding reestablish neutral lipid depots in the same locations as before starvation by expanding the lipid droplets. Such dynamics may be modulated by pharmaceutical treatments independently of the nutritional status.

Conclusion: Experiments with zebrafish are significantly easier, faster, and cheaper than those using rodents, currently the main model system for human drug target screening and prenatal developmental toxicology. Our data suggest that zebrafish larva and juvenile may be used as a promising model for screening molecules present in the environment, which have an obesogenic character.

11.30 - 11.45

Differential effects of conjugated linoleic acid (CLA) isomers on hepatic lipid droplet size and lipid droplet proteins in obese rats

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Background: CLA reduces hepatic steatosis in obese, insulin-resistant *fa/fa* Zucker rats. We proposed that the anti-steatotic effects of CLA may be due to altered expression of the perilipin family of lipid droplet proteins, which are required for formation and maintenance of cytoplasmic lipid droplets.

Objective: To determine the effects of CLA isomers on hepatic steatosis, lipid droplet size and lipid droplet proteins in *fa/fa* Zucker rats.

Procedure: *fa/fa* Zucker rats (6 wk old) were fed *cis-9,trans-11* (*fa9-11*) or *trans-10, cis-12* (*fa10-12*) CLA (0.4% wt/wt) or control (*faCTL*) diet for 8 weeks. Total hepatic lipids were extracted and quantified gravimetrically. Liver sections were stained with hematoxylin/eosin to assess the histological appearance and to quantify lipid droplet number, size and volume by image analyses. Western blotting was used to determine hepatic levels of lipid droplet proteins.

Results: CLA did not affect body weight, however, *fa10-12* rats had smaller livers (g/bwt) and less hepatic steatosis compared to *faCTL*. Although the *fa9-11* and *faCTL* rats had a similar total hepatic lipid concentration, the *fa9-11* rats had fewer cytoplasmic lipid droplets in hepatocytes and these droplets were larger in size compared to *faCTL* rats. The *fa10-12* rats had 65% less total liver lipids than *faCTL* rats, but there were more hepatic lipid droplets. However, this paradox could be explained by the smaller size of the droplets and the resultant decrease in lipid droplet volume. In parallel, the *fa10-12* rats had lower hepatic levels of adipophilin whereas other lipid droplet proteins (TIP-47, OXPAT, caveolin-1, perilipin, phospho-perilipin) were not changed by dietary CLA.

Conclusion: Improved hepatic steatosis by *trans-10,cis-12* CLA in obese, insulin-resistant rats is associated with smaller hepatic lipid droplets and lower levels of adipophilin.

11.45 - 12.00

Increased TAG and DAG contents paradoxically parallel the insulin sensitizing effect of DGAT1 overexpression in rat skeletal muscle

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Background: Fat accumulation in muscle is involved in the development of insulin resistance probably via inhibition of insulin signaling by lipid intermediates. The enzyme 1,2acylCoA:diacylglyceroltransferase-1 (DGAT1) esterifies a fatty-acyl-CoA on diacylglycerol to form triacylglycerol.

Objective: To investigate if unilateral overexpression of DGAT1 in adult rat skeletal muscle will increase conversion of the lipid intermediate diacylglycerol into inert triacylglycerol, thereby improving muscular insulin sensitivity under high-fat-fed conditions.

Procedure: The mouse DGAT1 gene construct was overexpressed by electroporation (one 800V/cm and four 80V/cm pulses) in left tibialis anterior muscle of male rats on chow or high-fat-diet (45%kcal) for three weeks, with the contralateral leg as sham-electroporated control. Seven days after electroporation, muscle-specific insulin sensitivity was assessed with a hyperinsulinemic euglycemic clamp using 2-deoxy-[3H]glucose.

Results: Oil-red-O staining showed a 2-fold increase in lipid accumulation in the DGAT1-overexpressing vs. control leg in animals fed a chow or high-fat-diet. Myocellular 2-deoxy-[3H]glucose incorporation was 20% higher in the DGAT1 overexpressing vs. control leg, both in chow (79.1±8.1 vs. 66.4±6.4 CPM/weight*ml, p=0.03) as in high-fat-fed rats (78.1±6.7 vs. 65.4±6.5 CPM/weight*ml, p=0.05). Muscular diacylglycerol content was elevated in DGAT1 overexpressing leg of animals compared to their control legs on both chow and high-fat-diet (HF: 1599±188 vs. 961±259 μM/g muscle, p=0.073 and chow: 373±15. vs. 243±49 μM/g muscle, p=0.045). Markers of lipolysis (ATGL and CGI58) and mitochondrial function (OXPHOS and PGC1α) were increased in the DGAT1 overexpressing leg of high-fat-fed rats compared to their control legs.

Conclusion: Unilateral DGAT1 overexpression increased muscle insulin sensitivity in parallel with increased DAG content. Interestingly, the increased TAG lipolytic capacity and the concomitant increase in lipolytic co-factors as and markers of oxidative capacity suggest that the balance between the supply and oxidation of fatty acids in skeletal muscle, rather than DAG or TAG levels per se is a determinant of lipid-induced insulin resistance.

Concurrent Session - 15

Lipids and Nutrition: Clinical nutrition

10.30 - 11.00

Invited contribution

Lipids in Critical Care Medicine

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While enteral nutrition is the basis for the critically ill, parenteral nutrition is often used in when a sufficient enteral nutrition is not or not fully achieved. Lipids are a mainstay of caloric supply in both cases as they combine the provision of building blocks for the membranes and are precursors for function molecules including lipid mediators bearing the ability to influence immunity. Pro-inflammatory lipid mediators as prostaglandins and leukotrienes are generated from arachidonic acid a key member of the n-6 polyunsaturated fatty acids (PUFA). In contrast, lipid mediators derived from the n-3 fatty acids eicosapentaenoic acid or docosahexaenoic acid may have reduced pro-inflammatory Resolvins and protectins derived from n-3 fatty acids have been identified as mediators to induce the resolution of inflammation. Modulating the amount of PUFA and the n-6/n-3 ratio was investigated as means to change the inflammatory response and improve the outcome of patients. Studies in patients undergoing major surgery with application of n-3 fatty acids demonstrated beneficial effects in terms reduced of length of stay and infectious complications. Experimental data shows that n-3 fatty acids may improve acute lung injury and sepsis. Clinical data hints that this concept may also improve outcome in critically ill. In addition, experimental and clinical data suggest that a reduction in n-6 PUFA due to inclusion of n-9 monounsaturated fatty acids may change the adaptive immune response and outcome of septic patients. In conclusion, modulating the amount of PUFA and n-6/n-3 ratio may be useful to improve outcome of critically ill patients.

11.00 - 11.15

Supplementation with a fish-oil enriched sip-feed leads to EPA incorporation into white blood cells and enhanced immune responses within one week

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Background: Immune modulatory effects of the n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are well described. However, EPA and DHA must be incorporated effectively into cell membrane phospholipids to modify cell function.

Objective: In order to address the absence of human data regarding short term incorporation in literature, the present study investigates the incorporation kinetics of EPA and DHA within one week of supplementation with a nutritionally complete sip-feed enriched with emulsified fish oil, specific oligosaccharides, high protein and leucine. Additionally, the effects on ex vivo immune function were determined.

Procedure: In a single-arm, open label study, twelve healthy volunteers (62.0 \hat{A} ± 4.5 years) consumed 400 ml of sip-feed providing 2.4 g EPA and 1.2 g DHA daily. Blood samples were taken at days 0 (baseline), 1, 2, 4 and 7. Ex vivo immune function was measured by the production of cytokines in LPS-stimulated whole blood cultures.

Results: After one week of supplementation, the percentage EPA in phospholipids of white blood cell membranes increased from 0.5% to 2.8% ($p < 0.001$), in red blood cell membranes from 0.8% to 2.2% ($p < 0.001$) and in plasma from 1.1% to 5.4% ($p < 0.001$). No change in the percentage DHA in phospholipids of white blood cell membranes was observed, however in red blood cells the percentage DHA increased from 3.5% to 3.9% ($p < 0.001$) and in plasma from 3.5% to 4.8% ($p < 0.001$). The ex vivo LPS-stimulated production of IL-1 \hat{I} ², IL-6, IL-8, IFN- \hat{I} ³ and TNF- \hat{I} [±] in whole blood cultures demonstrated a significant increase ($p = 0.008$) within one week of nutritional intervention.

Conclusion: In healthy volunteers, supplementation with a fish oil enriched sip feed significantly increased the percentage EPA in phospholipids of white and red blood cell membranes and plasma. In addition, the immune response towards LPS was enhanced significantly within one week.

11.15 - 11.30

Modulation by lipid nutrition of inflammatory cell phosphatidylcholine compositions in preterm infants

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Background: Preterm infants requiring intravenous total parenteral nutrition (TPN) to meet metabolic demand are at increased risk of sepsis. The lipid component of TPN influences inflammatory cell membrane lipid composition and could compromise their ability to combat sepsis. Additionally, lipid nutrition changes significantly during the first week of life as the body adjusts from a placental to enteral nutrition.

Objectives: To determine the effects of TPN with Intralipid and of preterm delivery on phosphatidylcholine (PC) compositions of blood lymphocytes and neutrophils.

Procedures: Blood samples were obtained from preterm infants <34 weeks gestation (250 µl) on either enteral or TPN nutrition. Control samples were obtained from term umbilical cords and adult volunteers. Lipids were extracted from purified peripheral blood mononuclear cells (PBMC) and neutrophils. Phosphatidylcholine (PC) molecular species were then analysed by electrospray ionisation mass spectrometry.

Results: PBMC PC compositions were very similar for preterm, cord and adult samples. In contrast PC from preterm neutrophils exhibited a different composition from either cord or adult cells, being enriched in sn-1 18:0 species (PC18:0/18:1, PC18:0/18:2) compared with sn-1 16:0 species (PC16:0/18:1) in controls. TPN was associated with significantly increased content of 18:2-containing species (PC16:0/18:2) at the expense of monounsaturated species (PC16:0/18:1) but no increased content of 20:4-containing species (PC16:0/20:4).

Conclusions: The unusual PC composition of preterm neutrophils was unexpected, and we are currently exploring both the functional implications and whether this was due to cell immaturity or the clinical status of these vulnerable infants. The increased content of 18:2-containing PC species was a direct reflection of the high content of 18:2 in Intralipid. The combined effects of prematurity and type of lipid nutrition are potential contributors to the compromised host defence responses that cause the increased risk of sepsis in this population of infants.

11.30 - 11.45

The effect of omega-3 fatty acids on prevention of vaso-occlusive crisis in homozygous sickle cell disease

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Background: The propensity of red blood cells (sickled and unsickled) to adhere to vascular endothelium and activation of platelets and leukocytes are the primary causative factors of vaso-occlusion in patients with sickle cell disease (SCD). Adhesion, aggregation, elasticity of blood cells and inflammatory response are strongly modulated by cell membrane lipids. Published studies have shown steady state patients with homozygous SCD have abnormal red blood cell fatty acid which is manifested primarily by higher arachidonic and lower eicosapentaenoic and docosahexaenoic levels. The cause of this abnormality and its effect on vaso-occlusion events is not fully understood.

Objectives: The aims were to investigate: (a) the effect of omega-3 supplementation on frequency and severity of vaso-occlusive crisis; (b) whether omega-3 fatty acid supplementation corrects red blood cell membrane fatty acid abnormality reported in previous studies.

Procedure: A double blind placebo controlled randomised trial has been conducted in SCD referral clinic, Khartoum, Sudan. All of the recruited patients (n=130) were supplemented with omega 3 fatty acid (n=65) or placebo (n=65) for one year. Painful crisis leading to hospitalisation, blood transfusion and neurological complications were recorded monthly. Red blood cell fatty acids of samples obtained at baseline and six month were analysed.

Results: Comparative (clinical severity score) and biochemical (red cell fatty acid profile) data will be presented.

Conclusion: The results of this study will help reveal the potential of omega-3 fatty acids for prevention of vaso-occlusive crisis and manifestations in patients with the disease.

11.45 - 12.00**Fish oil and the incidence of post-cardiac surgery atrial fibrillation: a double-blind randomised controlled trial**

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Objective: To examine the effects of dietary fish oil on the incidence of atrial fibrillation (AF) post cardiac surgery (coronary artery bypass grafting and/or valve repair/replacement).

Procedure: 200 participants were randomly allocated to ingest 15 mL citrus-flavoured fish oil (18%EPA, 12%DHA) or sunola oil (monounsaturated) each day commencing 3 weeks before the scheduled surgery date. The oils were continued during the post-operative in-patient period. The primary outcome measure was incidence of AF of at least 10 min duration or requiring intervention during the first 6 post-operative days or until discharge if earlier. This measure was assessed independently by two investigators. The primary endpoint was assessed by Fisher's Exact Test and was analysed on an intention-to-treat basis. Time to first episode of AF was assessed by Kaplan-Meier analysis and Log-rank test.

Results: 189 subjects completed the study (95 in the treatment group and 94 in the placebo group). The overall incidence of AF was 41.3%, with 35.8% in the treatment group and 46.8% in the placebo group ($p=0.14$, Fisher's Exact Test, RR 0.76, 95% CI: 0.54 to 1.08). Kaplan-Meier analysis showed fish oil supplementation resulted in a non-significant reduction in the time to first event ($p = 0.11$, log-rank test).

Conclusion: Fish oil was associated with a non-significant reduction in the incidence of post-operative AF.

Concurrent Session - 16**Lipids and Health: Cardiovascular health****14.00 - 14.30*****Invited contribution*****N-3 polyunsaturated fatty acid supplementation for the prevention and treatment of heart failure**

W Stanley

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The clinical syndrome of heart failure is complex and has multiple etiologies. Current treatments slow clinical progression, nevertheless prognosis remains poor for most patients. Epidemiological studies find high dietary intake of n-3PUFA from fish oils (docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)) is associated with decreased new onset heart failure, and supplementation with a low dose of DHA+EPA increases event free survival in heart failure patients. The underlying mechanisms responsible for these benefits are unclear. DHA and EPA can lower plasma triglyceride levels, and exert anti-inflammatory effects both systemically and in the heart by lowering inflammatory cytokines and depleting arachidonic acid in myocardial phospholipids. Animal studies show fish oil can favorably alter the function of cardiac mitochondria, which is associated with alterations in cardiac phospholipid, specifically an increase in DHA, depletion of arachidonic acid, and elevation of cardiolipin. Moreover, we recently showed improved tolerance to Ca^{2+} in cardiac mitochondria following supplementation with DHA. There is some evidence to suggest that high intake of α -linolenic acid is cardioprotective in heart failure, but the evidence is limited and not as compelling as with DHA+EPA. In summary, emerging evidence suggests that supplementation with n-3PUFA suppresses established pathophysiological mechanisms in heart failure, and may be effective for preventing and treating this malignant syndrome.

14.30 - 14.45

Induction of cardiac Angptl4 by dietary fatty acids is mediated by PPAR α and protects against oxidative stress

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Background: Little is known about the direct effects of dietary fatty acids on gene expression in the intact heart.

Objective/Procedure: In the present paper we show that oral administration of synthetic triglycerides composed of one single fatty acid alters cardiac expression of numerous genes, many of which are involved in the oxidative stress response.

Results: The gene most significantly and consistently upregulated by dietary fatty acids encoded angiotensin-like 4 (Angptl4), a circulating inhibitor of lipoprotein lipase expressed by cardiomyocytes. Induction of Angptl4 was specifically abolished in peroxisome proliferator activated receptor beta/delta (PPAR β/δ) -/- and not peroxisome proliferator activated receptor alpha (PPAR α) -/- mice. Consistent with these data, fatty acids stimulated binding of PPAR β/δ but not PPAR α to the Angptl4 gene. Upregulation of Angptl4 resulted in decreased cardiac uptake of plasma triglyceride (TG)-derived fatty acids and decreased fatty acid-induced oxidative stress and lipid peroxidation. In contrast, Angptl4 deletion led to enhanced oxidative stress in the heart, both after an acute oral fat load and after prolonged high fat feeding.

Conclusion: We conclude that stimulation of cardiac Angptl4 gene expression by dietary fatty acids and via PPAR β/δ is part of a feedback mechanism aimed at protecting the heart against lipid overload and consequently fatty-acid induced oxidative stress.

14.45 - 15.00

Eicosapentaenoic acid plus docosahexaenoic acid enhances aspirin's effect on platelet function

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Background: Aspirin inhibits platelet function by acetylating cyclooxygenase, downregulating the production of thromboxane from arachidonic acid. Although aspirin has been a stalwart drug for the prevention and treatment of cardiovascular disease, many individuals do not benefit from its use: a problem termed "aspirin resistance".

Objective: As eicosapentaenoic acid and docosahexaenoic acid (EPA/DHA) are competitive inhibitors for cyclooxygenase, we investigated whether EPA/DHA enhances the effect of aspirin on platelet function in a group of healthy adults.

Procedure: We conducted a blinded, randomized, placebo-controlled, clinical trial in 25 healthy individuals. Each subject received a single dose of all of the following agents at 4 study visits at least 4 weeks apart: placebo; 81 mg aspirin; 3.4 g of EPA+DHA (4 g Lovaza®; n-3 long-chain polyunsaturated fatty acid {LCPUFA}); and both EPA+DHA and aspirin. On the evening prior to each appointment, each ate a consistent low-fat dinner, fasted for 8 hours, then ate a low-fat breakfast after each agent dose. Platelet function was measured with the Platelet Function Analyzer-100 (PFA-100®), using closure time (CT) units (greater time indicates reduced platelet function), immediately before and 4 hours post agent. The Wilcoxon Signed Rank test was used to test for statistical significance.

Results: Compared to placebo, aspirin and Lovaza® alone had no significant effect on CT, but the combination of aspirin and Lovaza® increased CT by a mean of 34 seconds, from 131 to 165 (p=0.019).

Conclusion: Our data suggest that low-dose aspirin and EPA+DHA exert a synergistic downregulatory effect on platelet function. Perhaps what is known as "aspirin resistance" may be more accurately described as "EPA+DHA deficiency".

15.00 - 15.15

The effect of dietary fish oil on metabolic risk markers and body composition in slightly overweight teenage boys

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Objective: We hypothesized that intake of long-chain n-3 polyunsaturated fatty acid (n-3 LCPUFA) may have profound effects during growth and development, and we therefore investigated if fish oil (FO) affect markers of the metabolic syndrome, inflammation, and metabolic rate in teenage boys.

Procedure: Seventy-eight slightly overweight 13-15 year-old boys were randomized to FO (1.5 g/d of n-3 LCPUFA) or a mixture of typical vegetable oils (control) given daily for 16 weeks in two pieces of rye bread and a wheat roll. All boys were furthermore counselled to improve diet and exercise habits. Body composition (DEXA), metabolic rate (ventilated HOOD), glucose tolerance (OGTT), blood pressure (BP), and circulating markers of metabolic risk were determined at baseline and after 16 weeks.

Results: The boys had an average whole-body fat% of ~30 at baseline and their average vertical growth rate during the intervention was ~6 cm/year. The FO-group had higher erythrocyte (RBC) content of eicosapentaenoic acid (EPA) at 16 wks, 1.2 ± 0.5 vs. 0.6 ± 0.3 % in the control-group ($P > 0.001$). Body fat % was reduced in both groups during the intervention ($p < 0.02$), but this was not affected by FO. The FO-group had 3.8 ± 1.4 mmHg lower systolic ($p < 0.006$) and 2.6 ± 1.1 mmHg lower diastolic BP ($p < 0.01$). Plasma HDL- and non-HDL-cholesterol increased 5 and 7% in the FO-group and decreased 2 and 0% in the control group ($p < 0.01-0.02$). The change in RBC-EPA was inversely correlated with the change in systolic and diastolic BP, and directly correlated with the increase in HDL- and non-HDL-cholesterol. No significant effects of FO were found on any of the remaining parameters. The lifestyle counselling resulted in decreased sugar intake and increased fibre intake, but no change in physical activity level.

Conclusion: The results indicate that FO-supplementation has positive as well as negative effects on markers of metabolic risk in adolescent boys.

15.15 - 15.30

Systolic function is improved in overweight type 2 diabetic subjects after physical activity training without changes in cardiac lipid content

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Background: Excessive lipid storage in cardiac muscle has been suggested to hamper cardiac function via 'lipotoxic' pathways. We reported earlier that physical activity training in healthy overweight subjects improves systolic function and diminishes cardiac lipid content. It is yet unknown whether diabetic subjects respond similarly.

Objective: To investigate whether physical activity improves cardiac function and decreases cardiac lipid content in type 2 diabetic subjects.

Procedure: Eleven overweight male patients with type 2 diabetes mellitus (age: 59.5 ± 0.9 years, BMI: 30.5 ± 1.3 kg/m², fasting plasma glucose: 9.1 ± 0.6 mmol/l, HbA1c: 7.1 ± 0.3 %) followed a supervised 12-week physical activity training program (three sessions/week). Before and after training, systolic function was determined by CINE-MRI, cardiac lipid content was determined by image-guided Magnetic Resonance Spectroscopy (PRESS-sequence; TE=26ms; TR=4s, ECG-triggered and respiratory-gated with pencil beam navigator), and maximal oxygen uptake (VO₂max) was measured.

Results: VO₂max and ejection fraction were improved after training (VO₂max: from 27.1 ± 1.5 ml/min/kg to 30.1 ± 1.6 ml/min/kg ($p = 0.001$), EF from 50.5 ± 2.0 % to 55.6 ± 1.5 % ($p = 0.001$)). Surprisingly, cardiac lipid content did not decrease with training (from 0.80 ± 0.07 % to 0.96 ± 0.07 %, $p = 0.15$ (intensity of CH₂-resonance of lipids relative to water-resonance)).

Conclusion: While the currently employed training program increased cardiac function in this group of diabetic subjects, it did not affect cardiac lipid content. This is in contrast to our earlier findings in healthy overweight subjects, where the same training intervention diminished cardiac lipid content. These observations may indicate hampered exercise-induced lipid mobilization in the diabetic heart and reveal that reduction of cardiac lipids is not a prerequisite for the training-induced improvement in cardiac function.

Concurrent Session - 17

Biochemistry of Lipids : XXX-omics

14.00 - 14.30

Invited contribution

Lipidomics in health & disease

K Ekroos

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Lipidomics is defined as the systems-level analysis of lipid species, their abundance, biological activity, as well as sub-cellular localization and tissue distribution. Technological advances in mass spectrometry and associated method development have produced lipidomic techniques capable in characterizing and quantifying hundreds of molecular lipid species directly in total lipid extracts. Moreover, the high detection precision at the molecular lipid level prevents false-positive identifications, a necessity for quality lipid research and enables studies requiring GLP. The identification of lipid metabolic dysfunctions responsible for disease pathology also requires the coupling of customized bioinformatic tools to the analytical chemistry of lipidomics. Molecular lipids are the window to meaningful biological insight and a prerequisite for piecing together the molecular mechanisms of health and disease processes. Finally, molecular lipidomics is a valuable addition to the toolkits used both in biomarker discovery and in the identification of new drug targets.

14.30 - 14.45

Lipidomics and proteomics of some high density lipoprotein subclasses in healthy human subjects

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Background: High density lipoproteins (HDLs) have a role in the progression and regression of atherosclerosis, but their exact function is not well established yet. Measurement of plasma HDL cholesterol is of low predictive value and its use as a biomarker in assessing the individual's risk of cardiovascular diseases is now questionable.

Objective: We believe that some of HDL subclasses are better candidates as biomarkers especially the small ones. Alteration in the chemical composition of HDL subclasses may impair function and reduce their antiatherogenic properties. However, data about the proportions of lipid components and their fatty acid constituents in HDL subclasses are scarce in the literature and are not consistent.

Procedure: We analyzed the apolipoproteins and the lipid classes: triacylglycerols, cholesteryl esters and phospholipids, and their fatty acid composition in seven HDL subclasses isolated by the new method of electrofiltration.

Results: All the subclasses contained apolipoprotein A-I, cholesteryl esters, triacylglycerols, Phosphatidylcholine, and sphingomyelin, whereas other lipoproteins, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine were detected in some subclasses. There was a wide variation in the phospholipids distribution in the subclasses. We identified ten fatty acids in these lipid classes where six of them were saturated and four were unsaturated. The fatty acids 16:0 and 18:0 were the most abundant in all lipids of all the subclasses, whereas the other fatty acids showed variable distribution.

Conclusion: Our results show the variability of the chemical composition of HDL subclasses and explain the microheterogeneity of HDL particles due to differences in composition especially the fatty acids. We suggest that the proportion of phosphatidylethanolamine among HDL subclasses can be related to the impaired clearance of the small HDL

subclasses in cases of hyperlipoproteinemia, diabetes, and alcohol intake. Studying the detailed composition of HDL subclasses is crucial for better understanding of their role in atheroprotection and other pathological conditions.

14.45 - 15.00

Identification of on-off switch lipid genes in the intestine of a model system

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Background: The field of nutrigenomics seeks to identify the changes in gene expression that are elicited by nutrients.

Objective: The intestinal tissue is the first to experience altered dietary composition. The aim of the present study was to identify differentially expressed genes in the intestine after feeding in a convenient model system.

Procedure: Serial analysis of gene expression (SAGE) based on the enumeration of directionally reliable short cDNA sequences (tags), provides qualitative as well as quantitative analysis of a large number of genes in a defined tissue. Data analysis and tag-to-gene mapping were performed using a generic computer package as previously described (Knoll-Gellida et al., BMC Genomics 7: 46, 2006).

Results: Comparison of transcriptome data of zebrafish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*) anterior intestine in fed and fasted conditions using the SAGE method identified tightly regulated genes at the transcript level allowed for the identification of activated/deactivated pathways. Transcript levels of representative genes were validated by qPCR. Whole-mount in situ hybridization screening on zebrafish larvae identified a subset of lipid bimodal genes that were switched specifically in the enterocytes by nutritional factors. During the alimentary absorption peak, the level of up-regulation of some transcripts was found to be correlated to the lipid composition of the food.

Conclusion: The molecular phenotype described provides groundwork for future experimental approaches aimed at identifying functionally important nutrients involved in the development of the digestive system. In addition, it may facilitate further metabolic studies in zebrafish as a model for human diseases and the study of transcriptional events and molecular pathways disrupted by environmental toxicants.

15.00 - 15.15

A nutrigenomic approach reveals that LXR is required for hepatic steatosis induced by essential fatty acid deficiency

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Background: In mammals, dietary essential polyunsaturated fatty acids (PUFAs) influence the expression of genes involved in de novo hepatic fatty acid biosynthesis (lipogenesis). A deficiency in dietary PUFAs results in increased expression of lipogenic enzymes in the liver and ultimately in aberrant triglycerides (TGs) accumulation (hepatic steatosis). The Liver X Receptor (LXR) is a nuclear receptor that plays a part in the transcriptional control of lipogenesis. The hypothesis that LXR is involved in mediating the effects of dietary fatty acids on lipogenesis has been proposed but remains controversial.

Objectives: We used a nutrigenomic approach to investigate the role of LXR in the hepatic response to dietary lipids.

Procedures: We used wild-type (W-T mice) and transgenic mice lacking isoforms of LXR (LXR^{-/-} mice). These adult male mice were fed forband aboth nine weeks with three isocaloric diets containing similar amount of fatty acids. However, the three diets contained no PUFAs, standard PUFA proportion, or a high PUFA proportion.

Results: In mice of both genotypes the effect of the diets on hepatic PUFA levels was assessed through fatty acid profiling. We observed that the deficiency in dietary PUFAs induced an expected steatosis paralleled with a marked increase in lipogenic genes expression in W-T mice but not in LXR^{-/-} mice.

Conclusions: These data highlight for the first time the role of LXR in the response of hepatic lipogenesis to dietary PUFAs. Interestingly, we observed that dietary PUFAs markedly influence the expression of enzymes involved in both cholesterol and oxysterol metabolism. Oxysterols are described as natural activating ligands for LXR. Therefore, we postulated that the marked regulation of enzymes involved in oxysterol synthesis that occurs in response to dietary PUFAs may influence lipogenesis via LXR.

Concurrent Session - 18

Lipids and Nutrition : Maternal and infant nutrition III - Allergy and immune function

14.00 - 14.30

Invited contribution

Red blood cell levels of docosahexaenoic acid and other polyunsaturated fatty acid levels in pregnant women are modified by single nucleotide polymorphisms of the FADS gene cluster: results from the ALSPAC study

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Background: Long-chain polyunsaturated fatty acid (LC-PUFA) contents in blood and tissues are associated with early development of visual, cognitive and immune functions as well as lifelong health. LC-PUFA blood and tissue levels depend both on dietary intake and endogenous conversion of precursor polyunsaturated fatty acids (PUFA) by the enzymes delta-5 desaturase (D5D) and delta-6 desaturase (D6D). Previously, we and others have shown associations of polymorphisms (SNPs) of *FADS1* (D5D) and *FADS2* (D6D) with several n-6 and n-3 fatty acids, in particular with arachidonic acid (1-3). However, there is little evidence on genetic effects on levels of docosahexaenoic acid (DHA), which are considered particularly important for brain and retina function. Moreover, the functional role of *FADS3* is unclear. We explored the relationship between polymorphisms of the *FADS* gene cluster and red blood cell PUFA levels in pregnant women participating in the Avon Longitudinal Study of Parents and Children.

Methods: Red blood cell phospholipid FA were determined from 6711 samples of 4457 women obtained throughout pregnancy (gestational age 26.8± 8.2 weeks, mean±SD). We determined 3 SNPs of *FADS1*, 9 of *FADS2*, 4 intergenic SNPs and 1 of *FADS3*.

Results: Minor SNP alleles were consistently positively associated with the precursor FA and negatively with LC-PUFA as well as product/substrate ratios of the n-6 (AA/LA ratio) and the n-3 (EPA/ALA ratio) pathways. In contrast to previous studies, we also found strongly significant inverse associations to the DHA). A minor *FADS3* polymorphism was associated with increased PUFA and decreased LC-PUFA levels.

Conclusions: The results of this largest available cohort study on the relationship between the *FADS* polymorphisms and PUFA status markers shows a consistent association of the minor alleles of the tested SNPs in the *FADS1* *FADS2* gene cluster with increased levels of desaturase substrates and decreased levels of desaturase products of both the n-6 and the n-3 pathways, compatible with a decline in desaturase expression or activity due to the polymorphisms. In contrast to previous studies, we also found a consistent significant association of the rare SNP alleles with lower levels of DHA in red blood cell phospholipids of pregnant women, which might be due to the greater statistical power of this study and/or a higher rate of DHA synthesis in pregnant women compared to men or non-pregnant women. Given the strong association of an SNP in the *FADS3* gene with PUFA levels, we consider it highly likely that a gene product of *FADS3* has desaturating activity.

Supported in part by the UK Medical Research Council (MRC), the Wellcome Trust, the University of Bristol, Scotia pharmaceuticals, the Commission of the European Communities, Competence Network for Adiposity funded by the German Federal Ministry of Education and Research, the Munich Center of Health Sciences, a Freedom to Discover Award of the Bristol-Myers-Squibb Foundation, and NOAA.

References:

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14.30 - 14.45**Allergy and respiratory outcomes from the DINO (DHA for the improvement of neurodevelopmental outcome in preterm infants) trial**

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Background: Docosahexaenoic acid (DHA) may reduce childhood atopy. The DINO trial is the first long-term outcome study of DHA supplementation in preterm infants.

Objective: We report the effect on allergic and respiratory symptoms overall and in prespecified subgroups.

Procedure: DINO is a multicentre RCT designed to study the long-term efficacy of high-dose dietary DHA in infants less than 33 weeks' gestation. Lactating women received tuna oil supplements to increase the DHA content of their milk. Preterm infant formula with matching DHA composition was used if there was insufficient breast milk. Treatment continued until term.

Results: 657 infants were enrolled, 93.5% completed 18-month follow-up. DHA significantly reduced parental report of medically diagnosed hayfever at either 12 or 18 months of corrected age in male infants ($p=0.01$), and was associated with a borderline reduction in all infants with birth weight (BW) ≥ 1250 g ($p=0.05$). There was no effect on asthma, eczema, or food allergy. There was a reduction in supplemental oxygen use at 36 weeks corrected age in male infants ($p=0.03$) and all infants with BW <1250 g ($p=0.04$). There was no effect on duration of respiratory support, length of admission or requirement for home oxygen.

Conclusions: DHA supplementation for infants less than 33 weeks' gestation reduces hayfever in male infants. DHA reduces bronchopulmonary dysplasia in male infants, and in all infants <1250 g.

14.45 - 15.00**PUFA levels at 6 months of age after oil supplementation from birth predict symptoms of atopic dermatitis, cough and wheeze**

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Background: Epidemiologic studies suggest that the consumption of oily fish may protect against allergic disease, and this is further supported by the anti-inflammatory properties of n-3 polyunsaturated fatty acids (n-3PUFA).

Methods: Blood samples were collected from a subgroup of 115 children enrolled in a randomised control trial in which 420 children received either DHA enriched fish oil (280mg DHA per day) or a placebo (an equivalent capsule of oleic acid) from birth to 6 months of age. The occurrence of symptoms of eczema, wheezing and recurrent cough by the end of the supplementation period were assessed in relationship to PUFA levels in red cell membranes.

Results: After supplementation from birth, children who had received fish oil ($n=55$) had significantly higher levels of 22:6 n-3 DHA ($p=0.011$) and significantly lower 20:4n-6 AA ($p=0.001$) than the placebo group ($n=60$). In this subgroup with fatty acids levels measured, there was no difference in the development of symptoms between the groups. However, higher levels of total n-3 PUFA at 6 months were associated with reduced risk of persistent respiratory symptoms such as persistent and recurrent coughing (OR= 0.75; 95%CI 0.57-0.97; $p=0.029$). In contrast, higher levels of total n-6 PUFA were associated with an increased risk of developing symptoms of eczema (OR= 1.13; 95%CI 1.01-1.26; $p=0.037$) at 6 months.

Conclusion: Variations in n-3 and n-6 fatty acids levels at 6 months are associated with altered risk of cutaneous and respiratory symptoms. These effects were independent of postnatal supplementation, suggesting that maternal sources of PUFA in pregnancy and breast milk could be more important for determining infant PUFA status and risk of symptoms.

15.00 - 15.15

Salmon in pregnancy study (SIPS): effects of increased oily fish intake during pregnancy on neonatal immune responses and clinical outcomes

PS Noakes, M Vlachava, LS Kremmyda, ND Diaper, EA Miles, PC Calder
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Objective: To determine whether oily fish intervention in pregnancy modifies neonatal immune responses and early markers of atopy.

Methods: At 20 weeks gestation, 123 allergic, pregnant women with low habitual intake of oily fish (< 2/month) were randomised to consume two portions of salmon per week (each portion of salmon provided about 2 g of n-3 PUFA) or to continue their habitual diet of low oily fish consumption until delivery. Cord blood was collected at birth (n=101) and from the infants at 6 months of age (n=86). Neonatal PUFA levels and immunological responses (both innate and adaptive) to various stimuli were measured at birth. Total immunoglobulin (Ig) E was measured at birth and 6 months of age. Infants attended a clinic at 6 months of age for assessment of allergic sensitization (skin prick test [SPT]) and presence and severity of atopic dermatitis (AD).

Results: Oily fish consumption during pregnancy was associated with a significantly lower neonatal regulatory cytokine (IL-10) response to both LPS (p<0.05) and PHA (p<0.05). Similar trends were also seen for TNF- α (pro-inflammatory) and IFN- γ (Th1) responses, although the differences between groups were not statistically significant. There were no statistically significant differences in total IgE at birth or 6 months of age or in SPT positivity or AD at 6 months of age.

Conclusions: Oily fish intervention in pregnancy modified the pattern and magnitude of neonatal immune responses, but did not affect early markers of atopy. Further assessment of cellular immune function and clinical follow-up of these infants will determine if there are any effects of maternal oily fish intake on postnatal immune development and expression of allergic disease.

15.15 - 15.30

The effects of salmon consumption during pregnancy on breast milk fatty acid composition and immune function

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Background: Changes in breast milk long chain (LC) polyunsaturated fatty acid (PUFA) composition and immune function have been seen in intervention studies with fish oil supplementation during pregnancy and lactation.

Objective: To determine how consuming salmon during pregnancy may affect breast milk fatty acid composition and immune function.

Procedure: Women (n = 120) were recruited from a population who rarely ate oily fish and were at risk of having a child with atopy. They were randomly assigned to either remain on their normal diet or to include two portions of salmon per week from 20 weeks pregnancy until birth. Breast milk samples were collected on days 1, 5, 14 and 28 post-partum and analysed with gas chromatography for fatty acid composition and enzyme linked immunosorbent assay for sCD14, TGF-B1, TGF-B2 and sIgA.

Results: Day 5 breast milk samples from the salmon group had higher total saturated fatty acids (p<0.05), eicosapentaenoic acid (p<0.02), docosapentaenoic acid (p<0.001), docosahexaenoic acid (DHA; p<0.001) and lower total monounsaturated fatty acids (p<0.05). By day 28 the difference was only significant for DHA (p<0.01). Total n-6 PUFA did not differ significantly between groups. However the total n-6 PUFA / n-3 PUFA ratio was lower for the salmon group (p<0.01). All immune factors in breast milk decreased with time (p<0.001). Breast milk sIgA (p<0.01) and TGF-B1 (p<0.05) were lower in the salmon group. TGF-B1 correlated with TGF-B2 (r = 0.746, p< 0.001).

Conclusion: Salmon consumption during pregnancy significantly affects n-3 PUFA content of breast milk in early lactation and influences the breast milk content of some immunomodulatory factors.

Plenary: Diet and Nutrition 2 : Clinical nutrition (ESPEN)

16.00 - 16.45

Lipid droplets as modulator of inflammatory response

P Bozza

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Increased lipid accumulation within cytoplasmic lipid droplets (also known as lipid bodies or adiposomes) in nonadipocytic cells are commonly observed pathological features of a number of infectious, neoplastic and other inflammatory conditions. We investigated the mechanisms involved in lipid droplet formation and function in different infectious and neoplastic conditions. The mechanisms that govern lipid droplet biogenesis are highly regulated, and involve cell and stimulus-dependent pathways, that culminate in the compartmentalization of a specific set of proteins and lipids that may vary upon the stimulatory condition, providing evidence of functional heterogeneity of lipid droplets. In leukocytes and other cells of the inflammatory response lipid droplets are rapidly inducible organelles with roles in cell signaling and control of the synthesis and secretion of inflammatory mediators. Substantial progresses have been made demonstrating that enzymes involved in eicosanoid syntheses localize at lipid droplets and direct localization of sites of eicosanoid synthesis by EicosaCell technique has identified lipid droplets as major sites for eicosanoid generation in inflammatory and cancerous cells. Thus, favoring the heightened production of prostaglandins and leukotrienes and participating in the amplification of the inflammatory response. Close interactions of intracellular pathogens and lipid droplets have been observed, suggesting that pathogens may exploit lipid droplets for their growth. Moreover, inhibition of lipid droplet formation has roles in the modulation of host response to infection and cancer cell growth. Collectively, lipid droplets are emerging as organelles with major functions in cell signaling, critical regulators of different inflammatory diseases, and attractive targets for novel anti-inflammatory and anti-neoplastic therapies.

Financial Support: CNPq and FAPERJ

16.45 - 17.30

Fatty acids as amplifiers of anti-cancer therapy

P Bougnoux

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The rationale for increasing the activity of anticancer treatments through a dietary intervention results from a body of circumstantial facts: i) sensitivity of breast cancer cells to anticancer chemotherapy can be increased when cell membranes are enriched with docosahexaenoic acid¹ (DHA); ii) sensitivity of experimental, autochthonous rat mammary tumors to anticancer drugs is substantially enhanced by a dietary intervention with fish oil or DHA², and this observation also applies to ionizing radiation³; iii) in humans, during neoadjuvant chemotherapy for large breast malignant tumors, the efficacy of anticancer drugs on the tumors is greater in patients with elevated DHA level in adipose tissue than in patients with low DHA⁴; iv) we recently carried out a dietary DHA intervention trial in metastatic breast cancer patients, and found survival to be increased when DHA was incorporated, thus documenting the feasibility, safety and interest of this approach during chemotherapy⁵.

Dietary polyunsaturated fatty acids such as DHA integrate into cells, leading to an enrichment of their membrane phospholipids. Targets for their actions have been individualized both in vitro and in vivo. These include a direct action at the cell membrane, or an indirect effect through their peroxidizability. DHA may alter cell antioxidant defenses⁶ through the regulation of the transcription of their genes. Peroxidation of these highly unsaturated fatty acids by the oxidative stress resulting from chemotherapy leads to deep alterations of cellular pharmacodynamics of anthracyclines⁷. It also destabilizes membranes of cell organelles such as mitochondria, which may amplify cell death. DHA integrates into endothelial cells, translating in an increase in tumor microvessels permeability with subsequent entrance of oxygen and anticancer drugs. The integration of these fatty acids into other cells within the tumor microenvironment may account for the multiplicity of their effects on the host, whose nutritional status appears as a determinant of tumor sensitivity to anticancer agents. Clinical trials are needed to precisely evaluate the role of these dietary fatty acids in breast cancer treatments⁸.

Posters - Tuesday June 1

Lipids and Health

P186

The effect of PUFAs on bovine oocyte development: a mechanistic approach

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Background: The effect of dietary polyunsaturated fatty acid (PUFAs) on reproductive performance in cattle is diverse among different studies. Changes in dietary FAs are reflected in the composition of the follicular micro-environment and may exert a direct effect on oocytes maturation. PUFAs are precursors for PGE and are known to affect mitogen activated protein kinases (MAPKs) both of which are important for oocyte maturation.

Objectives: To examine the effect of linoleic acid (LA) and α -linolenic acid (ALA) on oocyte developmental potential. Involvement of PGE and MAPK pathways was also investigated.

Procedure: Different combinations of PUFAs (LA or ALA), PGE₂, specific COX-2 inhibitor (NS398) and/or MAPK-kinase inhibitor (U0126) were supplemented to oocyte maturation media. Stage of oocyte nuclear maturation was determined by aceto-orcein staining after 24hrs of culture. PGE was measured in the spent media by radioimmunoassay. MAPK phosphorylation was determined using western blotting.

Results: Maturation, cleavage and blastocyst rates were significantly lower in LA-treated and higher in ALA-treated groups. The concentrations of PGE were significantly increased by LA (>10X) and ALA (2-3X). Addition of 500 ng/ml PGE to FA-free and gonadotrophin free media increased maturation rate. NS398 significantly inhibited oocyte maturation; an effect which was completely abrogated in the presence of ALA, and resulted in increased PGE. ALA significantly increased MAPK 1 and 3 phosphorylation during the first 6h of maturation. However, LA significantly decreased phosphorylation levels after 24h. U0126 had an inhibitory effect on oocyte maturation associated with a significant decrease in PGE synthesis. ALA supplementation in the presence of U0126 failed to restore PGE synthesis and the maturation level was significantly inferior to the control.

Conclusion: PUFAs have a differential role in controlling oocyte maturation in vitro. This effect involves changes in the hormonal and molecular regulation of oocyte maturation mediated through a MAPK and PGE dependant pathway.

P187

Does altered EPA and DHA metabolism contributing to cognitive decline: impact of aging and ApoE4

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Background: How fish intake may protect humans against cognitive decline is actually not well understood, but one possibility is the incorporation into membranes of two fatty acids in fish - eicosapentaenoic (EPA) and docosahexaenoic (DHA). However, aging and apolipoprotein E ϵ 4 (ApoE4), the two most important non-modifiable risk factor of cognitive decline, seems to alter the metabolism in EPA and DHA.

Objective: To overview the evidences that aging and/or carrying an ApoE4 allele alter EPA and DHA metabolism and to evaluate whether it can impact on the risk of cognitive decline.

Results: Recent evidence suggests that blood EPA and DHA are age-dependent and may be modulated by ApoE4. In elderly, EPA is approximately 50% higher compared to young adults. After supplementation with EPA+DHA, the increase in DHA is higher while EPA remains about 50% higher in the elderly. In ApoE4 carriers, the concentration of EPA and DHA is higher in plasma triglycerides but the increase in EPA and DHA after supplementation is lower compared to the non-carriers. However, whether aging interacts with ApoE4 in the metabolism of EPA and DHA is still unknown. Our recent studies suggest that the triglycerides and the free fatty acids in plasma lipids are possibly the most altered by aging or ApoE4. Whether there is a direct link between higher EPA+DHA in blood and better scores on cognitive testing still needs further investigation but some studies already support such a direct link between the two.

Conclusion: Since fish intake lowers the risk of cognitive decline but age and genetics increase the risk and since age and genetics seems to alter EPA and DHA metabolism, then the latter may compromise transport, delivery and utilization of EPA and DHA and possibly contribute to alter brain functions.

P188

Omega-3 fatty acids in bipolar patients with a low omega-3 index and reduced heart rate variability: the 'Bipo-3 trial'

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Background: Patients with bipolar disorders are at an increased risk of cardiovascular morbidity and mortality. Even when euthymic a substantial proportion of these patients shows signs of autonomic dysregulation as reflected by decreased heart rate variability (HRV). Recent studies suggest that 1-2 g of omega-3 fatty acids are effective in the treatment of bipolar depression. At the same time several studies have shown the ability of these fatty acids to improve measures of HRV in different patient groups. Apart from treating conventional cardiovascular risk factors, there is no clearly effective treatment to lower the increased cardiovascular risk in patients with affective disorders.

Objective: To test the hypothesis that parameters of HRV (SDNN, LF/HF) can be improved by the addition of omega-3 fatty acids in euthymic bipolar patients with a low omega-3 index (<5%) and reduced HRV (SDNN < 60msec).

Procedure: Based on a formal power analysis we designed a randomized, double-blind, placebo-controlled study to test this hypothesis.

Results: 51 patients, fulfilling the DSM-IV criteria for bipolar disorders, will be treated in a 12-week parallel study design with omega-3 fatty acids or corn oil, in addition to usual treatment. At baseline and at the end of the study period HRV as well as the omega-3 index will be measured. During these 12 weeks, every three weeks, there will be an assessment of mood symptoms, using a variety of established rating scales. In addition blood will be drawn for safety reasons.

Conclusion: The results of this study will help answering the question whether a low omega-3 fatty acid status, as defined by a low omega-3 index, might be causally related to the increased risk of cardiovascular morbidity and mortality in patients with bipolar disorders.

P189

Intake of omega-3 correlates with changes in cognitive function and levels of eicosapentaenoic acid and omega index in elderly subjects

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Objective: To evaluate if four months supplementation can improve cognitive function in elderly subjects and if there is any correlation between cognitive function and the increase in omega-3 fatty acids in the blood.

Materials and Methods: 28 elderly subjects, age 70-82, were given 10 ml of omega-3 formulation daily for 4 months, containing 3.2 g omega-3 fatty acids (EPA 1.4 g, DHA 0.9 g), Oleic acid 1.2 g, GLA 100 mg, vitamin D 10 microgram, vitamin E 20 mg, coenzyme Q10 20 mg and a mixture of natural antioxidants.

Mini Mental State Examination test was used to evaluate if the participants had a normal cognitive function, and then with the Trial Making Test (seconds to complete test) TMT - A test for visual ability and TMT - B test for executive functions.

Whole blood content of EPA and the Omega index (EPA/AA x100) was measured according to Jabbar and Saldeen (2006).

Results: 26 subjects had normal MMSET tests, average 28 points. Two subjects had a low score, 24, indicating cognitive impairment before the supplementation. TMT - A score from 60.3 to 50.8 seconds after supplementation (p<0.01). TMT - B from 144.4 to 119.2 seconds after supplementation (p<0.05). Total score TMT A+B from 204.7 to 170.0 seconds after supplementation (p<0.001). Whole blood EPA was 0.33 % before supplementation and increased to 0.99 % after supplementation (p<0.001). Omega index changed from 7 to 20 after supplementation (p<0.001).

Cognitive function showed a positive correlation to increase in whole blood EPA (p<0.05), as well as increase in Omega index (p<0.01).

Conclusion: Intake of the omega-3 formulation improved cognitive function and correlated with whole blood EPA and Omega index. The positive correlations indicate a causal relationship between omega-3 and cognitive function in elderly subjects.

P190

Changes in omega-3 index in healthy individuals after intake of cod liver oil with different quality. a randomized controlled trial

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Background: The n-3 index has been suggested as a risk factor for coronary heart disease and cardiac death. To our knowledge, it is still not clear whether supplementation with adequate dose n-3 FA with different quality will influence the n-3 index. In addition, for later intervention with n-3 FA, it would be useful to investigate

to what extent abstaining from intake of marine n-3 FA in a four week period has on this biomarker.

Objective: The aim of this study was to investigate the effect on the n-3 index in healthy individuals 1) when abstaining from intake of marine n-3 FA for 4 weeks and 2) with respect to the intake of cod liver oil capsules with different quality.

Procedure: A double-blinded randomized controlled trial originally designed to investigate the health effects of cod liver oil with altered quality among healthy individuals, was performed. After the 4 week wash-out period the participants were randomized to three different groups receiving capsules containing either 9 g cod liver oil (1.5 g EPA and DHA/d) with highly different PV and AV values or 9 g sunflower oil as control oil for three weeks. Fasting blood samples were collected at week 0, 4 and 7, and FA from dried whole blood samples were measured by gas chromatography.

Results and Conclusion: Preliminary result shows that the n-3 index level at the screening visit was 5.1 % (95% CI (4.8,5.5)) from a total of 57 subjects (17 men and 40 women, aged 19-49 yr, BMI 17.3-30.1 kg/m²). The effect of abstaining from dietary intake of n-3 FA for four weeks and after three weeks of daily cod liver oil supplementation with different quality on the n-3 index level will be presented.

Abbreviations: EPA, Eicosapentaenoic acid; DHA, docosahexaenoic acid; FA, fatty acids; PV, peroxide value; AV, anisidine value

P191

Omega-3s: from science to policy

JT Winkler

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Background: In addition to a great deal of scientific research, many policy initiatives are in process regarding omega-3 fatty acids -- in the European Union, the United States and other countries. These cover the regulation of claims, the setting of dietary reference values, designing research programmes, risk-benefit analyses, and practical trials, sometimes supported by reviews of recent literature.

Objective: To review the scale of current initiatives, to analyze their content, to identify the implications for public health and research, then to suggest areas for improvement.

Procedure: A group of two dozen leading international lipid scientists have identified policy initiatives, collectively analyzed them, and agreed on key issues and actions.

Results: The group has selected three particular issues of concern. (1) Many initiatives imply equivalence between ALA and EPA/DHA in delivering health benefits, based on assumed high levels of conversion. This directly

contradicts ISSFAL's statement on conversion (Brenna et al, PLEFA, 2009). (2) Recommended intakes for EPA/DHA are commonly low, well below ISSFAL's 2004 recommendation, 500mg/day. (3) Evidence on the mental health benefits of EPA/DHA is not properly taken into account.

Conclusion: Current policy initiatives are not based on good science. If fully implemented, they would discourage adequate intakes of EPA/DHA. They would also seriously delay public health action on the mental health benefits of omega-3s.

Therefore, the group has begun an active campaign to improve policy initiatives. ISSFAL members will be given the opportunity to support this effort, if they wish.

P192

Effect of a fatty acid-vitamin D supplement on recurrent upper airway infections. A pilot study

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Background: Recurrent upper airway infections are common among certain children. Intake of omega-3 fatty acids as well as of vitamin D has been shown to be important for the immune defence. The aim of this study was to examine the effect of a fatty acid-vitamin D supplement containing fish oil, canola oil and vitamin D on recurrent upper airway infections.

Methods: This was a 4-month pilot study in 113 children, aged 1-15 years (mean age 7.5 years). Healthy children, children with recurrent upper airway infections and children with other health problems were included. Intervention consisted of intake of 5 ml of the fatty acid-vitamin D supplement daily (0.41 g EPA (eicosapentaenoic acid), 0.28 g DHA (docosahexaenoic acid) and vitamin D (cholecalciferol) 5 mcg).

Results: Oral intake of omega-3 and vitamin D showed a significantly negative correlation in recurrency rate of upper respiratory infections. Lowest EPA quartile (0-0.19 g) showed a recurrency rate of 77% versus 17% for the highest EPA quartile (0.60 - 0.78 g).

4 months of supplementation significantly decreased recurrent colds (21%) and recurrent throat (12%). Total omega-3 fatty acids, EPA and DHA in plasma increased by 56%, 138%, and 50%, respectively.

Conclusion: This pilot study indicates a clear beneficial effect of supplementation of especially omega-3 fatty acids and vitamin D in children with recurrent upper respiratory infections. Further studies are warranted.

P193**The effect of omega-3 polyunsaturated fatty acids on normal and neoplastic oral keratinocyte apoptosis**

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London, London, United Kingdom

Background: Squamous cell carcinomas (SCCs) of the aerodigestive tract often recur because of incomplete excision or the appearance of second primary or second field cancers. Recent evidence suggests that the omega-3 polyunsaturated fatty acids (PUFAs) have antitumorigenic activities.

Objective: In the present study we tested the potential of omega-3-PUFAs to act as selective chemopreventive and therapeutic agents against oral and epidermal SCCs.

Procedure: We investigated the effect of the long chain omega-3-PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on the malignant keratinocyte cell lines SCC-13 and SCC-25, the pre-malignant immortal line SVFHK and the normal keratinocyte lines, NHEK-131 and HEK-127. Cell viability was determined by the methyl tetrazolium (MTT) assay. The annexin-V/DAPI flow cytometric assay was used to assess apoptosis. Reactive oxygen species (ROS) formation was detected by H₂CFDA staining. Western blot analysis was performed to investigate the signaling pathways involved.

Results: Omega-3-PUFAs (1-5 μM) inhibited growth dose-dependently after 4 days. Under the same conditions, the PUFAs had little effect on normal keratinocytes. The apoptosis assay showed that the malignant cells were undergoing apoptosis after 48 h PUFA treatment. Western blot analysis confirmed that PUFAs cause apoptosis via caspase-3 cleavage. The cleavage of caspase 9 and 8 demonstrated the involvement of the intrinsic and extrinsic apoptotic pathways, respectively. Higher doses of PUFAs appeared to increase ROS production. Moreover, PUFAs increased ERK1/2 phosphorylation in SCC-25. This was apparent already during the first hours of treatment and continued to increase for 24 hours. The role of ERK1/2 activation in the apoptosis process and the involvement of other signaling pathways are under investigation.

Conclusion: The omega-3-PUFAs DHA and EPA display a marked anti-tumour effect against SCC keratinocytes at concentrations that do not eliminate normal cells, thus giving them a significant potential as future therapeutic and prophylactic tools against head and neck cancer.

P194**Contraction induced lipolysis despite inhibition of hormone-sensitive lipase in skeletal muscle**

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Background: In skeletal muscle Hormone-sensitive Lipase (HSL) has generally been accepted to be the rate limiting enzyme responsible for lipolysis of intramyocellular triacylglycerol (IMTG) during contractions and epinephrine stimulation. However, this notion is based on in vitro lipase activity data, which may not reflect the in vivo lipolytic activity. In addition, no study has investigated whether Adipose Triglyceride Lipase (ATGL) is activated during contractions.

Objective: This study investigated whether HSL is the only TG-lipase activated during contractions in skeletal muscle and if ATGL contributes to lipolysis of IMTG.

Procedure: In ex vivo incubated rat soleus muscles HSL was inhibited with a mono-specific, small molecule inhibitor (76-0079) and IMTG content was measured in basal and contraction stimulated muscles using BODIPY staining of lipid droplets and analyzed by confocal microscopy.

Results: Initially the experimental set up was evaluated in a time course study showing that IMTG content was unchanged after 5 min while a significant decrease in IMTG content was found after 20 minutes of contractions. HSL inhibition was measured as an increase in muscle diacylglycerol content during contractions that only occurred in the presence of the HSL-inhibitor. At maximal inhibition of HSL, lipolysis of IMTG was found to a similar extent as in the control situation without the HSL-inhibitor. In addition, the contraction induced lipolysis of IMTG independent of HSL occurred in both type I and II fibers.

Conclusions: The present study is the first to demonstrate that contraction induced lipolysis of IMTG occurred despite inhibition of HSL. These results suggest that HSL is not the only lipase activated by contractions and that ATGL most likely is activated as well and thus plays a major role in lipolysis of IMTG during contractions.

P195**Antibody synthesis enhancement by feeding conjugated linoleic acid during early life**

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Background: Conjugated Linoleic Acid (CLA) has been reported to modulate immune cell functions in experimental models, however, very little is known about this effect in early life.

Objective: To determine the effect of 80% c9,t11 and 20% t10,c12 CLA supplementation during gestation, suckling and/or early infancy on immunoglobulin production of rats.

Procedure: Pregnant Wistar rats were obtained on day 7 of gestation. Up to 8 experimental dietary groups were designed according to the route of supplementation (CLA by oral gavage, CLA through dam's milk or CLA fed dams or CLA incorporated in the pelleted diet), the length of the supplementation period (from 0 to 5 weeks) and the period in which CLA was given (gestation and/or suckling and/or early infancy). IgG, IgM and IgA serum concentrations were quantified in 14, 21 and 28 day-old animals by ELISA.

Results: In 14-day-old pups, animals receiving CLA during 4 weeks had ~83% and ~25% increase for IgG and IgM, respectively, when compared with those fed standard chow ($p < 0.05$). In 21-day-old animals, CLA supplementation during 5 weeks increased almost 4 times total Ig sera concentration, by means of IgG enhancement ($p < 0.05$). In 28-day-old animals, pups that received CLA diet during and after suckling showed higher IgG, IgM and IgA sera concentrations than those receiving CLA only during 1 wk after suckling and than animals fed standard chow.

Conclusion: In overall, the CLA mixture used exerts beneficial effects by enhancing the production of antibodies in suckling and early infant rats.

P196**Diabetes mellitus, not obesity, in pregnancy is associated with lower fetal long chain polyunsaturated and essential fatty acid status**

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Introduction: LCP, notably arachidonic (AA) and docosahexaenoic (DHA) acids, are important structural components of fetal brain and modulators of gene expression. (Gestational) diabetes mellitus [(G)DM] during pregnancy is associated with lower fetal LCP status, most likely due to augmented fetal glucose supply, causing dilution by de novo synthesized fatty acids, and possibly by decreased formation of fetal LCP. Obesity is associated with higher risk of insulin resistance and disturbed glucose homeostasis. We investigated the influence of obesity and (G)DM in pregnancy on fetal LCP status.

Procedure: We included 28 controls (BMI < 25 kg/m², no (G)DM), 16 obese women (BMI > 30 kg/m², no (G)DM) and 8 women with (G)DM in Curaçao. Maternal insulin sensitivity/glucose homeostasis (fasting glucose, insulin, HOMA-IR, HbA1c) was measured in week 36 of pregnancy. Umbilical veins (UV) and arteries (UA) were collected at birth for the determination of their fatty acid compositions.

Results: Compared to controls, women with (G)DM had higher BMI, fasting glucose and HbA1c, whereas obese women showed higher BMI and insulin.

UV of diabetic pregnancies showed lower AA, LCP ω 3+ ω 6 and higher 18:1 ω 9, 20:1 ω 9, 20:3 ω 9, 22:3 ω 9 and ω 9. UA showed lower DHA, LCP ω 3, AA, 22:4 ω 6, LCP ω 6, ω 6 and LCP ω 3+ ω 6, PUFA and EFA index, and higher 18:1 ω 9, 20:1 ω 9, 22:1 ω 9, ω 9, MUFA and DHA deficiency index.

Conclusion: Obesity, although associated with insulin resistance, is not associated with lower fetal LCP and EFA status. Lower fetal LCP and EFA status in pregnancies complicated by (G)DM seems rather due to augmented fetal glucose supply than to insulin resistance.

P197**Myocardial fatty acid utilization: regulation of subcellular CD36 distribution by vesicle-associated membrane proteins**

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Background: Upon stimulation of insulin signaling or contraction-induced AMPK activation, the long-chain fatty acid (LCFA) transporter CD36 and the glucose transporter GLUT4 similarly translocate from intracellular compartments to the plasma membrane of cardiomyocytes to increase uptake of LCFA and glucose, respectively. This similarity in regulation of CD36- versus GLUT4 traffic suggests that the same families of trafficking proteins, including vesicle-associated membrane proteins (VAMPs), are involved in both processes. While several VAMPs have been implicated in GLUT4 traffic, nothing is known about the putative function of VAMPs in CD36 traffic. Therefore, we compared the involvement of the myocardially expressed

VAMP-isoforms in insulin- or contraction-induced CD36- and GLUT4 translocation.

Objectives: Five VAMP isoforms were silenced in HL-1 cardiomyocytes, after which these cells were treated with insulin, the contraction-like AMPK activator oligomycin, or electrically stimulated to contract. Subsequently, CD36- and GLUT4 translocation as well as substrate uptake were measured.

Results: Three VAMPs were demonstrated to be necessary for both CD36- and GLUT4 translocation, either specifically in insulin- (VAMP2, VAMP5) or in oligomycin/contraction-stimulation (VAMP3). Additionally, there are VAMPs specifically involved in either CD36 traffic (VAMP4 mediates insulin- and oligomycin/contraction-induced CD36 translocation) or GLUT4 traffic (VAMP7 mediates basal GLUT4 retention).

Conclusions: The involvement of distinct VAMP isoforms in both CD36- and GLUT4 translocation indicates that CD36 translocation, just like GLUT4 translocation, is a vesicle-mediated process dependent on SNARE-complex formation. The ability of other VAMPs to discriminate between CD36 and GLUT4 allows the notion that myocardial substrate preference can be modulated by these VAMPs.

P198

Carbamazepine neuroprotective effect is higher in a model of epileptic-magnesium-deficient mice fed a chronic diet rich in omega3 (rapeseed oil)

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Background: We showed that the antiepileptic drug, carbamazepine (CBZ) was more efficient in Mg-deficient mice fed an omega3 diet (rapeseed: 9% alpha-linolenic acid) than in mice fed an omega6 diet (sunflower:corn), using different tests: Magnesium-Deficiency-Dependent-Audiogenic-Seizure (MDDAS), Maximum-Electroshock-Seizure (MES), PentylentetraZol (PTZ) and NMDA.

Objective: In order to validate the positive potential of omega3 and to exclude the putative negative effect of omega-6, controls were fed a monounsaturated diet, (High-Oleic-Sunflower-Oil: HOSO) similar to rapeseed oil but without omega3.

Procedure: Mice were fed, for 30 days, Mg-deficient diets (50 ± 5 mg/kg), containing 5% vegetable oils: omega3 (rapeseed) or HOSO or omega6 (sunflower:corn, 1:3). CBZ (25 mg/kg b.w. dissolved in DMSO:saline solution 1:1) was injected IP, once daily for 10 days. Audiogenic seizure and MES tests were performed as described previously (Bac et al., J Neurosci 1998). Chemical seizures were induced by subcutaneous doses of 85 mg/kg (lethal dose without CBZ) of PTZ or by increasing IP lethal dose of NMDA without CBZ.

Results: From the 2nd to the 8th day, the PTZ and MES tests were poorly impacted. The NMDA test was more improved after CBZ treatment in rapeseed oil group (168%) as compared to monounsaturated (HOSO) and omega6 (sunflower:corn) groups (143%). The beneficial effect of the rapeseed oil was still observable but decreased progressively in the 6 following days. The MDDAS test was less drastic after CBZ treatment (in terms of frequency and severity of seizures) in the rapeseed group as compared to HOSO and sunflower: corn groups.

Conclusion: The additional beneficial effect in CBZ treated mice is clearly attributable to the omega3 content of the rapeseed diet. The similar results obtained with monounsaturated and omega6 diets exclude a deleterious effect of omega6.

P199

Anticonvulsant evaluation of fatty 6-hydroxyflavanone against animal seizures induced by a diet deficient or not in magnesium

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Background: Flavonoids include natural fatty compounds known for their physical interactions with lipids and protection properties against lipid peroxidation. They interact among others with the benzodiazepine-binding site of GABAA receptor/chloride channel complex.

Objective: Three flavanoids, 6-hydroxyflavone (6HF), 6-hydroxyflavanone (6HFN) and flavanone (FN), were evaluated in animal seizures induced by anti-GABAergic compounds (bicuculline, picrotoxin or pentylentetrazol) or by acoustic (magnesium deficiency-dependent audiogenic seizure -MDDAS- test) challenges in order to account for the underlying anticonvulsant target(s).

Procedure: A 5% fat (Sunflower:corn) diet, deficient in magnesium was given to mice and MDDAS test was performed as described previously (Bac et al., J Neurosci 1998). Chemical seizures were induced in normal magnesium fed animals by subcutaneous doses of 85, 2.7 and 4.1 mg/kg of pentylenetetrazol, bicuculline and picrotoxin, respectively. The flavonoid compounds were administered via IP route 30 minutes prior to the seizure tests.

Results: 6HF and 6HFN but not FN provided protection against seizures induced by pentylenetetrazol; doses protecting 50% animals (efficient doses 50, ED50) were 13.6 and 5.6 mg/kg for 6HF and 6HFN, respectively. Seizures induced by bicuculline and picrotoxin were not affected by the flavonoids. 6HFN was the most active in the MDDAS test with an ED50 of 34 mg/Kg. At this dose, FN and 6HF protected only 36 and 25% of tested animals. Modulations by 6HFN ED50 of MDDAS (latency, wild running, seizure, recovery) phase durations are different from those induced by reference antiepileptic drugs phenytoin (sodium channel blocker) and diazepam (GABAergic protection), respectively, suggesting alternative mechanisms for the flavonoid.

Conclusion: 6HFN has a good anticonvulsant profile, being active in MDDAS and pentylenetetrazol seizure tests. In view of its remarkable efficacy against seizures induced by pentylenetetrazol, a chemical acting on GABAA and also NMDA receptor, 6HFN is planned to be further tested against NMDA-induced seizures

P200

Dietary fish oil has opposing effects on CCR2+ monocytes and CCL2 levels in blood of healthy and endotoxemic mice

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Background: Dietary fish oil, rich in n-3 PUFA, has diverse beneficial effects on many chronic inflammatory disorders and infections. The chemokine CCL2 (MCP-1), a ligand for the chemokine receptor CCR2, mediates the recruitment of monocytes both during steady state and upon infection or inflammation.

Objective: The aim of this study was to determine the effects of dietary fish oil on monocyte subsets in blood and on CCL2 concentration in serum from healthy and endotoxemic mice.

Procedure: Mice were fed Western-type diet with or without 2.8% fish oil for 6 weeks. Half of the mice were injected intraperitoneally with lipopolysaccharide (LPS). Blood cells were collected and surface molecules and chemokine receptors analyzed with flow cytometry. CCL2

concentration in serum was determined by ELISA.

Results: Healthy mice fed the fish oil diet had a lower proportion of monocytes expressing CCR2 (inflammatory monocytes) in blood than mice fed the control diet. Induction of endotoxemia increased the proportion of inflammatory monocytes in blood in mice fed the fish oil diet but not in mice fed the control diet. Healthy mice fed the fish oil diet had a lower serum concentration of CCL2 than mice fed the control diet, but in endotoxemic mice CCL2 concentration was higher in mice fed the fish oil diet than in mice fed the control diet.

Conclusion: These results indicate that dietary fish oil may attenuate the immune activation state during chronic inflammation and homeostasis but intensify the immune response upon infection.

P201

Dietary fish oil increases the proportion of neutrophils and the concentration of CCL3 in peritoneum and blood of endotoxemic mice

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Background: Dietary fish oil, rich in n-3 PUFA, has beneficial effects in many inflammatory disorders and infections. The chemokines KC (CXCL1) and MIP-2 (CXCL2) are ligands for the chemokine receptor CXCR2 and MIP-1 α (CCL3) is a ligand for CCR1. These chemokines and chemokine receptors play an important role in recruitment of neutrophils upon infection or inflammation.

Objective: The aim of this study was to determine the effects of dietary fish oil on the concentration of KC, MIP-2 and MIP-1 α in serum and peritoneal fluid, and on neutrophil populations in blood and peritoneum of endotoxemic mice.

Procedure: Mice were fed a Western-type diet with or without 2.8% fish oil for 6 weeks. Half of the mice were injected intraperitoneally with lipopolysaccharide. Blood and peritoneal exudate cells were collected and surface molecules and chemokine receptors analyzed with flow cytometry. KC, MIP-2 and MIP-1 α concentrations in serum and peritoneal fluid were determined by ELISA.

Results: Following endotoxin administration a specific population of neutrophils was observed in the blood. This neutrophil population was larger and less granulated than the neutrophils in blood from healthy mice. Dietary fish oil increased the proportion of these neutrophils in blood by more than twofold compared with that in mice fed the control diet 48h following endotoxin administration. It also increased the proportion of neutrophils in peritoneum 48 h following endotoxin administration.

Dietary fish oil increased the concentration of MIP-1 α in both serum and peritoneal fluid 3h and 48h following induction of endotoxemia. On the other hand, dietary fish oil decreased the concentration of MIP-2 in serum 3h after induction of endotoxemia.

Conclusion: These results indicate that dietary fish oil may augment neutrophil recruitment to blood and peritoneum following infection and that these effects may be mediated by an effect on MIP-1 α .

P202

Dietary supplementation with eicosapentaenoic acid decreases natural killer cell activity in broiler chickens

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Background: There has been interest in enrichment of poultry meat with long chain n-3 polyunsaturated fatty acids (PUFA) to increase their consumption by humans. There is concern that high levels of n-3 PUFA have detrimental effects on immune function in chickens.

Objective: determining effects of various sources of n-3 PUFA on natural killer (NK) cell activity of splenocytes and peripheral blood leukocytes (PBMC's) in chickens.

Procedure: One-day-old male Ross 308 broilers (n=20) were fed a common starter diet for 21 d. Then, birds were randomly allocated to 4 pens. Broilers were fed for 33 d on one of four sources of n-3 PUFA: echium-, algae-, fish oil (FO)-and linseed-enriched diets. Chickens were sacrificed between 41 and 43 d of age. NK activity assay was conducted on splenocytes and PBMC's.

Results: splenocytes and PBMC's from FO-fed chicks exhibited the lowest NK cell activity while those from linseed oil-fed chicks exhibited the highest NK cell activity.

Conclusion: Eicosapentaenoic acid (EPA), but not docosahexaenoic acid (DHA) or α -linoleic acid, maybe responsible for the suppressive effect of NK cell activity. These studies highlight the need for the poultry industry to consider the health status of poultry when poultry meat is being enriched with n-3 PUFA.

P203

Do high levels of n-3 PUFA in plasma phospholipids increase the risk of postoperative atrial fibrillation?

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Background: It has been postulated that the anti-arrhythmic and anti-inflammatory effects of n-3 polyunsaturated fatty acids (PUFA) may decrease the risk

of postoperative atrial fibrillation (POAF) following open heart surgery.

Objective: To examine the relationship between the levels of n-3 PUFA and n-6 PUFA in plasma phospholipids (PL) and POAF in patients undergoing coronary artery bypass graft (CABG) surgery.

Methods: The levels of fatty acids in plasma PL were measured preoperatively and on the third postoperative day in all patients. POAF was defined as an episode lasting more than 5 minutes, documented by continuous electrocardiographic monitoring. Wilcoxon-Mann-Whitney test was used to compare the groups who did and did not develop POAF. Multivariable logistic regression was used to examine the relationship between the levels of fatty acids in plasma PL and POAF.

Results: A total of 125 patients were enrolled with a median age of 66 (range, 45-82), 82% were males. The overall incidence of POAF was 49.6%. The patients in the POAF group were older (P=0.003) and they had a higher maximal serum concentration of C reactive protein following surgery (P=0.04). The plasma PL level of AA was lower, whereas DHA was higher in patients with POAF than those without POAF, both pre- and postoperatively (P<0.05). The EPA level was also higher postoperatively in the POAF group (P<0.05). After adjusting for confounding variables, the strongest relationship between POAF and the levels of different fatty acids in plasma PL was seen for the level of total n-3 PUFA postoperatively with an incremental Nagelkerke R² of 0.084, P=0.005, Odds ratio (95% confidence interval) 1.362 (1.096-1.694).

Conclusion: There was no evidence that relatively high levels of n-3 PUFA in blood lipids are preventive against POAF following CABG surgery. On the contrary, high levels of n-3 PUFA were associated with increased frequency of POAF.

P204

Inverse relationship between n-3 pufa in red blood cell membranes and inflammatory biomarkers in patients undergoing open heart surgery

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Background: Several studies suggest that inflammation may play a role in the pathogenesis of postoperative atrial fibrillation (POAF) but the relationship between POAF and preoperative C reactive protein (CRP) levels has been inconsistent.

Objective: To determine the relationship between n-3 polyunsaturated fatty acids (PUFA) in red blood cell (RBC)

membranes and circulating inflammatory biomarkers and the development of POAF following open heart surgery.

Procedure: RBC membrane phospholipid (PL) fatty acids and several circulating inflammatory biomarkers were quantified in patients immediately prior to open heart surgery. POAF was defined as an episode lasting more than 5 minutes, documented by continuous electrocardiographic monitoring.

Results: The median age of the patients (n=124) was 67 years (range 43-82), of whom 99 (79.8%) were males. POAF occurred in 55.6% of the patients. Their median age was higher compared to those without POAF, 70 (45-82) vs. 65 (43-79) years (P=0.02). The patients with POAF had higher EPA (2.04% vs. 1.79%, P=0.037) and DHA (7.86% vs. 7.11%, P=0.036) levels in RBC membrane PL and lower levels of AA (12.01% vs. 12.92%, P=0.006) compared to those without POAF. No difference was observed between preoperative levels of high-sensitivity CRP in patients with and without POAF (2.03 mg/L vs. 1.89 mg/L, P=0.99) nor was there a difference between preoperative levels of IL-6, IL-18, IFN- β and TGF- β in patients with and without POAF (P>0.05). The levels of EPA and DHA in RBC membranes were inversely associated with preoperative levels of IL-6, IL-18 and TGF- β (P<0.05) whereas the level of RBC AA was directly associated with IL-6 and TNF- β (P<0.01).

Conclusion: These results suggest that EPA and DHA have an anti-inflammatory effect but this effect does not appear to influence the occurrence of AF following open heart surgery.

P205

Low omega-3 fatty acid status and increased risk of suicide death among active duty U.S. military: a case control comparison

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Background: Suicide deaths among US military have increased to record numbers, but causal factors have not yet been identified. Deficiencies in neural active omega-3 essential fatty acids may increase risk of depression and suicide.

Objective: Determine if low omega-3 status is associated with increased risk of suicide death among active duty US military.

Methods: Serum fatty acid status was quantified among n=1,600 previously collected samples from US active duty suicide deaths (n= 800) and (n=800) controls matched for age, date of serum collection, sex, rank and year of incident (2002-2008). DD Form 2796 provided post deployment health assessment data.

Results Higher serum docosahexaenoic acid (DHA, % total fatty acids) correlated with decreased risk of suicide death. Among males, the adjusted odds of suicide death, was greater among the (n=195) with the lowest octile of DHA%, OR=1.73 (95% CI; 1.13-2.64, p< 0.02), and those (n=1,156) in the middle octiles, OR= 1.56 (95% CI; 1.12-2.18, p<0.008), compared to the top octile(n=179). By comparison, having observed a coalition soldier wounded or killed increased risk of suicide death by OR= 1.54 (95% CI; 1.12-2.12, p< 0.007).

Conclusion: These US military personnel (n=1,600) had low DHA status compared to US and world populations. Low DHA status is a significant risk factor for military suicide deaths after adjustment for ethnicity and branch of military service. Low DHA status may be a significant risk factor for military suicide deaths that is reversible by dietary intervention.

P206

FADS 1-2 polymorphisms modify the effect of formula feeding on child IQ at age 8 years

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Background: A recent study by Caspi et al has reported that the rs174575 polymorphism within the FADS 1-2 gene moderates the effect of breastfeeding so that those children with the GG genotype have similar IQs whether breastfed or not. For other genotypes a clear advantage was seen for breastfed children.

Objective: Determine if FADS 1-2 polymorphisms modify effects of breastfeeding on IQ.

Methods: Data from the Avon Longitudinal Study of Parents and Children were available for 5934 children with child IQ (WISC III) at 8y and genetic data. Genotype data for two polymorphisms (rs174575 and rs1535) were obtained for both the mother and child. Linear regression analysis was used to explore breastfeeding, genetic and interaction effects.

Results: Breastfeeding was strongly associated with full-scale IQ with a difference of 7.8 points compared to formula fed infants [95% CI 6.6, 9.0]. There was no evidence of a genetic main effect with IQ. An interaction with rs174575 was observed such that breastfed GG children performed better than their formula fed counterparts by an additional 5.8 points [1.4, 10.1] (interaction p=0.0091). A mother-child genetic interaction was also observed so that GG children of GG mothers performed better than expected by an additional 7.6 points [2.1, 13.0] compared to other GG children

($p=0.0070$). Similar results were found for rs1535 and for performance and verbal IQ although effect sizes were generally reduced for these other outcomes. Interaction results were largely unaffected by adjustment for 7 factors.

Interpretation: This study was unable to replicate the findings of Caspi et al. In contrast to their study, GG children exhibit the greatest difference between feeding methods such that breastfed children performed similarly irrespective of child genotype whereas formula fed GG children performed worse than other children on formula milk.

P207

Reduction of relapse among chronic alcoholics with n-3 HUFAs: a randomized placebo controlled trial

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Background: Chronic alcohol depletes DHA from neuronal tissues. Low DHA status predicts relapse in an observational study. Restoration of n-3 HUFAs may reduce relapse and reduce aggressive and depressive symptoms.
Objective: Determine if consuming 2 gm/d of EPA+ DHA (2:1), in comparison to a placebo, decreases relapse among chronic alcoholics.

Procedure: Chronic alcohol dependant subjects ($n=96$) were treated at the NIH research unit for 28 days. At baseline, cerebral spinal fluid, plasma, RBC's, whole blood, structural MRI, structured diagnostic interviews, NEO personality testing Hamilton depression ratings and other psychometric data were collected. After randomization, subjects received weekly outpatient therapy and research visits for 12 weeks. Subjects remaining in the study, ($n=74$) were readmitted overnight and baseline assessments were repeated. As substance abusing subjects are occasionally unreliable, changes in n-3 HUFA composition of 30% in CSF or plasma was selected a priori as indication of compliance.

Results: Compliant active assignment subjects ($n=28$) had fewer drinking days/ 90 d therapy (3.2 d, 95%CI 2.5-4.9) compared to subjects ($n=46$) with unchanged n-3 HUFA status (17.4 d/90, 95%CI 12.6-19.8) ($n=39$ placebo + $n=7$ non-compliant active) with an effect size of 0.84 (Hedges g), $p<0.005$. CSF neurotransmitter metabolites of serotonin (5-HIAA) and dopamine (HVA), depression, aggression and perceived stress scales were unchanged. Changes in CSF fatty acid composition did not correlate with changes in CSF neurotransmitter metabolites.
Conclusion: Restoration of n-3 HUFA status may improve relapse prevention among chronic alcoholics. Sobriety contributed substantially more to reductions in aggressive and depressive symptoms than supplementation.

P208

Human CD4⁺ and CD8⁺ T cells subsets stimulate endothelial prostacyclin I2 synthesis. Relation to age and gender

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Background: We have previously showed that cell-cell contact between human umbilical vein endothelial cells (HUVEC) and human peripheral blood lymphocytes (PBL) markedly enhance prostacyclin I2 (PGI2) synthesis through a Src kinase/ERK1/2- cPLA2-PGHS-1-PGIS pathway, in vitro. **Objective -** Using the same experimental conditions we determined here (1) whether the increment in endothelial PGI2 output was associated to a particular PBL subsets and (2) if the endothelial response varies according to the age or gender of the PBL donor. **Procedures:** Confluent HUVEC were incubated alone (control) or co-incubate with PBL (positive control), LT-CD4⁺, LT-CD8⁺, NK, and/or B in a in a serum-free medium for 4 h. HUVEC were then washed to eliminated lymphocytes and 6-oxoPGF1 α , the stable metabolite of PGI2, was measured by ELISA.

Results: Our results shows that PGI2 synthesis triggered by PBL-endothelial cell contact involves both, LT-CD4⁺ and LT-C8⁺ subsets but not the NK or B population. Interestingly, we observed no increment of PGI2 when HUVEC were coincubated with LTCD8⁺ from males donors over 50 years contrary to that observed with LTCD8⁺ from females of the same age.

Conclusions: The capacity of endothelial cell to increase PGI2 might be relevant in pathologic conditions involving LT-CD4⁺ and/or LT-C8⁺ subset and suggest that aging could affect T-CD8⁺ function in males.

P209

Maternal and foetal polyunsaturated fatty acids are compromised in growth restricted pregnancies and in pre-eclampsia

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Background: The growing foetus requires essential polyunsaturated fatty acids (PUFA) for growth and development.

Objective: To compare PUFA status in women who had a healthy pregnancy ($n=165$), women with intra-uterine growth restriction (IUGR, $n=23$) and women with pre-eclampsia (PE, $n=64$). Maternal and their respective foetal

PUFA were also compared on a subset (control=86, IUGR=13, PE=21).

Procedure: Maternal (third trimester) and cord blood (at delivery) erythrocyte fatty acids were extracted, identified using gas chromatography and quantitated by comparison to an internal standard.

Results: All erythrocyte omega-6 and omega-3 PUFA were approximately 40% lower in women with PE and IUGR compared to healthy controls ($p < 0.0005$). Apart from linoleic acid, the foetal PUFA were consistently 25-30% lower in PE and in IUGR compared to foetal controls ($p < 0.001$), but in IUGR the foetal/maternal ratios were significantly higher for 18:2n-6, 22:5n-6 and 22:5n-3 ($p < 0.05$) suggesting compensation by upregulation of PUFA transport.

Conclusion: There is a reduced supply of maternal PUFA in PE and IUGR, but at least in IUGR the foetus overcomes this partially by preferential accrual of PUFA suggesting there is no defect in placental transfer.

P210

Elevated serum sphingomyelin is associated with reduced gray matter density: evidence from twins discordant for schizophrenia

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Objective: Lipid abnormalities are an intrinsic feature of schizophrenia supported also by recent evidence from serum and brain tissue lipidomics. Associations between genetic and phenotypic data and more detailed molecular information may reveal clues about the pathophysiology of the disease.

Procedure: Global lipidomics approach using UPLC/QTOF/MS was applied to analyse serum samples from 19 twin pairs discordant for schizophrenia (mean age 51 +/- 10 years; 7 monozygotic pairs; 13 female pairs). A total of 360 molecular lipids were detected and quantified. Gray matter volume measurements taken from high-resolution magnetic resonance images were obtained from the same twin pairs.

Identification of the cortical surface areas in which gray matter density and selected lipids were associated was performed with earlier described methodology.

Results: Elevated serum sphingomyelins (SM) were dominating the lipidomic profiles of schizophrenic co-

twins as compared to their unaffected co-twins. The top ranked sphingomyelin was SM(d18:1/18:0), which was 27% elevated ($P < 0.01$, pairwise t-test). Its concentration was significantly associated with the reduced gray matter density including regions of the temporal and occipital lobes as well as corpus callosum, as confirmed by permutation testing and separately for each hemisphere ($P_s < 0.05$). The significant association remained if corpus callosum was excluded from the analysis, for the right hemisphere only.

Conclusion: The twin design allowed us to link abnormal serum SM changes to schizophrenia independent of the genetic background. SM is an abundant brain lipid which is also a precursor of lipotoxic ceramide which levels, in turn, have recently been shown to be elevated in white matter of schizophrenic subjects. SM is also an abundant lipid in circulation, found particularly in low- and high-density lipoprotein particles. Associations of circulating SM to specific brain regions of potential pathogenic relevance suggest SM may play a role in schizophrenia.

P211

9 Months fish oil supplementation does not affect immune markers in healthy infants

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Background: n-3 long-chain polyunsaturated fatty acids (LCPUFA) are thought to affect immune function and may affect immune maturation in early life.

Objective: To investigate whether 9 months fish oil supplementation in late infancy could modify immune function.

Procedure: A randomized controlled double-blinded trial with fish oil or sunflower oil (control) from 9 to 18 months of age in 106 healthy Danish infants. Before and after the intervention we measured fatty acid composition of erythrocytes (RBC), plasma IgE and C-reactive protein (CRP) as well as cytokine production in whole-blood cultures stimulated *ex vivo* with 20 mg/L lipopolysaccharide (LPS) or *Lactobacillus paracasei* for 24 h.

Results: The median (interquartile range) intake of intervention oils was estimated to 3.8 (3.2-4.2) mL/d, with no difference between the groups ($P = 0.17$), corresponding to 1.2 g/d n-3 LCPUFA and 2.6 g/d n-6 PUFA in the fish oil and control group, respectively. Fish oil supplementation effectively raised RBC n-3

LCPUFA ($P < 0.001$). Apart from *L. paracasei* stimulated IL-10 ($P < 0.001$), cytokine production did not significantly increase from 9 to 18 months. Preliminary analyses showed no effect of the fish oil intervention on IgE, CRP or *ex vivo* production of IFN- γ , IL-10 or IFN- γ :IL-10 after either LPS or *L. paracasei* stimulation.

Conclusion: Our earlier finding of increased *ex vivo* IFN- γ production after fish oil supplementation from 9 to 12 months was not reproducible in this age group. The supposed acceleration of immune maturation with fish oil may no longer be visible when infants reach 1½ years of age. An alternative explanation would be that both sunflower oil rich in n-6 PUFA and fish oil rich in n-3 LCPUFA affect the immune system early in life.

P212

What is the influence of hydroxyurea on the composition of red blood cells of patients with sickle cell disease?

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Background: Currently, hydroxyurea is the only disease-modifying therapy approved for sickle cell disease (SCD). Hence, there is a great interest in understanding deeply its use in treating patients with this disorder. We conducted a randomized comparison of hydroxyurea (HU) influence on membrane lipid perturbation in the treatment of SCD.

Objectives: The aim of the study was to investigate, whether (a) patients with homozygous sickle cell disease (SCD-HbSS) treated with hydroxyurea, have abnormal red blood cells (RBC) phospholipids (PLs) composition; (b) the abnormality, if exists, affects all the RBC phospholipids or is restricted to a particular PLs classes.

Procedure: Phospholipids composition of RBC of 20 steady-state HbSS patients treated with hydroxyurea and 21 healthy controls was analysed using ^{31}P NMR spectroscopy.

Results: The results indicate that red cells of sickle cell patients have an abnormal phospholipids profile. However, changes concern two phospholipids classes only, i.e. phosphatidylinositol (PI) and sphingomyelin (SM). The levels of PI and SM are higher ($P < 0.005$) and lower ($P < 0.05$) for HU treated patients, respectively. In the consequence, we observe an imbalance between PI/SM ($P < 0.0001$) ratio and phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylcholine (PC) to PI and SM as well (PE/PI $P < 0.05$, PS/PI $P < 0.001$, PC/PI $P < 0.001$, PC/SM $P < 0.05$, PS/SM $P < 0.05$). The results also show that ratios PE/PI, PS/PI, PC/PI decrease 1.7 times, whereas PC/SM and PS/SM ratios increase 1.1 times for HbSS patients relative to the controls.

Conclusions: We found the influence of HU on phospholipids composition of RBC of sickle cell disease patients that is manifested by increase of PI and decrease of SM concentrations simultaneously. These changes affect the ratios between PI or SM, and PE, PS and PC as well.

P213

Gestational weight gain related to intake of aquatic food and fatty acid status in blood

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Objective: To investigate Gestational Weight Gain (GWG) in coastal, riverine/lake and inland regions in China.

Procedure: Ninety healthy women in early gestation were recruited from coastal, riverine/lake, and inland regions (prepregnancy BMI 22.30 ± 0.49 , 20.16 ± 0.41 , 21.07 ± 0.32 kg/m²) in China. Obstetric and dietary data were collected at 28 weeks and 35 weeks with blood samples at 35 weeks.

Results: Intakes of aquatic food in the coastal (169.16 ± 22.83 g/day) and riverine/lake (145.96 ± 18.05 g/day) women were higher than that of the inland (26.78 ± 7.65 g/day) group ($p < 0.005$), in which The average intake of EPA&DHA of 54.0 & 54.7 mg/day for coastal area, was significantly higher than in riverine/lake (27.6 & 39.9 mg/day), or inland areas (2.27 & 1.35 mg/day). Pre-pregnancy body weight and BMI of riverine/lake women (51.67 kg, 20.16 kg/m²) were significant lower than that of coastal group (57.96 kg, 22.30 kg/m²) ($p < 0.005$). There were no statistic differences between their body weights by 28 to 35 weeks of gestation. However, the GWG of inland group was significant higher than that of coastal women after 28 weeks ($p < 0.005$) and up to 35 weeks ($p < 0.005$). The GWG was negatively correlated with the aquatic intakes ($r = 0.23$, $P < 0.05$). Several main fatty acids in maternal plasma choline phosphoglycerides (CPG) had weak but significant correlations with GWG at 35 weeks., i.e. negatively with DHA ($r = -0.24$, $P < 0.05$), EPA ($r = -0.22$, $P < 0.05$), Oleic ($r = -0.32$, $P < 0.005$), total n-3 ($r = -0.24$, $P < 0.05$), Mono ($r = -0.32$, $P < 0.005$), positively with n-6 ($r = 0.33$, $P < 0.005$), n-6/n-3 ($r = 0.27$, $P < 0.05$).

Conclusion: The coastal and riverine/lake women had lower GWG than that of inland women. The former had a higher consumption of aquatic food and subsequently higher n-3 LCPUFA and mono-unsaturated fatty acids in maternal plasma CPG.

P214

Phosphatidylcholine/phosphatidylethanolamine ratio determines bilayer phase structure between external and internal leaflet and hence the membrane curvature and its functional properties

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Background: The Phosphatidylcholine/phosphatidylethanolamine (PC/PE) ratio determines bilayer phase structure, therefore, virtually all functional properties of the membrane.

Objective: To investigate if there were differences in PC/PE ratios of maternal and neonatal erythrocyte membranes between three regions of different aquatic food consumptions.

Procedure: Ninety healthy women in early gestation were recruited from coastal (C), riverine/lake (R), and inland (I) regions in China. Obstetric and dietary data were collected at 28 weeks and 35 weeks with blood samples at 35 weeks and term from the cord. The fatty acids in phospholipid fractions and concentrations of PC and PE in erythrocytes were analyzed.

Results: Consumptions of aquatic food in the coastal (169.16±22.83 g/day) and riverine/lake (145.96±18.05 g/day) women was higher than that of the inland (26.78±7.65 g/day) group ($p<0.005$). The inland maternal erythrocyte PC contained the much lower DHA, n-3LCPUFA than coastal ($p<0.001$, $p<0.001$) and riverine/lake ($p<0.001$, $p<0.001$); and the inland maternal erythrocyte PE contained lower DHA, n-3LCPUFA significantly as well. But DHA, n-3LCPUFA didn't have obvious differences among the neonatal erythrocyte PC and PE in three regions. Despite these differences, the PC/PE ratios of maternal (I: 1.06±0.09, C: 1.11±0.06, R: 1.12±0.08) and neonatal (I: 0.90±0.11, C: 1.01±0.10, R: 1.19±0.06) erythrocytes was conserved and similar in the three regions.

Conclusion: Although different consumption of aquatic food affected n-3 LCPUFA in PC and PE of maternal and neonatal erythrocyte membranes, the PC/PE ratios were conserved in mothers and neonates suggesting a physiological mechanism to preserve the shape change responsiveness of the erythrocyte plasma membrane. China is a world leader in fresh-water aquaculture. It is interesting that the blood omega 3 status of the mothers in the fresh-water aquaculture region was similar to those in the marine fishing region.

P215

The effect of gestational age on red blood cell fatty acids during pregnancy

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Objective: Studies relating maternal prenatal fatty acid concentrations to child health and development outcomes rarely take account of the time-point at which maternal blood samples are taken. Here we assess variation in red cell fatty acids over gestation.

Procedure: As part of the UK's Avon Longitudinal Study of Parents and Children (ALSPAC) collaborating mothers gave blood samples at various time points during pregnancy. Red blood cells from 8012 samples from 5315 mothers were assayed for 39 different fatty acids. Of these, 7720 samples from 5119 mothers had valid results including gestational age at sampling. Statistical analyses employed multiple regression (with log transformations where appropriate). DHA status was calculated as the ratio of DHA and 22:5n-6.

Results: Of the 39 fatty acids tested, 27 (69 %) were significantly associated with gestational age at sampling at the 5% level, and 19 (49 %) at the 0.1% level. Of the latter, positive relationships were seen for 14:0, 16:0, 16:1n-7, 18:1n-9, 20:3n-9, 22:5n-6 and 18:4 n-3. Conversely, at $p<0.001$ there were negative associations with some saturates (15:0, 18:0, 19:0), some n-7 monounsaturates (17:1 and 18:1) and many n-6 (18:2, 20:2, 20:4 and 22:4) and n-3 (20:5, 22:5, 22:6) polyunsaturates, as well as with DHA status. With 10 additional weeks of gestation, fatty acids showing >10% increase were: 14:0 [b=0.114], 18:4n-3 [0.158], 18:1n-9 [0.206], 16:0 [0.343] and 16:1n-7 [0.393]. Decreases of >10% were seen for 17:1n-7 [-0.127], 22:4n-6 [-0.176], 22:5n-6 [-0.182], 20:4n-6 [-0.205], 20:5n-3 [-0.215], 18:1n-7 [-0.219] and 18:0 [-0.246]. Reductions in DHA and DHA status with increasing gestational age were less pronounced (b= -0.066 and -0.163 respectively) although still highly statistically significant.

Conclusion: Maternal prenatal blood fatty acid concentrations show highly significant variations with gestational age. This must be taken into account when studying maternal prenatal fatty acids in relation to child outcomes.

P216

Determination of heritability of fatty acid composition of adipose tissue as biomarker of fat intake in identical and non-identical twins

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Introduction: Biomarkers of fat are well known to reflect fat intake more accurately than traditional assessment methods such as FFQ. Adipose tissue is a reliable biomarker of long-term fat intake. Dizygotic (DZ) and monozygotic (MZ) twins are excellent sample to calculate the heritability and genetic variations in relation to fatty acid profile.

Objectives: To estimate heritability and dietary variation in the fatty acids composition of adipose tissue in MZ and DZ twins. **Procedure:** Abdominal adipose biopsies from a 530 Female Caucasian twins have been collected from the Twin's UK registry. Fatty acids were extracted and methylated from adipose tissue using acid-based catalysis. Fatty acid methyl ester (FAME) were analyzed by gas chromatography (GC) using a 60m capillary column (cpsil88). FAME peaks were identified using the retention times of known standards. The following fatty acids were identified C10:0, C12:0, C14:0, C16:0, C16:1n-7, C18:0, C18:1n-7, C18:1n-7, C18-trans, C18:2n-6, C18:3n-3, C20:0, C20:3n-6, C20:4n-6, C20:5n-3, C22:4n-6, C22:5n-3, and C22:6n-3.

Results: All fatty acids were significantly correlated between the twins ($P = 0.000$). The strongest correlation was for cis-vaccinic acid, 18:1n-7 ($r^2 = + 0.726$, $P = 0.000$) and the weakest was for linoleic acid, 18:2n-6 ($r^2 = 0.326$). MUFA constituted over half (52.36%) of the total fatty acids mainly as 18:1n-9 with a significant proportion of 16:1n-7. SFA constituted 25.51% mainly as palmitic acid. PUFA accounts for 15.38% of the fatty acids and the ratio of n-6/n-3 was 6.39. Trans fatty acids accounted for 1% of the fatty acids. **Conclusion:** Our results indicate that in this group of subjects, adipose tissue was a good biomarker for fatty acids that are not endogenously synthesized including essential fatty acids and TFA.

P217

Omega-3 blood concentrations and sleep problems in healthy children aged 7-9 years

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Background: Omega-3 and omega-6 fatty acids and their derivatives influence many aspects of brain chemistry and physiology, and there is some evidence that these include the regulation of arousal and sleep. Difficulties with sleep are prevalent in the general population and lead to a wide range of physical and psychosocial problems, but little is currently known about their possible links with omega-3/omega-6 status.

Objective: To investigate blood concentrations of omega-3 and omega-6 fatty acids in relation to reported sleep problems in a healthy child population.

Procedure: 178 children aged 7-9 years from the DHA Oxford Learning and Behaviour (DOLAB) Trial gave a fingerprick blood sample for fatty acid analysis (Martek Biosciences Inc.), and were also assessed using a well-validated subjective measure of child sleep habits (CSHQ, Owens 2000). Correlations were performed between CSHQ scores (8 sub-scales and a total sleep disturbance score) and blood concentrations of omega-3 and omega-6 fatty acids, controlling for age, sex and socioeconomic status.

Results: Problems with sleep duration were associated with lower blood omega-3 concentrations (ALA $p < 0.02$, EPA $p < 0.05$, DHA $p < 0.01$, total omega-3 $p < 0.001$) and with higher total omega-6/omega-3 ratios ($p < 0.001$), but not with any omega-6 measures. Other CSHQ sub-scale scores showed no consistent relationships with blood fatty acids, but children with lower DHA showed a slight tendency towards higher total sleep disturbance scores ($p < 0.09$).

Conclusion: Low blood concentrations of omega-3 appear to predict problems with sleep duration in otherwise healthy school children. These associations merit further investigation, ideally using objective measures of sleep. If low omega-3 is related to sleep insufficiency, well powered intervention studies could establish whether improving omega-3 status may reduce these sleep problems.

P218

Proteomics analysis of the regulatory changes in the neuronal and retinal cells caused by the eicosapentaenoic acid supplementation

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Background: Docosahexaenoic (DHA) is a vital component of cellular and subcellular membranes. It is specifically present at very high levels in the brain and retina. In the cerebral grey matter, it makes up to 30% of the fatty acid content of the amino phospholipids, ethanolamine and serine.

In contrast to DHA, eicosapentaenoic acid (EPA) is found in trace amount in cell membranes. However, it plays a critical role in the modulation of various physiological functions particularly immune and vascular. EPA is hardly detectable in the brain, nevertheless there is accumulating evidence which reveals that it is more effective than DHA for ameliorating neurological disorders such as attention deficit/hyper activity disorder (ADHD), Alzheimer disease, schizophrenia and depression. The molecular mechanism of the beneficial effect of EPA for treatment of neurological disorders and cancer is not well understood.

Objectives: Proteomic study was undertaken to identify the molecular targets of EPA in neuronal cells.

Procedure: Well characterised neuronal (neuroblastoma SH-SY5Y) and highly specialised in DHA metabolism retinal neuronal cell lines (ARPE19 and Y71) were supplemented with EPA (50µM) and incubated for 1, 2, 3 and 5 days. The changes in the protein expression were analysed by 2D protein electrophoresis. The proteins upregulated upon the EPA supplementation were spotted and subjected to the identification by MALDI-TOF mass spectrometry.

Results: The upregulation of up to 60 proteins especially on the second day of supplementation with EPA was detected. The results of mass spectrometry identification of some of regulatory targets of EPA in the neuronal cells will be presented.

Conclusion: Preliminary study suggests that beneficial effect of EPA on the neuronal cells is due to upregulation of vital proteins in the transcription apparatus, exocytosis machinery and the trophic signalling at the synapse.

P219

Antiproliferative glycolipids from a sea star and two marine sponges

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Objective: Glycolipids from marine sponges are known to possess immunomodulating and antitumor activity, whereas little is known about activity of those of the echinoderms. We report the isolation, characterization and biological activity of some glycosphingolipids (GSL) isolated from the sea star *Narcissia canariensis* (Senegal) and the sponges *Axinyssa djiferi* (Senegal) and *Amphimedon viridis* (Gulf of Aden), and their antitumor activities were studied within the "Canceropole du Grand Ouest, France".

Procedure: Glycolipids were separated from other lipids by column chromatography on silica gel with dichloromethane, acetone (glycolipids) and methanol. The major GSL were isolated by HPLC and identified by high resolution electrospray mass spectrometry and nuclear magnetic resonance experiments.

Results: A GSL isolated from *N. canariensis* contained a b-glucopyranoside as sugar head, a 4,8,10-triunsaturated long-chain sphingoid base and a series of 2-hydroxylated fatty acyl chains. This GSL presented a cytotoxic activity on cancerous cell lines with LD50 of 15µM on multiple myeloma, 20µM on colic carcinoma and 35µM on glioblastoma. The GSL from *A. djiferi* was a mixture (Axidjiferosides) including the same β-galactopyranose with C22-C26 α-hydroxy fatty acids and C15-C21 sphingoid bases (with an unusual double bond between C-6 and C-7). This GSL showed an antiproliferative activity against THP-1 human cancer cells (45% inhibition at 10mg.mL⁻¹)

and inhibited multiple myeloma cells (microinjection mode, 50% inhibition after 22h). Two GSL from *A. viridis* contained a b-glucopyranoside and a b-aminoglucopyranoside as sugar heads respectively, and unsaturated iso-C15:1-C18:1 sphingoid bases and mainly the α-hydroxydocosanoic acid. They also showed promising an inhibition of multiple myeloma cells.

Conclusion: This study shows a structural diversity of active glycolipids from marine invertebrates and confirms previous results that showed the interest of glycosphingolipids as potentially useful therapeutic agents. These GSL are currently submitted to further biological investigations.

P220

A critical examination of evidence from randomized controlled trials that omega-6 fatty acids are cardioprotective

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Background: Randomized controlled trials (RCTs) are cited as 'convincing' and 'decisive' evidence that dietary n-6 PUFA are cardioprotective and provide a basis for an advisory to consume 'at least 5 to 10% of energy'. A critical examination of RCTs evaluating these assertions has not been conducted.

Objective: Here we evaluated the postulation that increasing n-6 PUFA at the expense of other fatty acids is associated with decreased risk of nonfatal MI, cardiac deaths, and total deaths.

Procedure: Six RCTs, and one non-randomized crossover study reporting increased n-6 PUFA consumption were identified by searching MEDLINE and secondary referencing. A database was constructed on diets, clinical endpoints, and possible confounders.

Results: Mean dietary intervention periods ranged from 1 to 6 years. Two of 7 trials analyzed data from men and women separately, totaling 9 data sets. At least one definite clinical endpoint was available for each data set. One data set from the non-randomized study reported a significant reduction in MI and cardiac deaths (FMHS-M). Two reported borderline significant benefit in MI (ODHS; LA-Vet). One trial, which lacked MI data, reported significantly increased total deaths (SDHS). Two others reported a borderline significant increased MI (MCS-W; RCOT) and increased total deaths, without specific statistical analysis. Three remaining data sets (FMHS-W; MCS-M; BRC) reported no clear effect. High PUFA diets were critically confounded by simultaneous alterations in trans fatty acids (TFA), n-3 ALA, EPA+DHA, and neuroleptic medication use. In the few studies that supported benefit of increasing total PUFA, the intervention group received large quantities of ALA

(>2en%) and/or EPA+DHA (>2en%), and study oils displaced large quantities of TFAs (2-10en%). In the non-randomized study, dieters received considerably less psychotropic medications linked to cardiac events and deaths. **Conclusion:** RCTs provide no decisive evidence for concluding that n-6 PUFA are cardioprotective after confounding factors are considered.

P221

Treatment-resistant chronic daily headache: Baseline vs. low n-6 + low n-3 vs. low n-6 + high n-3 diets

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Background: Long-term changes in consumption of dietary fatty acids may alter tissue composition and physiology of the nervous system and immune system. We hypothesized that low n-6, and low n-6 + high n-3 diets would decrease the percentage of n-6 HUFA in total HUFA (%n-6 in HUFA), and alleviate physical pain in patients with treatment-resistant Chronic Daily Headache (CDH).

Objectives: To assess the extent and rate of changes in the %n-6 in HUFA elicited by diets that are low in n-6 PUFA, or low in n-6 + high in n-3 PUFA. We further sought to explore the association between changes in %n-6 in HUFA and pain frequency, intensity and quality-of-life.

Procedure: After a 6-week pre-intervention phase, 72 ambulatory CDH patients are randomized to 1 of 2 diets for 12 consecutive weeks. The low n-6 diet contains <2.0 en% LA and <60mg/d AA, with average US n-3 consumption. The low n-6 + high n-3 diet contains <2.0 en% LA, 1.5 en% ALA and >0.5 en% EPA+DHA. Fat sources, and foods sufficient for most meals and snacks, are provided at randomization and every 2-weeks. Subjects receive 6 dietitian-administered counseling sessions, and access to a comprehensive website to reinforce advice. Pain frequency, intensity and medication use are recorded daily in an electronic diary. Blood and psychometrics are collected at baseline and every 4 weeks.

Results: Of the first 12 randomized patients, 9 (75%) completed the intervention phase. Dropouts cited limited meat consumption. In preliminary blinded

analysis, %n-6 in HUFA decreased, and Omega-3 Index increased in RBC phospholipids in all 9 subjects.

Anecdotally, several patients reported decreased pain intensity and medication use.

Conclusion: Low n-6 and low n-6 + high n-3 research diets can be achieved and maintained for 12 weeks. Full analyses of metabolic and clinical outcomes will be performed at study completion.

P222

Dietary fatty acids quality in pregnant mice determine health or disease in adult offspring

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Background: The mammalian fetus is completely dependent on the fatty acids supplied by its mother. Disturbances in nutrient supply can induce lasting consequences for growth and metabolism of the offspring throughout life. The significance of fatty acids quality on the developmental origins of adult disease is still unknown.

Objective: To evaluate the impact of dietary fatty acids quality in pregnancy on developmental origins of disease in adult offspring.

Procedure: C57Bl6/J mice were fed experimental diets in pregnancy and lactation. Parameters of the metabolic syndrome: insulin resistance and body fat accumulation were measured in adult offspring after high fat diet (HFD, lard). Isocaloric diets contained 6% oil: control (C, soybean oil), saturated (SFA, coconut), n-3 (flaxseed) or n-6 (safflower). During HFD, glucose uptake (GTT), insulin levels and HOMA index were determined. Body fat (BF) was estimated by MRI.

After sacrifice, tissues lipid content and fatty acid composition were analyzed. $\Delta 6$ and $\Delta 9$ desaturase activities were determined.

Results: HOMA index was 10% higher in SFA vs C. In n-3 and n-6 it was 65% and 35% lower vs C. BF in n-3 was 50% lower vs C. Liver lipids in n-3 and n-6 were 20% and 40% lower vs C and in SFA 36% higher. Pancreas lipids in n-3 and n-6 were 48% lower than in C. Muscle lipids in n-3 and n-6 were 78% and 44% lower vs C; in SFA, 18% higher. Epididimal fat in n-3 was 75% lower vs C. $\Delta 6$ and $\Delta 9$ desaturase activities increased and decreased, respectively in n-3 vs SFA.

Conclusion: Dietary fatty acids quality in pregnancy rule the development or prevention of disease. Saturated fatty acids favor, whereas unsaturated prevent metabolic syndrome in adult offspring.

P223

The effect of a high fat meal on measures of endothelial function and leukocyte activation in healthy volunteers

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Background: Endothelial dysfunction (ED) is a hallmark for the initial stage of vascular dysfunction and has been associated with diet-related disorders such as cardiovascular disease. This makes prevention of ED an important health target. From previous studies we know that a high-fat (HF) meal temporarily impairs endothelial function (EF). Most intervention studies only evaluated the effect of a HF meal on Flow Mediated Dilatation (FMD), a measure of macrovascular EF. The question remains whether other types of macro- and microvascular EF measurements can be used to observe ED after a HF meal. In addition, it has been shown that consumption of a high fat meal results in an increased expression of activation markers on leukocytes, which might contribute to ED.

Objectives: The ultimate goal of this study is the establishment of a reliable model to investigate postprandial induced ED. Therefore, we examined the effect of a HF meal on several measures of EF. The secondary aim was to evaluate the effect of a HF meal on the expression of activation markers on leukocytes.

Procedure: The study was a double-blind randomized cross-over intervention with 20 healthy young male volunteers (age 18-28). Participants visited the university six times, where they consumed either a HF milkshake or a 'average breakfast' control milkshake. Both interventions were performed in triplicate. Measures of EF and blood samples were collected at baseline and at 3 and 6 hours after consuming the milkshake. EF was determined by measuring local arterial stiffness by echo-tracking of the carotid artery, regional arterial stiffness by pulse wave analysis/tonometry of the radial artery, micro-vascular skin blood flow by iontophoresis/ laser Doppler and macrovascular blood flow by FMD. Leukocytes activation markers were measured by flow cytometry analysis.
Results: The study has been finalized in March 2009 and data analyzes is ongoing.

P224

B-vitamin supplementation is necessary for the brain gamma-secretase activity reducing effects of docosahexaenoic acid and uridine monophosphate

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Background: Alzheimer's disease (AD) is characterized by neurodegeneration and deposition of beta-amyloid (Abeta) plaques in the brain. Abeta peptides may promote pro-inflammatory responses and activate neurotoxic pathways causing neuronal dysfunction characterized by a loss of spines and neurites leading to neuronal death. Abeta is produced by clipping of APP by beta- and gamma-secretase, membrane-bound enzymes. Previously, it was shown that specific nutrients may affect brain processes relevant to AD, including neuronal membrane synthesis. Docosahexaenoic acid (DHA) and uridine monophosphate (UMP) increased formation of neuronal membrane and dendritic spines.

Objective: In order to test the influence of nutrients on membrane-bound enzymatic activity relevant to AD, the effects of DHA and UMP on brain g-secretase activity as well as the role of B-vitamins in enabling these actions, were now assessed.

Procedure: EXPT1: Gerbils were fed with UMP enriched diet and gavaged daily with DHA from different sources. EXPT2: Rats were fed with DHA+UMP in combination with a B-vitamin deficient or a B-vitamin supplemented diet. After 4 weeks, brains were collected and homogenized. Activity of g-secretase was measured with a fluorescent assay using a g-secretase specific peptide. Rate of fluorescent signal increase caused by peptide breakdown was taken as a measure of g-secretase activity. Additionally, plasma homocysteine levels were determined.

Results: Combined dietary supplementation of DHA and UMP reduced brain g-secretase activity only in the presence of sufficient B-vitamins. Single nutrient supplementation had no effect on g-secretase activity. Plasma homocysteine levels were lowered in rats receiving B-vitamins in the diet.

Conclusion: These data show that only combined administration of B-vitamins, DHA, and UMP is successful in lowering g-secretase activity significantly, indicating that a multi-nutrient approach may offer the most effective method in the dietary management of AD.

With funding from the EU FP7 project LipiDiDiet, Grant Agreement NÂ° 211696.

P225

Eicosapentaenoic acid as an add-on treatment for co-morbid major depression in patients with diabetes mellitus: a randomized, double-blind placebo-controlled study

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Background: Depression is a common, burdensome psychiatric disorder in people with diabetes. A considerable percentage diabetes patients receiving antidepressant drug therapy do not achieve remission of major depression. It has been suggested that ω -3 polyunsaturated fatty acids, in particular ethyl-eicosapentaenoic acid (E-EPA), may be an effective add-on treatment for reducing depressive symptoms in patients with major depression.

Objective: The present study is the first to test the efficacy of E-EPA versus placebo in the treatment of co-morbid major depression in people with diabetes mellitus.

Procedure: We conducted a 12-week, placebo-controlled, double-blind study of E-EPA (1 gram/day) versus placebo in 25 diabetes patients meeting DSM-IV criteria for major depressive disorder, in addition to ongoing use of anti-depressant medication. Depressive symptoms were assessed with the Montgomery Åsberg Depression Rating Scale (MADRS) at baseline and for 12 weeks follow-up with two-weekly intervals. Blood samples were collected at baseline and at 12 week follow-up to determine EPA levels in the erythrocyte membranes. Data were analyzed with ANOVA for repeated measures.

Results: Thirteen participants were randomly assigned to E-EPA; 12 patients were given placebo. Patients receiving E-EPA had tripled levels of EPA in their erythrocyte membranes at 12 weeks follow-up, while this remained stable in control participants receiving placebo. In both groups, depressive symptoms significantly decreased over time ($F = 21.14$, $P < 0.001$), yet no significant differences were found between those treated with E-EPA versus placebo ($F = 1.63$, $P = 0.145$).

Conclusion: In this pilot study, no evidence was found

for the efficacy of E-EPA in reducing depressive symptoms in diabetes patients with co-morbid depression.

Clinical Trials Registration: ISRCTN 30877831.

Caption 1: Development of the MADRS score over time

P226

Docosahexaenoic acid and infant heart rate variability

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Objective: To examine the effects of infant formula with docosahexaenoic acid (DHA) on infant cardiac autonomic control.

Procedure: Infants enrolled in the DIAMOND study at the University of Kansas Medical Center were randomly assigned to be fed formula containing one of 4 different levels of DHA (0, 0.32, 0.64, and 0.96% of total fatty acids) from birth to 12 months. At 4, 6, and 9 months, infants ($N = 120$) participated in a visual habituation protocol in which behavioral and psychophysiological indices (EKG) were simultaneously measured. Interbeat-interval (IBI) series derived from the EKG were hand corrected for artifacts and ectopic beats (QRSTool software). A mixed-model analysis for Age and DHA was run on several different indices of heart rate variability (HRV): Standard Deviation of IBIs (SDNN), Mean Absolute Successive IBI Differences (MSD), a Cardiac Vagal Index (CVI), and a Cardiac Sympathetic Index (CSI: a ratio of the Toichi metrics).

Results: Age differences (varying from $p < .01$ to $p < .001$) were observed for all metrics. For two specific metrics, however, the DHA assignment group had a marginal or statistically significant effect on the HRV measures; the highest dose showed the most optimal HRV values. For MSD, the effect of Group trended toward significance, $F(3, 163.908) = 2.193$, $p = .09$. For CSI, the Group effect attained statistical significance, $F(3, 197.225) = 2.69$, $p < .05$. The highest dose condition showed the greatest balance among sympathetic/parasympathetic outputs, and a comparison of the highest dose group with the other three attained statistical significance ($p < .05$).

Conclusion: DHA may affect various metrics of heart rate variability. The data analysis presented here suggests that high doses of DHA in infant formula produced greater balance of sympathetic nervous system function, as indicated by its effect on CSI.

Supported by Mead Johnson Nutrition.

P227**Vitamin B6-mediated sphingosine 1-phosphate metabolism in the immunological homeostasis in the gut**

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Objective: Unique gut immune systems are equipped to maintain the immunological homeostasis at intestine, and its disruption results in the immune diseases. We previously reported that sphingosine 1-phosphate (S1P), a lipid mediator, regulates the trafficking of gut-associated lymphocytes including intraepithelial T lymphocyte and IgA-producing B cells (J Exp Med, 2007; Blood, 2007 & 2008; J Immunol, 2008). Additionally, we found that S1P also mediated the development of food allergy by the regulation of trafficking of pathogenic cells (J Immunol, 2007). Because S1P metabolism is mediated by vitamin B6, we aimed in this study to elucidate whether vitamin B6-mediated S1P metabolism was involved in the development of food allergy.

Procedure: We employed ovalbumin (OVA)-induced intestinal allergy model as food allergy model (J Clin Invest, 106:199-206, 2000). To prevent S1P-mediated signal and vitamin B6-mediated S1P metabolism, mice were treated with FTY720 and 4-deoxypyridoxine (DOP), a vitamin B6 antagonist, respectively. We compared diarrhea incidence and immunological phenotypes such as serum IgE production and mast cell infiltration.

Results: As treatment with FTY720, DOP treatment resulted in the inhibition of allergic diarrhea without affecting serum IgE production. Intriguingly, FTY720-treated mice showed reduced numbers of both mast cells and activated T cells in the large intestine, while only mast cells were inhibited to infiltrate into the large intestine of DOP-treated mice.

Conclusion: Our current data collectively indicate the critical role of vitamin B6-mediated S1P metabolism in the development of food allergy. It also suggested that mast cells and activated T cells showed different dependency on S1P in the development of food allergy, which provides a new potential target in the prevention of allergic responses in the intestine.

P228**Monoacylglycerols from echium plantagineum seed oil: inhibitory action on human cancer cell growth**

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Background: Nowadays there is an increasing interest towards the development and improvement of alternative therapeutic methods for the treatment of several types of cancer. Beneficial effects achieved by using n-3 polyunsaturated fatty acids (n-3 PUFAs) have been widely reported, but few evidences have been found related to the effects of monoacylglycerols (MAGs), a family of lipids with a single fatty acid moiety attached to the glycerol backbone.

Objective: The objective of this work was to assay the cytotoxic activity induced by MAGs compared with triacylglycerols (TAGs) and free fatty acids (FFAs) tested in a human colon cancer cell line.

Procedure: MAGs, TAGs and FFAs were obtained after enzymatic hydrolysis of Echium plantagineum seed oil. Cells were treated at various concentrations of MAGs, TAGs and FFAs, and results were obtained by means of the MTT test.

Results: Data obtained in this study suggest that inhibition rate of tumor cells for MAGs was higher than those for other products from enzymatic hydrolysis whereas negative effects against healthy colon cell line have not been detected.

Conclusion: Data obtained in this study suggest that only MAGs among all hydrolysis products tested could be considered as a bio-active compound for inhibiting cell growth in the cancer cell line assayed.

P229**Dual action of an omega 3 polyunsaturated fatty acid analogue on hyperglycaemia and diabetic nephropathy in experimental diabetes**

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Objective: The limitations experienced in omega3 fatty acid (FA) supplementation to treat human diseases led us to synthesize analogues of these FA to increase potency, tissue stability and target selectivity. One of these, B-oxa21:3n-3, showed such properties. Because of its ability to selectively inhibit the activation of protein kinase C (PKC)β, a signaling molecule involved in promoting hyperglycemia-induced nephropathy, over other PKC isozymes, the anti-diabetic effect of this FA was examined.

Procedures: Albino rats were made diabetic by an injection of streptozotocin (65mg/kg) and then treated with different doses of B-oxa21:3n-3. Blood glucose was measured using a glucometer and PKC B translocation by western blotting of particulate fractions. Glucose disposal was determined by a glucose tolerance test and in vitro glucose uptake was by a radiometric assay using L6 myotubes.

Results: The protective effects of B-oxa21:3n-3 was

evident in short (3 weeks) and longer (3 months) term experimental diabetes. There was significant reduction in polydipsia and polyuria, associated with marked reduction in microalbuminuria. Histological assessment of the glomeruli and surrounding tubulointestitium showed suppression of mesangial expansion and reduction in damage to the tubulointestitium in response to β -oxa21:3n-3. Liver function assessed by blood levels of AST and ALT, also improved in response to β -oxa21:3n-3. Quantification of the FA by GC-MS in rat blood and kidneys showed that β -oxa21:3n-3 reached levels which inhibited high glucose-stimulated PKCB activation in mesangial cells in vitro and in vivo. Unexpectedly, rats treated with β -oxa21:3n-3 showed significantly improved response to a glucose tolerance test, consistent with improved glycaemic control during the treatment period. β -oxa21:3n-3 showed insulin like activity in promoting glucose uptake by L6 myotubes.

Conclusions: The omega3 fatty acid analogue, β -oxa21:3n-3, shows two major properties relevant to protection against diabetes, an ability to act like insulin and reduce hyperglycaemia and protect against complications such as nephropathy.

Biochemistry of Lipids

P230

Regulation of human lymphocyte signaling by oleic and linoleic acids: role of PKC-zeta

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Background: Previous studies of our group showed that oleic (OA, C18:1, n-9) and linoleic (LA, C18:2 n-6) acids promoted a stimulatory effect in human lymphocyte proliferation at 25 μ M. This effect did not involve JAK-STAT pathway activation. Others studies have shown that phosphatidylinositol-3,4,5-triphosphate (PIP3) generated by phosphoinositide 3-kinase (PI3K) also play a critical role in lymphocyte activation and proliferation.

Objective: In the present study, we investigated the role of OA and LA on lymphocyte signaling pathways related to cell proliferation and the involvement of PI3K with these effects.

Procedure: Human lymphocytes were isolated from normal donors from heparinized venous blood by density-gradient sedimentation using Histopaque. Cells were stimulated with concanavalin A (ConA) for 24 hours and cultured with fatty acids in the presence and absence of

PI3K inhibitors wortmanin (100 nM) and LY294002 (10 μ M). Cell proliferation was determined by thymidine incorporation. Before protein analysis, the cells were stimulated with ConA and treated with wortmanin or LY294002 for 30 minutes. After this period cells were incubated with fatty acids for one hour. Afterwards, PKC-zeta and ERK1/2 phosphorylation were analyzed by Western Blotting and PKC-zeta activity was determined by an immunocomplex kinase assay.

Results: OA and LA at 25 μ M pronounced the stimulatory effect of IL-2 in lymphocyte proliferation and ERK1/2 phosphorylation, but this effect was abolished in the presence of the inhibitors. These fatty acids also increased PKC-zeta activity, but in the presence of wortmanin and LY294002 no effect was observed. The total expression of all these proteins was not altered by the fatty acids tested.

Conclusion: In conclusion, OA and LA stimulate lymphocyte proliferation by increasing PKC-zeta activity and consequently ERK 1/2 phosphorylation. Probably, these effects involves PI3K activation and PIP-3 generation.

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P231

Cow milk lipid-droplets organization modulates triglycerides digestion by gastric and pancreatic lipases in vitro in conditions close to human physiology

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Background: Dairy lipids are consumed as lipid-droplets surrounded by phospholipids layers in native milk, or having a size and a surface composition modified by treatments. This organization might modulate fatty acid bioavailability and thus postprandial lipemia, which the impact in cardiovascular diseases is strongly suggested.

Objective: We aimed to determine the effect of industrial technological process on triglycerides digestibility from differently-organized cow milk lipid-droplets.

Procedure: Lipid-droplet from native milk (4.1 μ m), pasteurized milk (4.7 μ m), or homogenized-pasteurized milk (0.6 μ m), and big-sized lipid-droplets (BLD: 10 μ m) or small-sized lipid-droplets (SLD: 1.6 μ m) were digested *in vitro* under gastric (pH 4.5, 60 min) and duodenal conditions (pH 7, 30 min) close to human physiology. Generated free fatty acids were quantitated after lipid extraction.

Results: Pasteurization enhances gastric (18% vs 4%) and duodenal triglycerides digestibility (63% vs 48%). Surprisingly, homogenization, that markedly decreases lipid-droplet size increasing consequently the lipid/water interface, does not improve gastric (9%) or duodenal (51%) lipolysis probably due to an important change in droplet surface composition (micelles of casein). SLD are 2 to 2.5 times more efficiently hydrolyzed than BLD by gastric (18% vs 8%) and pancreatic (76% vs 31%) lipases, in accordance with published data on artificial lipid-droplets (enteral emulsions) (Armand *et al.* 1999); this can be explained by the fact that small-sized droplets offer a higher lipid interface area that allows to 1) delay the inhibition phenomenon of gastric lipase by the released long-chain fatty acids, and 2) increase the number of lipases molecules able to bind at the lipid/water interface and to hydrolyze triglycerides.

Conclusion: The size of dairy lipid-droplets is not the sole important parameter for fatty acid bioavailability; other key factors (type of proteins or of phospholipids surrounding the lipid-droplet) might play a determinant role that needs to be deeply explored. ANR-06-PNRA-012 AGILAIT.

P232

Barramundi desaturase and elongase prefer omega 3 fatty acids as substrates and the delta 6 desaturase has delta 8 activity

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Background: Barramundi (*Lates calcarifer*) are diadromous fish inhabiting the freshwater and saltwater estuarine regions of northern Australia.

Objective: To examine the enzymic capability for conversion of 18 carbon omega 3 (n-3) alpha-linolenic acid (ALA, 18:3n-3) to n-3 long chain polyunsaturated fatty acid (LCPUFA) in barramundi.

Procedure: We performed 5' and 3' rapid amplification of cDNA end to identify the full sequences of barramundi putative delta 6 desaturase and elongase and cloned the full length open reading frame (ORF) of the two enzymes into *Saccharomyces cerevisiae* to test for substrate specificities and activities.

Results: Functional expression of barramundi delta 6 desaturase (barraD6D) and elongase (barraElo) revealed that the barraD6D is not only capable of desaturating ALA to 18:4n-3, LA to 18:3n-6 and 24:5n-3 to 24:6n-3 but also showed delta 8 desaturase activity which synthesises 20:4 n-3 and 20:3n-6 from 20:3 n-3 and 20:2 n-6, respectively. The BarraElo showed a broad range of fatty acid substrate

specificity from 18 to 22 carbon PUFA with the greatest activity with n-3 eicosapentaenoic acid (EPA). However, the conversion rate of desaturation was generally lower than elongation in yeast system. In addition, both barraD6D and barraElo showed a preference for n-3 substrates over n-6.

Conclusion: These findings show that barramundi have the desaturation and elongation capacity for LCPUFA biosynthesis using ALA and LA as substrates. Also, the barraD6D possesses delta 8 desaturase activity providing an alternate route for LCPUFA synthesis.

P233

Subcellular localization of FABPs from parasite helminths

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Background: FABPs are highly conserved proteins for which the elucidation of their functional role is one of the issues still unresolved. These proteins are key molecules in the biology of cestodes as these organisms are unable to synthesize de novo most of their own lipids. Our group has isolated four genes encoding fatty acid-binding proteins: EgFABP1 and EgFABP2 from *Echinococcus granulosus* (Platyhelminth, Cestoda), the parasite responsible of Hydatid disease and MvFABPa and MvFABPb from *Mesocostoides vogae*, another cestode used as a model organism.

Objective: We set as our objectives to determine the intracellular location of EgFABPs and MvFABPs to approach to the understanding of their functions.

Procedure: The following techniques were applied: differential centrifugation, western blot, mass spectrometry, confocal fluorescence immunomicroscopy, parasite culture.

Results: Using subcellular fractionation techniques coupled with western blot of one and two dimension electrophoresis and mass spectrometry we identified the proteins under study in different subcellular compartments: cytosol, nucleus, mitochondria and microsomal fraction. In toto immunomicroscopy of *M. vogae* larvae allowed us to verify the previous results using commercial fluorescent markers. Colocalization with Bodipy FL-C16 and FABPs signal suggested in vivo binding. In vivo uptake was also demonstrated.

Conclusion: Unlike the expected, *M. vogae* FABPs seems to have an ubiquitous localization, since both proteins are present in all the compartments studied. However, a small difference in their relative concentrations on the different compartments could indicate a differential behavior. The colocalization of these proteins with nucleic acids suggests that MvFABPs

might be involved in regulating gene expression as occurs with L-FABP (liver) of mammals. In this sense we have begun the search for transcription factors of the type of PPARs. Concerning EgFABPs localization of EgFABP2 has not been identified in any of the subcellular fractions.

P234

Adipocytes and J774 cells co-cultivation regulate pro-inflammatory mediators expression

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Background: The obese state is characterized by inflammation of low intensity and this increased production of pro-inflammatory molecules by adipose tissue has been associated with the accumulation of macrophages in this tissue. The molecular signals that initiate macrophages activation in obese adipose tissue are not yet known, but there are several candidates.

Objective: In order to determine which molecules (proteins or lipids) may be involved in this activation process, we used a model of co-cultivation between adipocytes and monocytes. **Procedure:** J774 cells were maintained in DMEM containing 10% fetal calf serum. We extract the adipocytes of epididymal adipose tissue of mice C57black using collagenase type II and incubated with J774 cells for different periods of time (1, 3, 4, 6, 12, 18 and 24 hours) to evaluate the gene expression of TNF- α , IL-1 β , IL-6, IL-10, MRS-1 and MIP-1 by real-time PCR in both cell types. Total RNA was extracted using Trizol reagent. The expression of genes was compared with that of the constitutive Hprt1 gene whose expression was not modulated by treatment.

Results: The co-culture with adipocytes stimulated the expression of TNF- α , IL-1 β , IL-6, IL-10 and MRS-1 cells by J774, at specific periods of incubation. The expression of TNF- α increased about 4 times after 1 hour of incubation. All other genes had their expression increased after longer periods of co-cultivation besides MIP-1. In adipocytes we also observed an increase in TNF- α and IL-1 β expression after short periods of co-cultivation. The expression of IL-6 was stimulated mainly after 12 hours and the expression of MCP-1 was increased in almost all periods of incubation.

Conclusion: The results suggest that adipocytes and monocytes contact may lead to time dependent changes in expression of many proteins involved in inflammation. Financial support: FAPESP and CNPq.

P235

Effect of fasting and diet on fatty acid metabolic alterations in very-long chain acyl-CoA dehydrogenase (VLCAD) null mouse heart

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Background & Objective: VLCAD deficiency is one of the most common fatty acid (FA) oxidation disorders in humans and has been linked to cardiomyopathy development. The VLCAD deficient (VLCAD^{-/-}) mouse shows a milder phenotype than that of humans. Further to our findings that VLCAD^{-/-} mouse hearts can efficiently oxidize exogenous long chain FA, but display other lipid metabolic alterations, we conducted a targeted metabolomic and gene expression profiling study to better understand the role of VLCAD on FA metabolism.

Procedure: Using fed (standard or high fat (HF) diet) and 24 h fasted 15, 30 and 45 week-old VLCAD^{-/-} mice and their control VLCAD^{+/+} littermates, we assessed: (i) the FA profile of triglycerides (TGs) and phospholipids (PLs) as well as, (ii) citric acid cycle (CAC) intermediates levels by gas chromatography-mass spectrometry, and (iii) the expression of selected metabolic genes using qPCR in the heart.

Results: FA profiling revealed the following alterations in VLCAD^{-/-} hearts: (i) in PLs, levels of docosahexaenoic acid were lower under all conditions tested, while those of arachidonic acid were higher albeit only with the HF diet; and (ii) in TGs, there were age- and condition-dependent accumulation of FAs, more especially, palmitate, oleate and linoleate. Cardiac CAC intermediates levels were also decreased in VLCAD^{-/-} mouse in an age-dependant manner. qPCR analysis revealed also cardiac alterations in mRNA levels for metabolic genes, which were exacerbated by fasting or HF diet, specifically as protein/enzyme involved in: (i) FA β -oxidation (Acadl and Acadm), and (ii) TG hydrolysis (Lipe), (iii) anaplerosis (Pcx and Pccb) or (iv) mitochondrial functions (Ppargc1a and Ucp3).

Conclusion: Collectively, our results demonstrate that VLCAD^{-/-} mouse display metabolic alterations despite their ability to oxidize long chain FAs. These alterations are exacerbated by aging and FA overload and could be involved in cardiomyopathy development.

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P236

Characterization and differences of human facial and abdominal subcutaneous preadipocytes

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Background: The loss of subcutaneous fat in facial skin is associated with aging. In contrast, abdominal fat tends to increase with age

Objective: Understanding the molecular and cellular pathways that regulate the metabolism of subcutaneous fat in these anatomical areas could identify distinct targets for therapies for these conditions.

Procedure: Mesenchymal progenitor cells (preadipocytes) were isolated from human facial and abdominal skins obtained with informed consent, and were compared for their capacity to differentiate, to synthesize lipids, and to produce lipid droplets.

Results: As expected, the combination of IBMX, dexamethasone, and insulin induced the production and accumulation of lipid droplets in both types of preadipocytes. The inclusion of rosiglitazone, a PPAR-g agonist, further increased the number of differentiated adipocytes and the size of lipid droplets in both cases. The expression of several lipogenic genes (FABP4, FAS, PPAR-g, and GLUT4) was similarly induced in facial and abdominal cells. However, a greater proportion of facial preadipocytes were induced to differentiate in vitro. In addition, facial cells retained their ability to differentiate through a greater number of sub-passages. Finally, isoproterenol, a beta-adrenergic receptor agonist, differentially affected facial and abdominal cells, stimulating lipolysis in abdominal cells but not to the same extent in facial cells.

Conclusion: The loss of facial subcutaneous fat with aging does not seem to be secondary to a loss of preadipocytes or their ability to differentiate. The observed differences could be due to a tissue-specific mechanism, such as the lack of an adipogenesis signal or possibly changes due to the increased UV-exposure of facial skin compared to abdominal skin.

P237

Sunlight-induced IL-11 may cause photoaging-induced loss of subcutaneous fat

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Background and Objective: Sun-exposed areas of the body tend to lose subcutaneous fat with aging. IL-11 is known to inhibit the differentiation of adipocytes in vitro, and particular cytokines (eg IL-1 and TGF- β) stimulate fibroblasts to secrete IL-11. We hypothesize that

ultraviolet light may stimulate the production of IL-11 by skin dermal fibroblasts in vivo, and that this mechanism may explain the loss of facial subcutaneous fat with aging.

Procedure: Human fibroblasts, epidermal-dermal equivalents, epidermal equivalents, and explants from human abdominal skins were exposed to radiation from a Newport solar simulator and/or treated with IL-1 +/- TGF- β . IL-11 secretion was measured by ELISA. Human subcutaneous preadipocytes were isolated from samples of abdominal and facial surgeries (with informed consent) and were induced to differentiate in vitro under standard conditions.

Results: Preliminary experiments confirmed that recombinant IL-11 inhibits adipocyte differentiation in vitro. Radiation from a solar simulator (2.5 - 10 J/cm²) stimulated IL-11 secretion from fibroblasts, epidermal-dermal equivalents, and explants, and the combination of cytokines and solar irradiation resulted in an approximately additive effect. Epidermal equivalents failed to produce IL-11 in response to solar irradiation (5 J/cm²), confirming that dermal cells are required for IL-11 secretion.

Conclusion: These results support the hypothesis that solar radiation-induced IL-11 may be involved in the photoaging-induced loss of subcutaneous fat. Inhibition of this process might slow or reverse age-induced changes in facial contouring.

P238

Serum lowers anabolic processing of highly unsaturated fatty acids and conversely favours their catabolic retroconversion in differentiated Caco-2 cells

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Background: Dietary long-chain polyunsaturated fatty acids (PUFA) play key roles in cell physiopathology. In addition to its role in the uptake of dietary fatty acids, the intestinal epithelium plays an important role in their pre-systemic biotransformation. In vitro culture of differentiated Caco-2 cells is widely used as an appropriate model of the human absorptive enterocytes.

Objective: Our aim was to further elucidate the ability of Caco-2 cells to process long-chain PUFA and to sort them into triglycerides and phospholipids.

Procedure: Caco-2 cells were cultivated for 14 days either in DMEM containing 10% of FBS or in serum-free hormone-defined BDM. They were then incubated for 7 days in the presence of either ω -6 (C18:2, C20:4, C22:6) or ω -3 (C18:3, C20:5, C22:5) fatty acids. Cells were then harvested, fat was extracted and separated into TG and PL before GC determination of fatty acid profiles.

Results: Conversion of C18:3 ω -3 or C18:2 ω -6 into longer and/or more unsaturated fatty acids, as well as elongation of C20:5 ω -3 or C20:4 ω -6 was much more efficient in the serum-free BDM condition. By contrast, a significant retroconversion of C22:6 ω -3 into C20:5 ω -3 was detected in cells cultivated in FBS-DMEM, but not in serum-free BDM. The proportion of fatty acids incorporated into phospholipids was higher in FBS-DMEM than in serum-free BDM.

Conclusion: The presence of serum in the culture medium appears to influence the biotransformation of long chain PUFA by down-regulating the activity of Δ 9, Δ 6, Δ 5 desaturases, promoting their retroconversion and favouring their incorporation into phospholipids.

P239

Activity of muscle lipoprotein lipase after acute exercise

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Background: Lipoprotein Lipase (LPL) plays a major role in the metabolism of lipids and is responsible for the hydrolysis of triacylglycerol from circulating chylomicrons and very low-density lipoproteins. We have previously shown, using subcellular fractionation, that in rat skeletal muscle, in the basal state, muscle LPL (mLPL) was located within the cytosol. The site of activation is the luminal surface of endothelial cells in the capillaries. An increase in mLPL activity is thought to be mediated at least in part by a translocation of the enzyme from the cytosol to the site of activation.

Objective: The objective of the present study was to investigate the regulation of muscle LPL activity after acute exercise.

Procedure: Male Wistar rats were subjected to one of two exercise protocols; 1) Four hours of swimming exercise (n=8) or 2) Ten minutes of intense in situ electrical stimulation (n=8).

Results: In the present study short term in situ electrical stimulation of rat hindlimb muscles did not increase the activity of mLPL above resting levels, whereas prolonged swimming exercise induced increased activity of mLPL. Both exercise protocols resulted in decreased muscle glycogen content and increased plasma adrenaline concentrations.

Conclusion: Taken together these results indicate that muscle glycogen or the level of circulating adrenaline per se seem not to be responsible for the activation and translocation of mLPL in response to acute prolonged exercise. Total energy turnover during the exercise bout could be an important factor in regulating mLPL activity as total energy turnover is higher during the prolonged swimming exercise compared to the in situ stimulation, though based on the present study a time effect cannot

be excluded. (Signalling parameters are under investigation.)

P240

Quantifying *de novo* lipogenesis with deuterated water - estimating hepatic acetyl-CoA precursor enrichment

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Background & Objectives: In lipogenesis, the fatty acid methyl hydrogens (FA-methyl) are directly derived from acetyl-CoA. FA-methyl enrichment from $^2\text{H}_2\text{O}$ may be quantified by ^2H NMR analysis of triglyceride, thus the fractional synthetic rate (FSR) of fatty acids may be derived from the FA-methyl to acetyl-CoA ^2H -enrichment ratio. Currently, it is assumed that acetyl-CoA hydrogens are equilibrated with bulk water (BW) allowing FSR to be estimated as FA-methyl enrichment/BW. There are no measurements of hepatic acetyl-CoA enrichment from $^2\text{H}_2\text{O}$ therefore our objective was to quantify this by glutamine ^2H -enrichment analysis. Glutamine ^2H -enrichment is derived via the Krebs cycle. The position 3 hydrogens pair are directly derived from BW while position 4 hydrogens originate from acetyl-CoA. Hence the ratio of glutamine positions 4 to 3 enrichments (H4/H3) is equal to that of acetyl-CoA/BW.

Procedures: Livers from 8-wk fed Wistar rats (n=16) were perfused in recirculating mode for 2 hours with Krebs-Henseleit bicarbonate buffer enriched to 5% with $^2\text{H}_2\text{O}$ and supplemented with 11 mM glucose, 0.1 mM glycerol and 10 units/l insulin. Glutamine was isolated from the perfusion medium, hydrolyzed to glutamate and analyzed by ^2H NMR. The ratio of acetyl-CoA to body water enrichment was estimated as the ratio of glutamine position 4 to position 3 enrichments (H4/H3).

Results: Glutamine positional enrichments were 2.7 ± 0.3 % for hydrogen 2, 2.1 ± 0.1 % for each of the hydrogens in position 3 and 1.1 ± 0.1 % for each of the hydrogens in position 4. Glutamine H4/H3 was 0.53 ± 0.03 , thus acetyl-CoA enrichment was 53 ± 3 % that of body water.

Conclusions: In $^2\text{H}_2\text{O}$ -perfused livers, hepatic acetyl-CoA enrichment was only one-half that of BW. Thus, to obtain better estimates of fatty-acid FSR with the ^2H NMR method in this setting, hepatic acetyl-CoA ^2H -enrichment needs to be measured.

P241

Specific contribution of micelles versus vesicles in the absorption rate of free fatty acid and cholesterol in TC7-Caco2 cell

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Background: Free fatty acid (FFA), 2-monoglycerides and cholesterol (CH) are absorbed by enterocytes thanks to specific structures allowing their solubilisation in the lumen of the intestine, bile salt mixed-micelles and vesicles (liposomes). Both structures co-exist in the human intestinal lumen and their respective importance in the absorption of the lipid nutrients is not fully understood yet.

Objective: We aimed to compare the absorption rate of FFA and CH by human intestinal cell depending on the structure type, and to evaluate the impact on the gene expression of cell key transporters.

Procedure: Oleic acid (OA), 2-monoolein (2-MO) and CH were solubilised in classic bile salt-mixed micelles (BSM), bile salt-mixed micelles containing lysophospholipids (BSML), liposomes containing phosphatidylcholine (L), and liposomes containing phosphatidylcholine plus different species of lysophospholipids (LL). Absorption rate of OA and CH by Caco-2 TC7 clone was measured after 3 hours incubation time using radiolabelled molecules (3H OA and 14C CH). Gene expression of specific transporters of FA and CH was determined by Q-PCR and micro-arrays.

Results: OA is 4 to 7 times more efficiently absorbed when vehicled by L compared to BSM and BSML, and the presence of lysophosphatidylcholine containing oleic acid in LL enhances further the absorption rate. CH is 3.5 or 2.5 times more efficiently absorbed as BSML compared to BSM, and L or LL, respectively, and the species of lysophospholipid does not seem crucial. Expression of L-FABP and NPC1-L1 is significantly enhanced whatever the structure used by about 2.5 fold, whereas SRB-1 mRNA level is not increased.

Conclusion: Liposomes containing oleic-lysophospholipid represent the optimal structure for free oleic acid absorption, meanwhile cholesterol is preferentially absorbed as bile salt-mixed micelles containing lysophospholipids. The importance of the role of liposomes in the absorption of other species of free fatty acids needs to be further explored.

P242

Lipids with polyunsaturated fatty acids as cofactor of activation of G protein-coupled membrane receptors

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Background: G protein-coupled membrane receptors (GPCR) are often found in membranes that contain high

concentrations of lipids with polyunsaturated fatty acids.

Objective: We considered the issue of whether or not shifts between the active and inactive states of GPCR from lipid composition are fully explicable by differences in bilayer curvature elastic stress. Rhodopsin was chosen as typical representative of a GPCR of class A. **Procedure:** A series of six lipids with known spontaneous radii of monolayer curvature and bending elastic moduli were added at increasing concentrations to the matrix lipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and the metarhodopsin-I (MI) - metarhodopsin-II (MII) equilibrium measured by flash photolysis followed by recording UV-vis spectra. The average area per lipid molecule and the membrane hydrophobic thickness were derived from measurements of the 2H NMR order parameter profile of the palmitic acid chain in POPC.

Results: Large shifts in the MI-MII equilibrium towards MII correlated with introduction of docosahexaenoic hydrocarbon chains and the capability of the ethanolamine headgroups to engage in hydrogen bonding with rhodopsin, independent of the state of ethanolamine methylation. Hydrocarbon chain and headgroup effects were independent and additive. A quantitative comparison with previously reported spontaneous radii of curvature and bending elastic moduli of lipid monolayers of the investigated lipids indicated that changes of elastic parameters of the lipid matrix explain only a fraction of the shift towards MII.

Conclusions: The properties of lipids in the first layer surrounding GPCR, in particular the ability of docosahexaenoic acid chains to interact with GPCR and to adjust to GPCR conformational changes and the ability of ethanolamine headgroups to engage in hydrogen bonds with GPCR play a dominant role in the stabilization of the structural intermediates that activate G-protein.

P243

Cloning and functional characterisation of the fatty acyl Elovl2 and Elovl5 from rat

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Background: The fatty acyl elongase enzymes are required for the synthesis of long chain polyunsaturated fatty acids (LCPUFA). Luthria and Sprecher (1997) demonstrated that rat liver had approximately 10-fold greater elongation activity for C18 and C20 fatty acids substrates compared with C22 fatty acids.

Objective: To examine the substrate specificity of the rat elongases, Elovl2 and Elovl5.

Procedure: Two putative elongase sequences were cloned from rat liver tissue. The full length open reading frames

(ORFs) of the two genes were then cloned into *Saccharomyces cerevisiae*. Recombinant *S. cerevisiae* cells were cultured in the presence of various C18, C20 and C22 polyunsaturated fatty acid (PUFA) substrates to determine the substrate specificity of the enzymes.

Results: Elov12 was active with stearidonic acid (18:4n-3;SDA), eicosapentaenoic acid (20:5n-3;EPA), and docosapentaenoic acid (22:5n-3;DPA) with conversion rates of 55%, 55% and 35%, respectively. Compared with Elov12, Elov15 was similarly active with SDA and EPA but had little or no detectable activity with DPA. The potential for each enzyme to be active with more than one substrate has prompted substrate competition studies. To date, we have observed that SDA causes modest inhibition of Elov15 metabolism of EPA to DPA.

Conclusion: There is the potential for competitive n-3 substrate inhibition in the elongase reactions involved in metabolism of SDA to LCPUFA.

Luthria DL and Sprecher H. *Biochim Biophys Acta* 1346:221-30,1997.

P244

Fatty acid composition of Malaysian giant mudskipper (*Periophthalmodon schlosseri*) fish oil

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Background: The mudskipper (*P. schlosseri*) belongs to the Periophthalmalmin spp. within the family Gobiidae. They live in the mangrove swamps and intertidal habitats which are found only in tropical and subtropical regions. In some countries, the mudskipper provides a valuable source of protein throughout the Asian and African regions.

Objective: The main aim of the present study is to look into the composition of the Malaysian giant mudskipper lipid which might be useful to human health by looking into the ratio of ω - fatty acids present in *P. schlosseri* oil.

Procedures: The fish oil from Malaysian mudskipper fish was extracted using a modified Folch method. After methylation, the fish oil was analysed by GC and GC-MS. An HP-5 non-polar capillary column was used and the temperature was initially kept at 50°C for 2 min and then programmed at 5°C min⁻¹ to 250°C. The injector and detector temperatures were 220°C and 250°C respectively and He gas was used as carrier gas with a flow rate of 1.2 ml min⁻¹.

Result: The GC and GC-MS results showed that the major chemical constituents of *P. schlosseri* oil were palmitic (16:0) (23.0%) and stearic acid (C18:0) acid (29.2%), oleic acid (C18:1), (12.6%). The total saturated fatty acid (SFA) was also found to be high (48.3%). The ratio of the total saturated (SFA) and unsaturated acids (USFA) was 1:0.9 and it is considered that the giant snakehead fish oil is good for human consumption.

Conclusion: The result shows that the total ω -6 content of *P. chhlosseri* was 15.4% and it has ω -3 and ω -6 ratio of 1:1.

P245

Purification processes of 2-monoacylglycerols obtained from hydrolysis of *Echium plantagineum* seed oil

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Background: Triacylglycerols (TAGs) containing specific fatty acids in designated positions are called structured lipids (SLs), and they have become of interest because beneficial effects of these TAGs are closely associated to their structures. Mammalian pancreatic lipase hydrolyzes the ester linkages at the sn-1 and sn-3 positions with a preference for medium-chain over long-chain fatty acids. This way, MLM-type SLs containing medium-chain fatty acids (M) at sn-1 and sn-3 positions and long-chain fatty acids (L) at the sn-2 position could provide an improved absorption of the resulting sn-2 monoacylglycerol (sn-2 MAG) through the intestinal mucosa.

Sn-2 MAGs are convenient reagents for the synthesis of structured lipids. However, the yields in the synthesis and purification methodologies depend on the impact of acyl migration which isomerizes the sn-2 MAGs to sn-1 MAGs. This way, that factor should be taken into account when obtaining and purifying the desired sn-2 MAGs.

Objective: The objective of this study was the obtaining of sn-2 MAGs from *Echium plantagineum* seed oil containing gamma-linolenic acid (GLA, 18:3n-6) and stearidonic acid (SDA, 18:4n-3) as the most relevant components. Synthesis yield and acyl migration rate caused during purification processes have been reported.

Procedure: *Echium plantagineum* seed oil was hydrolyzed and the resulting sn-2 MAGs were purified by chromatographic column and by liquid-liquid extraction. Recovery yield and acyl migration rate (via 1H-NMR analysis) were determined.

Results: High acyl migration rates have been obtained when both purification methods are employed. This way, sn-1 MAGs are 1.5 times more abundant than sn-2 MAGs after the purification processes have been carried out. Chromatographic column purification offer better results for MAG purification after hydrolysis (more than 95%) than liquid-liquid separation (up to 50%).

Conclusion: None of the methodologies assayed for sn-2 MAG purification offer acceptable results due to the high acyl migration rate during the processes.

P246

Effects of dietary lipids on fatty acids composition of muscle of tilapia (*Oreochromis sp.*)

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Background: One of the mayor troubles related to the fish production is to find an adequate source for the fish diet. Substitution of the usual fish-based feed by other protein source is difficult due to the amino acid and fatty acid composition. Sources of protein from animal origin supply a suitable amino acid composition. However, their use for animal feeding purposes is forbidden, being only allowed some sources as feathers.

The insect-based meal could be a good alternative source of protein. Also, although the nutrient composition shows an adequate content in amino acids, the fatty acid profile shows a high rate between n-6 polyunsaturated fatty acids (n-6 PUFAs) and n-3 PUFAs.

Objective: The objective of this work is to compare fatty acid profiles in muscle between fishes fed with insect-based meal and fish-based meal.

Procedures: Tilapias (*Oreochromis sp.*) (n=90) with 30 ± 4.80 g of initial mean body weight were employed. Fishes were separated into 3 groups and fed with three different experimental diets (similar composition and different protein source) based on fish meal, insect meal and insect, blood and soya meal respectively. As lipid source fish oil and borage oil were used. After a two-month treatment fishes were slaughtered and the muscle was removed and frozen until fatty acid profile analysis.

Results: Muscular fatty acid profile is directly related to the diet composition: fishes which were fed with insect meal showed higher n-6 PUFA levels than the fish fed with fish meal. However, levels of n-3 PUFAs detected in fishes fed with insect-based meal were higher than what could be expected according to the n-3 PUFA content in insect-based meal.

Conclusion Results obtained in this work indicate that insect-based meals could be an alternative source of protein for fish feeding.

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Maternal and infant erythrocyte fatty acid changes during 3 months lactation are likely to be driven by insulin and DHA

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Background: Changes in maternal glucose and lipid fluxes, and de novo lipid synthesis (DNL) from pregnancy to lactation are driven by hormones, such as insulin, but also by nutrients. For instance, insulin induces, and docosahexaenoic acid (DHA) reduces, nuclear abundance of sterol regulatory element binding protein-1 (SREBP-1). SREBP-1 promotes DNL, fatty acid (FA) elongation/ desaturation and lipid synthesis.

Objective: With the influences of insulin and DHA in mind we tried to interpret maternal and infant erythrocyte (RBC)-FA changes from delivery to 3 months postpartum in 3 Tanzanian groups differing in freshwater fish intakes.

Procedure: RBC-FA were determined in mother-infant pairs at delivery and after 3 months exclusive breastfeeding in Maasai (no fish), Pare (low freshwater fish) and inhabitants of Sengerema (daily freshwater fish).

Results: The maternal RBC-FA courses exhibited signs of decreasing activities of DNL (16:0) and $\Delta 9$ -desaturase 9w9), but maternal and infant RBC-18:0 increased. RBC-18:1w7, 18:1w(e.g. 16:1 decreased in the mother, but increased in the infants. The maternal RBC-16:0 decrease and RBC-18:0 increase were more pronounced in the order Maasai>Pare>Sengerema, which is in the same order of increasing fish intake (all p<0.05) and RBC-DHA status (Maasai vs. Pare at delivery p=0.064, all other p<0.001). Postpartum RBC-14:0 increased in infants; but not in their mothers.

Conclusion: The postpartum restoring insulin sensitivity in the mother is likely to explain the RBC-FA signs of decreasing maternal DNL/ $\Delta 9$ -desaturase activities. The paradoxical maternal and infant RBC-18:0 increases suggest that the $\Delta 9$ -desaturase activity decline is more pronounced than the elongase-6 (Elovl6) decline. Oleic acid seems preferentially transferred to the child. Increasing infant RBC-14:0 derives from postpartum increasing insulin sensitivity of the breast, which promotes local DNL. The between-group differences in postpartum courses of RBC-16:0 and RBC-18:0 might well be related to differences in the status of DHA, which is a strong suppressor of SREBP-1.

P248**Maternal and infant docosahexaenoic acid (DHA) equilibrium during pregnancy and lactation**

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Background: Low-fish consumption is related to suboptimal neurodevelopment, coronary artery disease and (postpartum) depression. The courses of maternal long chain polyunsaturated fatty acids (LCP) in pregnancy and lactation in Western countries, and the higher LCP in infant serum lipids at birth compared with maternal lipids (biomagnification), suggest maternal depletion.

Objective: We investigated the LCP status at which the mother and infant reach an LCP equilibrium.

Procedure: Erythrocyte (RBC) and milk FA were determined in Tanzanian mother-infant pairs with low (Maasai), intermediate (Pare) and high (Sengerema) fish consumption. RBC were collected at delivery and after 3 months exclusive breastfeeding. Milk was collected at 3 months postpartum. We also used historical data from The Netherlands.

Results: At 3 months PP, there were no correlations for: maternal RBC-AA vs. breastmilk AA, breastmilk AA vs. infant RBC-AA or maternal RBC-AA vs. infant RBC-AA. There were positive correlations for: maternal RBC-DHA vs. breastmilk DHA, breastmilk DHA vs. infant RBC-DHA and maternal RBC-DHA vs. infant RBC DHA. Maternal RBC-DHA at 3 months was lower than the corresponding infant RBC-DHA up to a maternal RBC-DHA of 7.88 g%. A maternal RBC-DHA at delivery of 8.07 g% corresponded with an equal maternal RBC-DHA at 3 months. This maternal RBC-DHA equilibrium corresponded with an infant RBC-DHA of 7.21 g% at delivery, an infant RBC-DHA of 7.88 g% at 3 months and a milk DHA of 1.03 g% at 3 months.

Conclusion: Maternal and infant AA statuses seem independently regulated in these populations. At steady state intakes, the mother seems to lose DHA during lactation, when her RBC-DHA is below about 8 g% ('infant DHA biomagnification via breastmilk'). This maternal equilibrium corresponds with an infant RBC-DHA of about 7 g% at delivery and 8 g% at 3 months and a mature milk DHA of about 1 g%.

P249**The level of tristearin in high fat diets determines the hepatic insulin sensitivity**

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Introduction: High fat diets rich in saturated fat are commonly used to generate obese and insulin resistant mouse models. However, relatively little is known about the role of specific saturated fatty acids (FA) in the development of pathology. Here, we have studied the role of specific FA on tissue specific insulin sensitivity and whole body energy metabolism.

Methods: C57Bl/6 mice were fed diets based on palm oil (HFP) and lard (HFL) or palm oil. Since the level of the saturated FA stearate (C18:0) is strikingly different, we also used a HFP diet supplemented with tristearin (HFP+S) to the level present in HFL. Tissue specific insulin resistance was determined by hyperinsulinemic-euglycemic clamp analysis. Whole body metabolism was assessed by indirect calorimetry employing metabolic cages.

Results: Clamp analysis revealed significantly lower insulin mediated repression of endogenous glucose production in mice fed the HFL and HFP+S diets. In contrast, insulin stimulated glucose disposal was higher in mice fed HFL and HFP+S diets. Metabolic cage analysis revealed higher food intake, lower caloric energy expenditure and a preferential oxidation of carbohydrates over fatty acids in lard fed animals compared to palm oil. Dietary tristearin supplementation resulted in lower caloric energy expenditure and preferential oxidation of carbohydrates versus fatty acids compared to palm oil. DEXA scan analysis revealed a higher fat to lean mass ratio in animals fed a stearate rich diet.

Conclusion: Elevated levels of dietary stearate result in increased hepatic insulin resistance and an adverse metabolic phenotype characterized by low levels of fat oxidation and accumulation of adipose tissue mass.

P250**Increase of ALA bioconversion to LC-n-3 in plasma and liver of hamsters fed decholesterolized compared to normal milk fat diets**

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In the athero-thrombotic process, saturated fat (SFA) associated to cholesterol are known to increase the risk of atherogenesis by inducing the deposit of lipids in aorta

and vessels, while omega3 could reduce the final thrombotic phase by limiting the aggregation mechanisms. Milk fat, rich in SFA and cholesterol, contains limited ALA levels(0.4-0.8%), but, with a very low omega6/omega3 ratio (LA/ALA:2.5) the potential of bioconversion to LCn-3 could be preserved.

Objective: Evaluation of the impact on fatty acids, (i) of milk fat diets with or without cholesterol and (ii) of increasing dietary ALA by using blends including linseed oil.

Protocole: 10weeks-old hamsters received 20%fat diets for 12weeks. Two groups (n=8) received either normal milk fat with normal cholesterol (A1:280mg/100gfat) or low cholesterol levels (B1:50mg/100g fat), both of them having normal low ALA level (0.4-0.8%FA). In 4other groups, linseed oil was added to normal and decholesterolized milk fat to increase ALA levels to 3%FA (A2,B2 respectively) and 8%FA (A3,B3 respectively). Plasma and Liver FA were analysed.

Results/Conclusions: For equivalent dietary content of ALA (0.8%, 3% or 8%), a reduction of dietary cholesterol did not change ALA biodisponibility but induced a better bioconversion, increasing EPA+DHA-LCn-3. Compared to classical pure milk fat, cholesterol removal increased EPA+DHA in plasma and liver by 50% and 66% respectively. For the 3%ALA-blend groups, lowering the cholesterol content induced an EPA+DHA increase by 50 and 42% and for the 8%ALA-blend groups the increase reached 35 and 20% in plasma and liver respectively.

However the best bioconversion to DHA was obtained from pure decholesterolized milk fat, indicating that (i) the negative impact of cholesterol is stronger than the positive one obtained by artificial ALA supplementation, (ii) that the n6/n3ratio play a role and (iii) that desaturases activities (SCD1,Delta6,Delta5 determined by FA-Delta-Index) are participating to these modifications. Grants from SB-Alliance(F), Corman(Be)

P251

The relation between DHA and AA is synergistic at low DHA status and antagonistic at high DHA status

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Background: Both docosahexaenoic acid (DHA) and arachidonic acid (AA) are important across the entire lifecycle. Current data on the relation between DHA and AA are, however, inconsistent.

Objective: Investigate the relation between AA and DHA in populations with a wide variety of AA and DHA intakes.

Procedure: We determined erythrocyte (RBC) AA and DHA in male and non-pregnant female adults (n=212), women at delivery and 3 months postpartum (n=355), and infants from delivery until 3.5 years of age (n=1,157). We also determined AA and DHA in arterial (n=788) and venous (n=785) umbilical vessel walls. Populations were from the The Netherlands, Curaçao, Tanzania, Israel and Pakistan. In Tanzania we included populations with very high fish intakes. We also included populations with no fish intakes from Pakistan and Tanzania (Maasai, Iraqw, Barabaig).

Results: In each of the investigated compartments the relation between DHA and AA proved bell-shaped. This became supported by positive linear relationships in populations with low DHA status, and negative linear relationships in populations with high DHA status.

Conclusion: The relation between AA and DHA has mostly been studied in Western populations with low-moderate fish intakes. In the present study we included populations with both very low and very high fish intakes. Our genus evolved in a water-land ecosystem with ample availability of both DHA and AA. The present data suggest that at these high DHA and AA intakes, there is antagonism between AA and DHA, resulting in AA suppression. The synergism between AA and DHA at low DHA intakes suggests that AA will be adjusted to lower values, even if AA intake is high, such as by Maasai. Our results underscore regulation of the AA status by the concurrent DHA status. The AA status is for instance suppressed at an RBC-DHA status of 8 g%; the optimum for CAD prevention.

P252

Fatty acid binding proteins expression in zebrafish gut

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Background: Dietary fatty acids (FAs) are absorbed by the enterocyte. Once inside the cell, FAs are bound reversibly by transporters like Fatty Acid Binding Proteins (FABPs). FABPs are small proteins expressed in specific tissues, probably involved in the transport of FAs from the plasma membrane to cellular compartments among other possible functions. However, the precise role of each type of FABP remains to be elucidated. In teleost fish as in mammals, the proximal third of the intestine is the major site of fat absorption. Zebrafish appears as a model organism due to the large amount of information available at the molecular, cellular and anatomical level. It has been recently demonstrated that zebrafish enterocytes strongly expressed fabp1b and fabp2 mRNAs. However, no data are available at the protein level.

Objective: The aim of the present work was to study the protein expression pattern of FABP1b and FABP2 in the enterocyte and their dynamics after feeding.

Procedure: Zebrafish FABP1b and FABP2 recombinant

proteins were expressed, purified and used as antigens for antiserum production. The antibodies produced were used in immunohistological analyses. Gene expression pattern was concomitantly performed by whole-mount in situ hybridization. Both procedures were conducted on larvae after the first feeding under different diets.

Results: FABP1b and FABP2 proteins are expressed in soluble form in *E. coli*. The proteins are obtained in high purity after a first step of saline precipitation followed by molecular exclusion chromatography. The antibodies generated recognize a single band of appropriated weight in Western blot without cross reaction. Immunodetection on larvae revealed a different expression pattern along the intestine and a strong protein level in the enterocytes after feeding that was correlated to diet composition.

Conclusion: These data indicate that these FABPs play common and also specific roles in lipid-metabolic processes in the zebrafish gut.

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Islet transplant patients do not respond to hypolipidemic dietary intervention - role of hepatic de novo lipogenesis

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Background: Type 1 diabetes is associated with increased cardiovascular risk, yet typically normal blood lipid levels. Islet transplant (ITX) can restore insulin independence but is associated with life-long immunosuppressive drugs, which can be hyperlipidemic.

Objective: The objective of this study was to use stable isotopes to investigate the effect of ITX and a hypolipidemic dietary intervention on de novo lipogenesis (DNL).

Procedures: ITX (n=4) and non-diabetic control (CTL; n=4) subjects underwent testing before and after a dietary intervention incorporating a variety of hypolipidemic nutrients (fish oil, phytosterols, soy protein, and fibers). Deuterium-labeled water was used to estimate hepatic total and individual fatty acids (FA) DNL by measurement of deuterium enrichment in FA from VLDL in fasting baseline and 24h blood samples.

Results: In this group of patients, hepatic DNL of total and individual (14:0, 16:0, and 18:0) FA was significantly lower in ITX patients vs. CTL (p>0.05). The diet lowered LDL-cholesterol (LDL-c; p=0.04), and showed a trend towards reducing triglyceride (TG) 22% in CTL patients. Conversely, the diet increased TC 13% (p=0.04) and LDL-c 24% (p=0.04) in ITX patients. Following dietary intervention, hepatic total DNL showed a trend towards

reduction in the CTL patients but an increase in ITX patients. There were no significant changes in DNL of individual FA after diet intervention within each group, though there was a trend towards reduction in hepatic DNL of all major FA in the CTL patients.

Conclusion: A hypolipidemic diet effective at reducing blood lipids and DNL in healthy patients has surprisingly contrasting results in ITX patients. This indicates that ITX patients may not respond to conventional dietary therapy as would be expected. Mechanisms responsible may be related to adverse and unpredicted responses such as an increase in hepatic DNL, and may be influenced by immunosuppressive medications.

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The effects of iron and omega-3 fatty acid deficiencies, alone and in combination, on brain monoamines and cognition in rats

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Background: Iron as well as n-3 fatty acids (FAs) play an important role during brain development. Combined deficiencies in iron and n-3 FAs may have interactive effects on the structure and function of the central nervous system.

Objectives: To investigate the effects of iron and n-3 FA depletion, alone and in combination, on brain monoamine metabolism and spatial learning and memory function in rats.

Procedure: During a 5 week depletion period, male Wistar rats at 3 weeks of age were fed either a control, an iron deficient (ID), n-3 fatty acid deficient (n-3 FAD) or an ID+n-3 FAD diet. Spatial learning and memory were assessed at the end of the depletion period using the reference- and working-memory version of the Morris Water Maze (MWM) task.

Monoamine levels as well as total phospholipid (TPL) FA composition and iron concentration were examined in various brain regions.

Results: Working memory-related performance in the MWM was impaired in rats receiving an ID and n-3 FAD diet alone, but not in the combined group. In contrast, the ID+n-3 FAD rats had significantly altered monoamine levels in various brain regions compared to control rats,

whereas ID and n-3 FAD alone did not result in significant changes. After depletion brain iron levels were significantly reduced in ID and ID+n-3 FAD animals and TPL FA composition was significantly altered in different brain regions of n-3 FAD and ID+n-3FAD animals.

Conclusion The results show that a combination of ID and n-3 FAD may have additive effects on monoamine metabolism in rats. However, in the MWM task the occurrence of a combined deficiency seems to outweigh the effects of the single deficiencies.

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P255

Contaminants from paper can cause erroneous results in rapid testing for blood fatty acids

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Background: A newly developed method for determining the fatty acid composition in samples of whole blood via spotting on chromatography paper is faster and less expensive than existing methods.

Objective: To determine whether contaminants from paper influence the fatty acid composition analyses of whole blood using the chromatography paper method.

Procedure: Blood was collected by venapuncture from a healthy human volunteer. Blood (60 µl) was analysed either directly or from 20 µl or 60 µl whole blood applied to a 1.2 x 1.2 cm strip of chromatography paper. All samples were methylated with 1ml of 1% MeOH/H₂SO₄ for 3h. Fatty acid methyl esters were analysed by capillary gas chromatography. Each sample was repeated in triplicate.

Results: Compared with direct transmethylation of whole blood, spotting blood samples to paper resulted in an elevation in the percentage of saturated fatty acids and a reduction in the level of polyunsaturated fatty acids (table). Methylation of paper samples from 3 sources showed that peaks corresponding to 16:0 and 18:0 that ranged in total area from 2-12 picoamps.

Fatty acid composition (%) determined from whole blood directly or spotted on paper

Fatty acid*	whole blood 60 µl	Paper with blood 60 µl	Paper with blood 20 µl
16:0	22.6±0.04 ^a	24±0.06 ^b	28.2±0.76 ^c
18:0	11.6±0.02 ^a	11.5±0.06 ^a	12.1±0.22 ^c
18:2n-6	22.3±0.03 ^a	20.8±0.11 ^b	19.2±0.28 ^c
20:4n-6	9.3±0.02 ^a	8.7±0.03 ^b	8±0.11 ^c
20:5n-3	2.6±0.02 ^a	2.4±0.02 ^b	2.2±0.04 ^c
22:6n-3	5.1±0.02 ^a	4.8±0.03 ^b	4.4±0.04 ^c

*Value represent mean ± SD (n=3), different superscripts indicate significant difference between groups, P<0.05

Conclusion: These results suggest that contaminants from paper have the potential to alter the results of fatty acid analyses, especially when the amount of blood on the paper is low. There is a need to exercise caution when interpreting results obtained using the chromatography paper method.

P256

Australian children are not consuming enough long chain omega-3 PUFA

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Background: The Kids Eat Kids Play survey is the first national survey of Australian children's nutrition since 1995 and physical activity survey since 1985. The National Health & Medical Research Council (NHMRC) Nutrient Reference Values recommend Adequate Intakes (AI) for linoleic acid (LA), alpha-linolenic acid (ALA) and long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) ranging from 5-12 g/day, 0.5-1.2 g/day and 40-125 mg/day respectively, depending on age and gender. The NHMRC also recommend Suggested Dietary Targets (SDT) for optimising diets for lowering chronic disease risk for adults and adolescents 14 years and over. The SDT for LC n-3 PUFA is 610mg/day for males and 430mg/day for females.

Objective: To determine the current PUFA intakes, including the LC n-3 PUFA, with comparison to AI and SDT and to determine potential differences in intakes between children of different body weight and physical activity levels.

Procedure: The demographic and nutrient data files were merged for 4,487 children aged 2-16 years.

Results: The mean (median) PUFA intakes for 2-3 yrs, 4-8 yrs, 9-13 yrs, 14-16 yrs are as follows: linoleic acid (g) 5.2 (4.3), 6.7 (5.7), 8.3 (6.9), 9.7 (8.3) respectively; alpha-linolenic acid (g) 0.85 (0.71), 1.03 (0.86), 1.16 (0.99), 1.36 (1.17) respectively; LC n-3 PUFA (mg) 116 (47), 124 (55), 168 (67), 168 (78) respectively. Children met the AI for linoleic acid and alpha-linolenic acid, but only approximately 50% of children met the AI for LC n-3 PUFA. Furthermore, only 7% of the children met the SDT for LC n-3 PUFA per day. Comparison of LC n-3 PUFA tertile intakes showed no differences in 1) LC n-3 PUFA intakes in underweight, normal weight, overweight and obese children and 2) mean physical activity levels.

Conclusion: The majority of children need to increase their LC n-3 PUFA intakes for optimal health.

P257**Comparative analysis of target gene regulation by the transcription factor PPAR α between mouse and human**

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Background: Studies in mice have shown that PPAR α is an important regulator of hepatic lipid metabolism and acute phase response. However, little information is available on the role of PPAR α in human liver.

Objective: Here we compare the function of PPAR α in mouse and human hepatocytes via analysis of target gene regulation.

Procedure: Primary hepatocytes from 6 human and 6 mouse donors were treated with PPAR α agonist Wy14643 and gene expression profiling was performed using Affymetrix GeneChips followed by a systems biology analysis.

Results: Baseline PPAR α expression was similar in human and mouse hepatocytes. Depending on species and time of exposure, Wy14643 significantly induced the expression of 362-672 genes. Surprisingly minor overlap was observed between the Wy14643-regulated genes from mouse and human, although more substantial overlap was observed at the pathway level. Xenobiotics metabolism and apolipoprotein synthesis were specifically regulated by PPAR α in human hepatocytes, whereas glycolysis-gluconeogenesis was regulated specifically in mouse hepatocytes. Most of the genes commonly regulated in mouse and human were involved in lipid metabolism and many represented known PPAR α targets, including CPT1A, HMGCS2, FABP1, ACSL, and ADFP. Several genes were identified that were specifically induced by PPAR α in human (ALAS1, CYP1A1, TSKU) or mouse (Fbp2, Igals4, Cd36). Furthermore, several putative novel PPAR α targets were identified that were commonly regulated in both species, including CREB3L3, KLF10, KLF11 and MAP3K8.

Conclusion: Our results suggest that PPAR α activation has a major impact on gene regulation in human hepatocytes. Importantly, the role of PPAR α as master regulator of hepatic lipid metabolism is generally well-conserved between mouse and human. However, PPAR α regulates a mostly divergent set of genes in mouse and human hepatocytes.

P258**Colostrum PUFA composition and fish, seafood products, linoleic acid consumption during the third trimester of pregnancy in the EDEN cohort**

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Background: Colostral bioactive factors as long chain polyunsaturated fatty acids (LC-PUFA), docosahexaenoic (DHA) and arachidonic (ARA) acids, can exert regulatory functions in neonates early during the first days of life. LC-PUFA composition results from mother's nutrition, mobilization from storage and *de novo* synthesis.

Objective: We aimed at investigating the contribution of mother's nutrition during pregnancy, especially through fish and seafood products, and linoleic acid (LA) consumption, on colostrum LC-PUFA levels.

Procedure: Colostrum fatty acid composition of 379 lactating mothers (29 yr \pm 4.8 [17-45]) from the EDEN mother-child cohort (study of pre- and early postnatal determinants of the child's development and health) was performed by direct transmethylation and gas chromatography. Mothers usual diet during the last trimester of pregnancy was determined with a food frequency questionnaire. Relations between colostrum fatty acids and mother's fish and seafood products intake were analysed by multivariate regression adjusted on maternal age, body mass index (BMI), and n-6 intakes. Data are Mean \pm SD [min-max].

Results: The frequency of fish and seafood products intake was 9 \pm 7 [0-41] per month, and only 25% of the mothers consumed marine products three times a week as recommended. The LA, ARA, α -linolenic acid and DHA levels in colostrum were, respectively, 10 \pm 2% of total fatty acids [5-17], 0.84 \pm 0.18% [0.09-1.57], 0.64 \pm 0.23% [0.28-1.78]) and 0.66 \pm 0.26% [0.20-2.71]). Colostrum DHA level was positively associated to mother's age irrespective of marine products consumption (P<0.001), and to fish and seafood intakes after adjusting on maternal age (P<0.001). On the contrary the intake of n-6 exhibited a negative effect on milk DHA level (P=0.04) whereas maternal BMI had no effect.

Conclusion: Both increasing fish and seafood intake in pregnant mother and limiting n-6 intake are potential means to increase DHA level in colostrum. The reason for higher DHA level in older women has to be further investigated.

P259**High fat feeding blunts the insulin stimulated capillary recruitment in rats**

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Background: Colostral bioactive factors as long chain polyunsaturated fatty acids (LC-PUFA), docosahexaenoic (DHA) and arachidonic (ARA) acids, can exert regulatory functions in neonates early during the first days of life. LC-PUFA composition results from mother's nutrition, mobilization from storage and *de novo* synthesis.

Objective: We aimed at investigating the contribution of mother's nutrition during pregnancy, especially through

fish and seafood products, and linoleic acid (LA) consumption, on colostrum LC-PUFA levels.

Procedure: Colostrum fatty acid composition of 379 lactating mothers (29 yr \pm 4.8 [17-45]) from the EDEN mother-child cohort (study of pre- and early postnatal determinants of the child's development and health) was performed by direct transmethylation and gas chromatography. Mothers usual diet during the last trimester of pregnancy was determined with a food frequency questionnaire. Relations between colostrum fatty acids and mother's fish and seafood products intake were analysed by multivariate regression adjusted on maternal age, body mass index (BMI), and n-6 intakes. Data are Mean \pm SD [min-max].

Results: The frequency of fish and seafood products intake was 9 \pm 7 [0-41] per month, and only 25% of the mothers consumed marine products three times a week as recommended. The LA, ARA, α -linolenic acid and DHA levels in colostrum were, respectively, 10 \pm 2% of total fatty acids [5-17], 0.84 \pm 0.18% [0.09-1.57], 0.64 \pm 0.23% [0.28-1.78]) and 0.66 \pm 0.26% [0.20-2.71]). Colostrum DHA level was positively associated to mother's age irrespective of marine products consumption (P<0.001), and to fish and seafood intakes after adjusting on maternal age (P<0.001). On the contrary the intake of n-6 exhibited a negative effect on milk DHA level (P=0.04) whereas maternal BMI had no effect.

Conclusion: Both increasing fish and seafood intake in pregnant mother and limiting n-6 intake are potential means to increase DHA level in colostrum. The reason for higher DHA level in older women has to be further investigated.

P260

Single ion quantification of plasma, red blood cell and brain phospholipids with GC-EI-MS

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Background: Gas chromatography (GC) with flame ionisation detecting (FID) has been the method of choice for quantification fatty acids (FA) since GC analysis of FA has been developed. Instrument response and retention time information are utilized with FID detection, while the use of mass spectrometry as means of detection also has the advantage of identification by means of mass spectral information. This feature is advisable specifically in complex biological samples where contaminants, artifacts or co-eluting related compounds is still of concern when using FID detection. When doing single unique ion quantification with GC-MS, individual

calibration is done for each FA, which eliminates the need to determine and calculate response factors and therefore further enhance the validity of the method over a wide concentration range.

Objective: To evaluate the use of single ion quantification of FA with GC-EI-MS.

Procedure: Plasma, red blood cell (RBC) and brain samples from rats in an iron and n-3 FA depletion-repletion study were quantified with GC-EI-MS. The unique ions for each FA were identified and a quantification method was optimized for each matrix.

Results and Conclusion: Movement in FA composition of the total phospholipid fraction due depletion and repletion with n-3 FA and iron, alone and in combination, could accurately be detected with this method.

P261

The effect of omega-3 fatty acid intervention in children with higher compared to lower baseline cognitive scores: a secondary analysis

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Background: In humans n-3 long-chain polyunsaturated fatty acids play a well-documented role in brain development and function of the fetus and child. Although several studies have been conducted to determine the effects of n-3 fatty acid supplementation on cognition, more research needs to be done in school age children on the effects of n-3 fatty acids on cognitive development.

Objective: To determine whether cognitive development improves more in school children with lower cognitive baseline scores than in children with higher scores, when consuming increased amounts of n-3 fatty acids.

Procedure: The study design was a secondary analysis nested within a randomized controlled study done by Dalton et al. (2009). Subjects (n = 183) were randomly assigned to an experimental (n = 91) and control group (n = 92), receiving either a fish flour spread or a placebo spread for 6 months in a single blind study. Subjects were stratified according to their median baseline scores of the Hopkins Verbal Learning Test (HVLT) total, recognition and discrimination scores, as well as spelling and reading scores.

Results: Cognitive development improved significantly more in subjects with lower baseline cognitive and spelling scores, than in subjects with higher baseline scores in both the experimental and placebo groups, with the exception of the reading test. When comparing the changes in cognitive scores in the experimental with that

of the placebo group no significant differences were found, except in HVLT recognition ($p < 0.05$) and spelling ($p < 0.05$) in groups with higher baseline scores.
Conclusion: This explorative study indicates that children with lower baseline cognitive scores tend to respond higher to intervention compared to children with higher baseline scores. These findings should be further investigated in a study with enough power.

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Identification of a lipidomic signature sensitive to palm or butter-fat based diets in the brain of omega3 deficient young rats

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There is an increased demand for n-3 LC-PUFA, particularly DHA, during pregnancy, lactation and the first years of life, to support optimal visual and cognitive development for infant. Dietary strategies aiming at improving the w3-LCPUFA in brain may also influenced further the brain lipid species.

Objective: To examine how nutritional strategies improving the w3LCPUFA status of young rat pups can impact further the lipidomic profile of the brain, by using diets prepared with Butter-blends or Palm-blends (+Sunflower-Rapeseed) differently enriched in ALA or DHA.
Procedure: Dams were fed a 5%fat (w/w) diet for 6weeks before mating, during gestation and lactation, with a minimal-omega3 level (ALA0.4%). After weaning, 4 post-weaning-groups (females and males n=10each) omega3-deficient received six-week 10%fat (w/w) diets. Two groups received diets including rapeseed to provide the same ALA level (1.5% of FA) respectively blended with Palm (P1) and Butter (B1). The 2 other groups, P2 similar to P1 (Palm-1.5%ALA) was supplemented with DHA and AA (0.12% and 0.4%FA respectively) and B2 (Butter-blend) was enriched in rapeseed to increase ALA level from 1.5% (B1) to 2.3%.

Brain lipidomics was performed after the post-weaning-6week-diets using a LC-ESI-QToF.

Results: From the 880 lipid features detected, we identified 98 ones that were sensitive to dietary treatment. These can be clustered into 6 blocks of lipid species, which characterized the lipidomic signature of each treatment. Among the 6 blocks, 3 were modified by adding ARA+DHA in P1. We also found that 3 others were modified by increasing ALA in butter based-diet. Also importantly, the palm and butter-based diets induced an inverse lipidomic signature in the post-weaning young rats.

Conclusion: Besides influencing FA profiles, dietary fat can also dramatically influence the lipid species profiles in brain. Functional consequences cannot be disregarded and optimal dietary conditions should be sought.

With Grants from Lactalis

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Dietary intakes of EPA and DHA among soldiers deploying to combat areas

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Background: Psychological health problems and human error are leading causes of death and disability among military service members. One theory gaining acceptance is the postulation that omega-3 fatty acids are deficient and that ensuring adequate intakes may mitigate the growing psychological health crises in the US military. Despite the enthusiasm and vast potential, very little is known about the current level of omega-3 intake and tissue status among US military personnel.

Objective: To evaluate dietary intakes of omega-3 fatty acids in soldiers prior to deployment to combat areas.

Procedure: A food frequency questionnaire (FFQ) was used to measure consumption of foods rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Items to capture intake of EPA and DHA from fortified foods and supplements were included. Two hundred fifty soldiers scheduled for deployment to Iraq completed the FFQ. Ninety-one soldiers were assigned to a military post in a non-coastal area (Kansas), while 159 soldiers were assigned to units in a coastal state (Texas).
Results: Overall, 22.8% (n = 57) consumed one or more types of seafood at least twice per week, 11.6% (n = 29) reported taking a fish oil or omega-3 supplement, and 18.0% (n = 45) reported they had eaten an omega-3 enriched food at least once in the previous six months. Frequent seafood consumption was more common among the Texas group, with 30.2% (n = 48) consuming one or more types of seafood at least twice weekly compared to only 9.9% (n = 9) among the Kansas group. Omega-3 supplement use was slightly higher among the Kansas group (13.2%; n = 12) compared to the Texas group (10.7%; n = 17).

Conclusion: Although dietary intakes vary by geographic location, it appears that many deploying soldiers have low intakes of foods rich in EPA and DHA.

P264**Fatty acids and postprandial inflammation**

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Background: Consumption of a high-fat meal can lead to postprandial dyslipidemia. The magnitude of this postprandial response can be influenced by the presence of obesity or type 2 diabetes. In addition, the type of dietary fat consumed (i.e. saturated, monounsaturated and polyunsaturated) may influence the response in plasma triglyceride (TG), free fatty acid (FFA) and in expression of several lipid metabolism-related genes in peripheral blood mononuclear cells (PBMCs).

Objective: To elucidate the acute effects of an oral intake of either saturated, monounsaturated or polyunsaturated fatty acids on postprandial response of normal weight, obese and type 2 diabetic obese subjects.

Procedure: In a double blind cross-over dietary intervention study 18 lean healthy, 18 healthy obese and 6 obese diabetic men (50-70 yrs) consumed three high-fat shakes containing a high amount of saturated (SFA), monounsaturated (MUFA) or polyunsaturated (n-3) (PUFA) fatty acids. Before and 2 and 4 hours after shake consumption blood was drawn for measurement of plasma FFA, TG, glucose and insulin and PBMC gene expression of lipid metabolism-related genes.

Results: At the moment of submission of this abstract the study had just ended and analysis is still ongoing. We hope to present our results at the ISSFAL meeting.

Acknowledgements: This project is financially supported by the Dutch Diabetes Fund

P265**Diet contaminant - sphingolipid mycotoxin fumonisin B1, causes the brain pathology in experimental animals**

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Background: Fumonisin is a mycotoxin produced by fungi *Fusarium moniliforme* and some relative species. Fumonisin B1 (FB1) contaminates the corn all round the world and it is the carcinogen for some animals and people. Simultaneous exposure to FB1 and aflatoxins cause high-rate liver cancer in China and South America. It is known that high doses of FB1 disrupt the sphingolipid metabolism. We found that low doses of FB1 altered an immune response.

Objective: In this work we have shown an autoimmune reaction and increased apoptosis in brain of the experimental animals exposed to FB1.

Procedure: FB1 [0,5mM - 20mM] was fed to mice C57Bl/6

(12-weeks-old) and Wistar rats (10-weeks-old) in the different regimes. Animal were killed by guillotine; organs were removed. One part of organs was frozen for immunohistochemistry, another one was homogenized, stained with antibodies (mAb), and measured by flow cytometry.

Results: FB1 causes the systemic autoimmune reaction that was confirmed by staining to Ig+ cells and nuclear antigens. Brain, liver, spleen, and kidney of experimental animals were filled by B-lymphocytes. B-cells were increased in brain beginning from 4h after FB1 administration; the peak of B-cell reaction was on 4th day. Brain B-cells were contacted with CD8+ cytotoxic lymphocytes bearing the activation markers. There was clear difference in expression of such receptors as IRB, GLUT1, GLUT2, GLUT4, HSP25, HSP70, HSP90, and markers of activation on brain lymphocytes. Staining with propidium iodide, bromo-deoxyuridine, and annexin V has indicated the increased the number of apoptotic and necrotic cells in brain microglia.

Conclusion: Contamination of corn by mycotoxin Fumonisin B1 may cause the pathological alteration in brain local immune system, autoimmune reaction, and increased level of cells death that may be coupled with cognitive diseases.

P266**Combination of conjugated linoleic acid and trans-11 vaccenic acid reduces body weight and raises FA oxidation in the JCR:LA-cp rat**

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Background: *cis*-9, *trans*-11 conjugated linoleic acid (CLA) and *trans*-11 vaccenic acid (VA) are ruminant-derived trans fatty acids (rTFA). CLA has been associated with improved dyslipidemia and insulin sensitivity. We have shown that VA has novel hypo-lipidemic properties in the JCR:LA-cp rat. Therefore, we hypothesized that supplementation with both CLA and VA may have complementary effects to improve lipid and/or insulin metabolism.

Objective: To evaluate potential synergistic effects of purified forms of both VA and CLA in an animal model of the metabolic syndrome (JCR:LA-cp rat).

Procedure: Male obese JCR:LA-cp rats (n=24) were randomized and assigned to one of three iso-caloric diets for 16 weeks: control diet with 1% w/w cholesterol (CD), CD with 1% w/w CLA alone (CLA) or CD with both 1% VA and 1% w/w CLA (VA+CLA).

Results: Rats fed VA+CLA showed a higher food intake but a lower body weight than rats fed CLA. Fasting triglyceride (TG), cholesterol, LDL-C, as well as insulin

area under the curve were reduced in obese rats fed either CLA ($p < 0.05$) or the VA+CLA ($p < 0.001$) diet compared to obese controls. Liver weight and hepatic TG were also lower in rats fed either CLA ($p < 0.05$) or the VA+CLA diet ($p < 0.001$) relative to controls. Data is consistent with a decreased relative protein abundance of hepatic acetyl-CoA carboxylase in both dietary groups relative to control. Interestingly, we also observed an increase in citrate synthase activity in both liver and adipose tissue of rats fed either CLA or VA+CLA diets ($p < 0.001$) compared to control, suggesting increased mitochondrial fatty acid oxidative capacity.

Conclusion: Increased dietary intake of a combination of VA+CLA has a synergistic hypo-lipidemic effect compared to either FA alone in conditions of dyslipidemia. Increased loss of body weight by rTFA might be explained by an increase in the oxidation of FAs in the JCR:LA-cp rat.

P267

Effects of conjugated linoleic acid (CLA) isomers on hamster renal eicosanoids

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Background: Dietary conjugated linoleic acid (CLA) has been reported to have many potential health effects, but the data are often conflicting and the mechanism(s) by which CLA mediates its effects are not clear. One of the reasons for the different effects may be the differences in effects of specific isomers on eicosanoid metabolism.

Objective: We therefore used a lipidomics approach to examine the effects of CLA isomers on eicosanoid metabolism using normal hamster kidneys.

Procedures: Male Syrian golden hamsters were given atherogenic diets (5% dietary fat and 0.25% cholesterol) containing CLA isomers for 28 d. Diets were supplemented at the 2% level with either no CLA (control), cis9, trans11 (c9, t11) CLA, trans10, cis12 (t10, c12) CLA, or a mixture of trans8, cis10 (t8, c10) + c9, t11 CLA. Eicosanoids were extracted from kidneys and quantified using HPLC-tandem mass spectrometry.

Results: Of the more than 20 eicosanoids screened for, five prostanoids, two hydroxyoctadecadienoic acid isomers, leukotriene B₄, six hydroxyeicosatetraenoic acid (HETE) isomers and 5,6-epoxyeicosatrienoic acid were detected. The major effects of CLA were on the HETEs, which were elevated in kidneys from animals given CLA compared to control animals. For 5- and 20-HETE, these were higher in kidneys from hamsters given all 3 CLA diets compared to controls. For 9-, 11- and 15-HETE, these levels were higher in the 9, 11 compared to control kidneys. There also were minor

differences due to specific CLA diets on individual prostanoids, leukotriene B₄ and 5,6-epoxyeicosatrienoic acid.

Conclusions: CLA effects on normal renal eicosanoid metabolism appears to be mediated primarily via elevation of HETE isomers. The effects of individual isomers on specific eicosanoids may help explain the varying physiologic effects of specific CLA isomers.

P268

Seminolipids and genes involved in their synthesis are increased by dietary conjugated linoleic acid in obese Zucker rat testis

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Background: Seminolipids (SL, 3-sulfogalactosyl-1-alkyl-2-acyl-sn-glycerol) are the major sulfoglycolipids in the mammalian testis and their deficiency results in male infertility by arresting spermatogenesis. Male reproductive dysfunction is common in obese men indicating SL production may be altered in obese states.

Objective: To investigate whether SL and genes involved in SL synthesis are influenced by obese states and dietary fat treatment. Conjugated linoleic acid (CLA) was chosen since this fat exists in human testis and is also known to have anti-obesity effects. **Procedure:** Male obese (fa/fa) Zucker rats were fed a 8.5% (w/w) fat diet containing control (0%, w/w), c9t11-CLA (0.4%, w/w), or t10c12-CLA (0.4%, w/w) for 8 weeks. The lean animals were fed only the control diet. Cauda epididymal sperm morphology, CLA and SL content were analyzed. The genes involved in SL synthesis, ceramide galactosyltransferase (CGT) and cerebroside sulfotransferase (CST) were measured by RT-PCR.

Results: Obese rats fed a control diet had significantly ($p < 0.05$) higher numbers of abnormal sperm, 50% lower SL content, and lower CST and CGT gene expression in the testis in comparison to the lean rats. Both CLA isomers provided in the diets were incorporated into triglycerides (TG) but not into phospholipids (PL) in the testis. The c9t11-CLA diet significantly ($p < 0.01$) increased the level of C22:5n-6, a major fatty acid in rodent testis, in TG and PL. Both CLA diets significantly ($p < 0.05$) improved sperm morphology to be similar to the lean rats. The t10c12-CLA diet significantly increased SL as well as the CGT and CST gene expression. **Conclusions:** These results indicate that SL plays a role in male reproductive function in obesity. Dietary CLA, especially t10c12-CLA appears to be a possible dietary strategy for improving male infertility by increasing SL synthesis. (Supported by University of Manitoba Health Research Council & The Natural Sciences and Engineering Research Council of Canada)

P269

The long-term effects of infant formulae enriched with docosahexaenoic acid on the declarative memory abilities of toddlers

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Objective: To test the long-term effects of infant formulae containing docosahexaenoic acid (DHA) on the development of memory.

Procedure: Infants enrolled in the DIAMOND study at the University of Kansas Medical Center received one of 4 levels of DHA (0, 0.32, 0.64, and 0.96% of total fatty acids) in infant formula from birth to 12 months. Memory ability was measured in an imitation paradigm: participants were tested on their ability to imitate event sequences they had seen modeled twice in succession. Participants (n=70) were tested for immediate recall and 12-minute delayed recall at 10 months of age. Participants were tested at 24 months of age in a similar imitation protocol a) immediately, b) after a 20-minute delay, and c) after a 6-month delay (i.e., at 30 months of age). In addition, at 30 months of age, they were exposed to and tested on two events that they had not seen at 24 months of age.

Results: At 10 months of age, ordered recall was higher in the group that consumed formula containing 0.64% fatty acids as DHA compared to the other three groups, $F(3,231)=3.01$, $p<0.05$. At 24 months of age, memory abilities were significantly different among the groups, with those fed formula with 0.64% DHA during infancy again showing higher memory performance after the 20-minute delay, $F(3,137)=3.17$, $p<0.05$. Analyses of the novel events presented at 30 months of age showed a trend toward differences between the groups on immediate recall, $F(3,132)=2.12$, $p<0.10$, with the group consuming formula containing 0.64% fatty acids again performing best.

Conclusion: DHA may have differential, long-lasting effects on immediate and long-term memory. Specific effects on memory abilities were seen in infants who consumed formula containing twice the higher amount of DHA available in US formulas for term infants.

* Study support provided by Mead Johnson Nutritionals

P270

Change in fatty acid composition in adipose tissue of obese subjects after weight loss and maintenance. Diogenes study

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Introduction: Fatty acid composition in adipose tissue reflects dietary fat but also metabolic processing of fat. Aim of the study was to assess differences in fatty acid composition in adipose tissue triglycerides in overweight and obese subjects before and after weight loss in different European centres participating in the Diogenes project.

Methods: Obese or overweight adults from Maastricht and Copenhagen (n 66), from Sofia (n 61) and from Prague (n 40) were examined and adipose tissue biopsy was obtained before and after of the weight management regimen. Fatty acid composition was examined by gas chromatography. Data were analyzed by Kruskal-Wallis test.

Results: Before the treatment significantly lower proportion of saturated fatty acids and monounsaturated fatty acids was found in Sofia ($p<0.001$), higher proportion of n-3 PUFA in the group from Maastricht/Copenhagen ($p<0.001$) and higher proportion of n-6 PUFA was shown in subjects from Sofia. After weight maintenance significant increase in proportion of saturated fatty acids was found in subjects from Sofia, significant increase in n-6 PUFA was shown in subjects from Maastricht and Copenhagen.

Conclusion: The results show significant differences in fatty acid composition of subjects with overweight and obesity from 4 European countries before and after weight management regimen resulting partly from different dietary habits in individual regions of Europe. Supported by the European Community (Contract no. FP6-513946), <http://www.diogenes-eu.org> and by the grant of the IGA of Ministry of Health CR No. IGA NS/9830-4.

1. Conflict of Interest: None Disclosed

P271**Behavioral, genetic, and epigenetic implications of dietary supplementation with alpha-linolenic acid in humans**

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Background: Alpha-linolenic acid (ALA, 18:3n-3), an essential n-3 fatty acid, is a precursor for eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). n-3 fatty acids are integral to brain and cognitive development. Of the major n-3 fatty acids (ALA, DHA, and EPA), most studies have focused on DHA and EPA establishing their functional importance in neurons. Fewer studies have focused on the role of ALA, because, although EPA and DHA are metabolized from ALA, the conversion rate is low. Methylation of related genes could increase this rate. In the human diet, ALA is more readily available, more easily consumed, and less expensive relative to animal sources of DHA.

Objective: It is important that we determine the effects of ALA on the ability of the human system to metabolize DHA and EPA and subsequent effects on cognitive development. We hypothesized that ALA supplementation would increase EPA and DHA through methylation and allelic variations, subsequently improving memory.

Procedure: A sample of 16-month-olds provided baseline memory data and baseline fatty acid, methylation, and allele data for the FADS2 gene (coding the enzyme responsible for ALA to EPA to DHA conversion). They then began a 4-month ALA supplementation regimen in which flaxseed or control oil was mixed into their food. At 20-months of age, supplementation stopped and fatty acid profiles, methylation, and memory abilities were re-assessed.

Results: The results will show the utility of ALA as a means to DHA. Genetic information will be related to memory data.

Conclusion: Conclusions drawn from this work will allow us to work toward individualized nutrition: we will be able to educate parents on the individual fatty acid needs of their children. Children who don't convert will be counseled to consume animal sources of preformed DHA; converters will be counseled to consume plant sources of ALA.

P272**Lipid, fatty acid and sterol compositions of the red seaweed *Grateloupia turuturu***

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Objective: The alga *Grateloupia turuturu* is an invasive species from Brittany, France. A recent investigation dealt with various products such as proteins and lipids (1). The lipid, fatty acid (FA) and sterol compositions were investigated during 4 periods of 2009 in order to identify products with a potential value for use in nutrition and determine if there is a best period to collect regarding lipids.

Procedure: Total lipids were subjected to a saponification and/or subjected to a lipid class separation. FA were identified as methyl esters and N-acyl pyrrolidides, and sterols were identified by gas chromatography-mass spectrometry.

Results: The lipid content was found between 3% and 5% of the dry weight, and no significative variation during the four seasons was observed. Interestingly, lipids contained particular FA and sterols with high added value for applications in nutrition. In January, polyunsaturated FA accounted for 25-30%, including eicosapentaenoic acid 20:5n-3 (EPA) at 17-20% of total FA. Several minor FA were identified, including 3-hexadecenoic and 3-hydroxyheptadecanoic acids. Sterol composition showed significative levels of phytosterols, known as hypocholesterolemiants agents.

Conclusion: Considering these properties, the invasive red macroalgae *G. turuturu* of the coastal area of Brittany (France) may have interesting potentialities for food use.

P273**Anti-obesity effects of seaweed lipids with n-3 polyunsaturated fatty acids and fucoxanthin**

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Objective: Edible seaweeds such as *Undaria pinnatifida* and *Sargassum fulvellum* have unique lipids containing mainly eicosapentaenoic acid (EPA) and 18:4n-3 as fatty acid. Fucoxanthin, which is a carotenoid with allenic bond and epoxy group, is also contained in total lipids. In this study, we investigated anti-obesity effects of seaweed lipids on high fat-fed mice.

Procedure: The C57BL/6J and A/J mice (4 weeks old, Male) were fed the experimental diets containing 2-4% seaweed lipids for 4 weeks. AIN-93G containing 23% lard and 7% soybean oil was used as the basal diet in this study. Seaweed lipids used in this study were 68% glycolipids+phospholipids, 5% fucoxanthin and 2% fucosterol. Fatty acid composition of seaweed lipids were 44% 18:4n-3 and 16% EPA.

Results and Discussion: Dietary seaweed lipids attenuated body weight gain of high fat diet-fed C57BL/6J and A/J mice after 4 weeks feeding. Perigonadal and mesenteric white adipose tissue (WAT) weights were significantly lower in mice fed seaweed

lipids than those of control mice. In addition, down-regulation of monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis) mRNAs were observed in WAT of C57BL/6J and A/J mice. Thesea (TNF-afactor-adipocytokines are considered to induce insulin resistance in obese mice. In A/J mice fed seaweed lipids diet, blood glucose level also decreased. Interestingly, uncoupling protein 1 (UCP1) mRNA expression was detected in WAT of mice fed the seaweed lipids, although there is little expression of UCP1 in WAT of mice fed the control diet. UCP1 is usually expressed only in brown adipose tissue and a key molecule for metabolic thermogenesis to avoid an excess of fat accumulation. These results suggest that seaweed lipids exhibit anti-obesity effects through the reduction of WAT weight by up-regulation of UCP1 mRNA expression in WAT.

P274

Purification of AA-triacylglycerols from ARASCO by gravimetric column chromatography

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Background: Arachidonic acid (AA, 20:4n-6) is found in human breast milk and can directly affect infant growth. So the existence of AA in infant formulas is very important, especially for the purpose of supporting an adequate n-6/n-3 balance for the correct development of formula fed infants. Because of that, there is a high demand of AA from pharmaceutical and infant alimentary industries.

Purification of AA from different sources has been widely reported, and in most cases AA is obtained in the free fatty acid form or as methyl and ethyl esters. It seems that acyl groups in triacylglycerols (TAGs) show more metabolic activity and also human absorption is improved if acyl groups are supplied as TAGs.

Objective: The aim of this work was to purify an AA-enriched fraction of TAGs from commercial single cell oil named ARASCO® (38-44% AA content) by employing food-safe methodologies.

Procedure: TAG profile of ARASCO was obtained by RP-HPLC and the fatty acid (FA) profile of each cluster was determined by GLC. Later, normal-phase chromatography was used to purify the fraction with the highest AA content by employing different mixtures of solvents (n-hexane, acetone and ethanol) with increasing polarity.

Results: An AA-enriched fraction of TAGs with an AA content of 90.9% was obtained with a 56.3% purification yield (referred to the target peak).

Conclusion: This purification method allows the obtaining of a highly enriched AA fraction from a natural and renewable source, with the advantage of being available in the TAG form. It is also feasible to scale up and could be applied with alimentary purposes.

P275

Effects of dietary lipids on fatty acids composition of muscle of tilapia (*Oreochromis sp.*)

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Background: One of the mayor troubles related to the fish production is to find an adequate source for the fish diet. Substitution of the usual fish-based feed by other protein source is difficult due to the amino acid and fatty acid composition. Sources of protein from animal origin supply a suitable amino acid composition. However, their use for animal feeding purposes is forbidden, being only allowed some sources as feathers.

The insect-based meal could be a good alternative source of protein. Also, although the nutrient composition shows an adequate content in amino acids, the fatty acid profile shows a high rate between n-6 polyunsaturated fatty acids (n-6 PUFAs) and n-3 PUFAs.

Objective: The objective of this work is to compare fatty acid profiles in muscle between fishes fed with insect-based meal and fish-based meal.

Procedures: Tilapias (*Oreochromis sp.*) (n=90) with 30 ± 4.80 g of initial mean body weight were employed. Fishes were separated into 3 groups and fed with three different experimental diets (similar composition and different protein source) based on fish meal, insect meal and insect, blood and soya meal respectively. As lipid source fish oil and borage oil were used. After a two-month treatment fishes were slaughtered and the muscle was removed and frozen until fatty acid profile analysis.

Results: Muscular fatty acid profile is directly related to the diet composition: fishes which were fed with insect meal showed higher n-6 PUFA levels than the fish fed with fish meal. However, levels of n-3 PUFAs detected in fishes fed with insect-based meal were higher than what could be expected according to the n-3 PUFA content in insect-based meal.

Conclusion: Results obtained in this work indicate that insect-based meals could be an alternative source of protein for fish feeding.

P276**Effects of zinc and DHA on the epigenetic regulation of human neuronal cells**

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Background: Dietary intake of zinc and omega-3 fatty acids (DHA) have health benefits for a number of human diseases. However, the molecular basis of these health benefits remains unclear. Recently, we reported that zinc and DHA affect expression levels of histones H3 and H4 in human neuronal M17 cells.

Objective: Here, we aimed to investigate the effect of zinc and DHA on post-translational modifications of histone H3 in M17 cells.

Procedure: We used immunoblotting and densitometric analysis of M17 cells to determine changes in acetylation, deacetylation, methylation and phosphorylation of human histone H3 in response to zinc and DHA treatment.

Results: In response to increase in zinc concentration, we observed significant increases in deacetylation, methylation and phosphorylation of H3 and significant decreases in acetylation. To investigate the role of zinc in apoptosis, we investigated the levels of Bcl-2 and caspase-3. Indeed, zinc reduced the levels of the anti-apoptotic marker Bcl-2 while increasing the apoptotic marker caspase-3 levels. Conversely, DHA treatment resulted in a significant increase in acetylation of H3 and Bcl-2 levels and a significant decrease in deacetylation, methylation, phosphorylation of H3 and caspase-3 levels, suggesting that DHA promotes gene expression and neuroprotection.

Conclusion: In conclusion, our novel findings show the opposing effects of zinc and DHA in the epigenetic regulation of human neuronal cells and highlight the potential benefit of dietary intake of DHA for the control and management of neurodegenerative diseases.

P277**DHA acts through a zinc-dependent mechanism to prevent neuronal cell death**

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Background: The essential fatty acid DHA (docosahexanoic acid) comprises 3% of the dry mass of the human brain. Dietary deficiency of DHA is associated with increased incidence of neurodegenerative disorders including Alzheimer's disease and subsequent brain cell death. Nutritional DHA deficiency also affects brain zinc homeostasis. Our previous work showed that DHA deficiency increased hippocampal zinc levels and raised the expression of the ZnT3 putative zinc transporter in the rat brain.

Objective: Here, we hypothesize that DHA deficiency induces neuronal cell death through zinc-induced apoptosis.

Procedure: To elucidate the link between DHA, zinc and brain cell death, we grew cultured human neuronal M17 cells in DHA-deficient and DHA-enriched culture medium and assessed zinc transporter and caspase-3 protein levels with immunoblot analysis. Zinc transporter protein levels were also assessed by immunofluorescence and mRNA levels by real-time PCR. Zinc flux studies were performed with radiolabelled zinc.

Results: Exposure of M17 cells to DHA-deficient medium increased the levels of active caspase-3, relative to levels in DHA-replete cells, confirming the adverse effects of DHA deficiency in promoting neuronal cell death. To investigate the role of zinc in DHA-induced apoptotic cell death we grew cells in DHA-deficient and DHA-replete culture medium and measured zinc uptake using radiolabelled zinc. In DHA-treated M17 cells, zinc uptake was 65% less when compared with that of DHA-deficient cells. Furthermore, in DHA-treated cells, ZnT3 mRNA and protein levels were reduced in comparison to the levels in DHA-deficient cells.

Conclusion: Based on previous reports showing free zinc mediates brain cell death through apoptosis, we propose that neuroprotective function of DHA is exerted through a reduction in cellular zinc levels that in turn inhibits apoptosis.

Abstracts - Wednesday June 2

Plenary: Hot Topics

08.30 - 09.15

Transgenic nutritional enhancement: the production of omega-3 long chain polyunsaturated fatty acids in plants

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There is now considerable evidence as to the importance of omega-3 long chain polyunsaturated fatty acids (LC-PUFAs) in human health and nutrition. Unfortunately, current sources are either in severe decline (fish oils) or expensive (via microbial fermentation), leading to the search for an alternative source. We have been evaluating the possibility of producing omega-3 LC-PUFAs in transgenic plants, to provide a sustainable source of these important nutrients, since no native higher plant species synthesise these fatty acids. We have transgenically assembled the primary biosynthetic pathway for LC-PUFAs in both model plants and crop species. Our data indicate that whilst the transgenic synthesis of C20 LC-PUFAs such as arachidonic acid and eicosapentaenoic acid is clearly feasible, a number of factors may limit the efficient heterologous reconstitution of this pathway. We have attempted to address this problem in a systematic manner by firstly identifying different metabolic “bottlenecks” and then seeking genetic interventions to overcome them. It seems likely that a generic bottleneck resides within the primary LC-PUFA biosynthetic pathway as a result of the “substrate dichotomy” between the lipid-dependent desaturases and the acyl-CoA-dependent elongases which catalyze the reactions. Attempts to overcome this bottleneck, through the use of acyl-CoA dependent desaturase or acyltransferases will be presented. In addition, the impact (in terms of substrate-channelling) of endogenous plant lipid metabolism on the heterologous LC-PUFA pathway will be considered. The outcomes from our recent iterations of this transgenic metabolic engineering will be presented, and the future prospects for GM-derived LC-PUFAs will be discussed.

09.15 - 10.00

Results of the DOMInO Trial on DHA in pregnancy

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Background: Recommendations for DHA intake during pregnancy have been made by a number of organisations including ISSFAL in the absence of robust and conclusive data from randomised controlled trials.

Methods: Pregnant women were randomly allocated to consume either 3 x 500mg capsules of DHA rich fish oil concentrate (Incomomega 500 TG from Croda Chemicals, UK), providing 900mg of DHA per day, or 3 x 500mg vegetable oil capsules without DHA. The vegetable oil capsules contained a blend of three non-genetically modified oils representing the polyunsaturated, monounsaturated and saturated fatty acid profile of the average Australian diet. Women took their allocated capsules from 20 weeks gestation until birth. Symptoms of postnatal depression, assessed as a score >12 on the Edinburgh Postnatal Depression Scale, to 6 months post partum and neurodevelopment of the children, as assessed by the Bayley III Scales of Infant Development, at 18 months of age were the primary outcomes of the trials. Secondary outcomes included duration of gestation, preterm birth, prolonged gestation, as well as birth size.

Results: 2399 women (1197 DHA group and 1202 control group) were enrolled. Women had a mean age of 29±6 (±SD) years are trial entry and for 40% of women this was their first birth. 24% of women reported a previous history of depression. 98% of all women were assessed to 6 months post-partum and 95% of eligible children were assessed at 18 months of age. The demographic characteristics of women participating in the trial were typical of the Australian population and this together with the high retention rate ensures high validity and generalisability of the results. Final data for primary and secondary outcomes will be presented and discussed.

Concurrent Session - 19

Lipids and Health: Lipids, ageing and longevity

10.30 - 11.00

Invited contribution

Integrating metabolism by NAD⁺ sensors

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A century after the identification of a coenzymatic activity for NAD⁺, NAD⁺ metabolism has come in the spotlight again due to the potential therapeutic relevance of a set of enzymes whose activity is tightly regulated by the balance between the oxidized and reduced forms of this metabolite. In fact, the actions of NAD⁺ have been extended from being an oxidoreductase cofactor for single enzymatic activities to acting as substrate for a wide range of proteins. These include NAD⁺-dependent protein deacetylases, poly(ADP-ribose) polymerases, and transcription factors that affect a large array of cellular functions. Through these effects NAD⁺ provides a direct link between the cellular redox status and the control of signaling and transcriptional events. Of particular interest within the metabolic/endocrine arena are the recent results, which indicate that the regulation of these NAD⁺-dependent pathways, for instance by activation of the AMP-activated protein kinase, may have a major contribution to oxidative metabolism and lifespan extension. Here, I will provide an integrated view on: 1) the signaling and transcriptional pathways controlled by NAD⁺; and 2) novel data which show how modulation of NAD⁺-producing and -consuming pathways have a major physiological impact and hold promise for the prevention and treatment of metabolic disease.

11.00 - 11.15

N-3 polyunsaturated fatty acid (PUFA) deficiency aggravates the age-related impairment of astroglial function in rat brain

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Background: The unbalance between n-3 and n-6 PUFA in western diet may result in low brain DHA status that is supposed to participate to the decline of brain function with aging and to the occurrence of associated neuropathologies. However, the mechanisms linking DHA status in brain cells (neurons and astrocytes) and brain age-related disorders are still questioning.

Objective: We have shown that DHA regulates several functions of the astrocytes (Champeil-Potokar et al 06, Grntal et al 09). Because the neuroprotective role of astrocytes determines, in a large part, the brain resistance to age-related damages, we have investigated the possible consequences of an n-3PUFA deficiency on astroglial function in aging rats, focusing on glutamate scavenging and age-induced astrocytes hypertrophy (astrogliosis).

Procedure: Glutamate transport was investigated by measuring D-3H-Aspartate uptake by freshly isolated brain homogenates and by quantifying astroglial glutamate transporters by western blotting. Astrogliosis was evaluated by immunohistological and western blotting determination of GFAP (Glial Fibrillary Acidic Protein). These parameters were compared in young (4 month-old) and old (22 month-old) rats receiving an n-3PUFA balanced or deficient diet. Their brain fatty acid status was evaluated in the main phospholipid classes.

Results: n-3 PUFA intakes had no influence on astroglial glutamate uptake or GFAP in young rats. Old rats exhibited a 30% reduced glutamate uptake, and a marked increase in GFAP, as compared to young ones. These age-related changes were exacerbated by n-3 PUFA deficiency: glutamate uptake was decreased by 20% and GFAP was increased by 40% in n-3 PUFA deficient old rats as compared to n-3 PUFA balanced old rats.

Conclusion: These results indicate that n-3PUFA deficiency aggravates the impairment of astroglial glutamate transport

and the astrogliosis in aged rats. They suggest that a low brain DHA status contributes to brain aging through the alteration of astrocyte function.

11.15 - 11.30

Cognitive benefits of docosahexaenoic acid in age-related cognitive decline

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Background: Docosahexaenoic acid (DHA), principle omega-3 fatty acid in brain, plays an important role in neural function. Higher DHA intake and plasma levels are correlated with lower risk of cognitive decline and Alzheimer's disease (AD). Algal-DHA supplementation reduces amyloid and tau in transgenic AD mouse models. We examined effects of DHA clinically as a nutritional supplement for age-related cognitive decline (ARCD).

Objectives: Memory Improvement with DHA Study (MIDAS) was a randomized, double blind, placebo controlled, multi-centered trial to determine effects of 900mg/d DHA on improving cognitive functions in healthy elderly with ARCD over six months.

Procedure: 485 subjects (242 DHA; 243 placebo) with baseline Mini-Mental State Exam >26 and Wechsler Memory Scale III Logical Memory score >1 SD below younger adults were randomized in MIDAS. Primary outcome: change from baseline in CANTAB Paired Associate Learning (PAL), a visuospatial episodic memory test.

Results: MIDAS completion rate: 90% with 58% female, mean age: 70±9, mean education: 14.6±2.6 years, family history of dementia: 36%. ITT analysis demonstrated significantly fewer PAL 6 pattern errors with DHA versus placebo at six months (diff. score -1.63±0.76, p=0.03). Verbal Recognition Memory showed greater DHA immediate and delayed responses (p<0.02). Executive Function, Working Memory tests and Activities of Daily Living scale showed no differences between groups. Plasma DHA levels (active arm) significantly increased (by 3.2 weight%, p<0.001) and were correlated with the PAL response (p<0.04). Adverse events and serious adverse events were equivalent across groups.

Conclusion: Administration of 900mg/d DHA over 6 months improves episodic memory and learning in ARCD and may provide benefit to those with gradual memory loss.

11.30 - 11.45

Association of marine omega-3 fatty acid levels with total mortality and telomeric aging in patients with coronary heart disease

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Background: Increased intake of marine omega-3 fatty acids (EPA+DHA) is associated with prolonged survival in patients with coronary heart disease, but its mechanism is unclear.

Methods: Prospective cohort study of 957 outpatients with stable coronary artery disease from the Heart and Soul Study. Participants were followed up for a median of 6.0 years. Leukocyte telomere length was measured in a subset of 608 subjects at baseline and 5 years. Standard cardiovascular risk factors, demographics, socioeconomic status, health behaviors, and inflammatory markers were collected at baseline. Multivariable linear, logistic regression, and Cox proportional hazards models were used to investigate the association of baseline blood levels of EPA+DHA with death rates and changes in telomere length.

Results: There were 237 deaths. Compared with patients having baseline EPA+DHA levels below the median (<3.6%), those at or above the median had a 27% decreased risk of death [Hazard Ratio 0.73, 95% CI 0.55 to 0.99] after adjustment for confounders and mediators. Individuals in the lowest quartile of EPA+DHA experienced the fastest rate of telomere shortening (0.13 T/S units over 5 years; 95% CI 0.09, 0.17), whereas those in the highest quartile experienced the slowest rate of telomere shortening (0.05 T/S units over 5 years; 95% CI 0.02, 0.08); p<0.001 for trend. Each standard deviation increase in DHA+EPA levels was independently associated with a 29% reduction in the odds of telomere shortening (adjusted OR 0.71; 95% CI 0.52, 0.96).

Conclusions: Among patients with stable coronary artery disease, there was an inverse relationship between baseline blood

levels of EPA+DHA and both the risk for death and the rate of telomere shortening over 5 years. These data suggest that higher n-3 FA levels may slow cellular aging and thereby postpone death.

11.45 - 12.00

Dietary medicament for the treatment of neurodegenerative disorders

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Background: Alzheimer's Disease, Leukodystrophy (X-ALD) and other neurodegenerative disorders have different etiopathogenesis but they do share similar clinical manifestations and common metabolic pathway alterations. Dietary therapy is very important in neurodegenerative disorders due to the modulation of nutritional bioactive compounds at different levels.

In particular X-ALD neurodegeneration is associated to lipid metabolic alterations. As of date, the treatment available for patients suffering from X-ALD is a therapy based on Lorenzo's Oil, a 4:1 mixture of glyceryl trioleate (GTO) and glyceryl trierucate (GTE) which, combined with a reduction of the saturated fatty acids in the diet, seems to normalize lipid metabolic alterations in patients.

The problem associated to this type of therapy lies in the fact that the main constituent: erucic acid, hardly passes through the blood-brain barrier.

Objective: We aimed to investigate the effect of a mixture of GTO, GTE (4:1) and conjugated linoleic acid isomers (CLA: c9,t11 and t10,c12), in X-ALD patients.

Procedure: After the approval of the Ethics Committee, the mixture of GTO and GTE and CLA isomers was given to five X-ALD female carriers for two months. Plasma and liquor were drawn before and at the end of the treatment, lipid composition was analysed. The patients underwent also somatosensory evoked potential (SEP) evaluations.

Results and Conclusion: After the treatment, CLA, but not erucic acid, was measured in the liquor of the patients, indicating the passage through the blood-brain barrier. Moreover the patients exhibited improved SEP values. Our results suggest that the GTO, GTE and CLA mixture may be a novel promising therapeutic strategy for X-ALD.

Concurrent Session - 20

Biochemistry of Lipids: Hepatic lipid metabolism

10.30 - 11.00

Invited contribution

Transcriptional regulation of hepatic fatty acid metabolism

S Kersten

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The liver serves as a major sink for dietary fatty acids and endogenous fatty acids derived from the adipose tissue. Within the liver, incoming fatty acids can either be oxidized, stored within lipid droplets, secreted within very low density lipoproteins, or shuttled into alternative pathways. Accordingly, hepatic fatty acid metabolism need to carefully governed to balance the needs of the various pathways. Most of the short term regulation occurs via allosteric mechanisms and via phosphorylation/dephosphorylation of key enzymes. More gradual adaptations of hepatic fatty acid metabolism, including those related to feeding and fasting, are achieved by altering gene transcription. Several transcription factors are involved in the regulation of hepatic fatty acid metabolism. Many of these transcription factors are under hormonal control and/or are activated via changes in intracellular concentration of specific lipid species. Two important transcription factors are the sterol regulatory element binding protein 1 (SREBP-1) and the carbohydrate responsive element binding protein (ChREBP). While SREBP-1 mediates the suppressive effect of fasting and poly-unsaturated fatty acids on lipogenesis, ChREBP mediates the stimulatory effect of glucose on the same pathway. Another important transcription factor is the

Peroxisome Proliferator Activated Receptor α , which induces the expression of numerous genes involved in fatty acid oxidation in liver. Binding of ligands to PPAR α , which include numerous fatty acids and fatty acid-derived compounds, results in the recruitment of co-activators and dissociation of co-repressors, leading to chromatin remodeling and subsequent initiation of DNA transcription. Co-regulatory proteins that play a key role in PPAR α -dependent regulation of hepatic fatty acid metabolism include PGC1 α , Baf60a and Sirt1. Detailed whole genome expression profiling studies have shown that PPAR α governs the expression of genes involved in virtually every aspect of hepatic fatty acid metabolism. These studies also revealed that PPAR α is the principal mediator of the effects of dietary fatty acids on hepatic gene expression. Recently, profiling of promoter occupancy by PPAR α in human hepatoma cells via ChIP-chip analysis indicated important cross-talk between PPAR α and SREBP signaling. Overall, these analyses point to PPAR α as master-regulator of hepatic fatty acid metabolism.

11.00 - 11.15

SIRT4 suppresses hepatic fatty acid oxidation by signalling through PPAR α

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Background: Sirtuins connect metabolism with longevity in yeast, flies, and worms via their NAD- dependent deacetylase or ADP-ribosyltransferase activities. Mammals contain seven homologs of yeast Sir2 (SIRT1-7) of which SIRT3, SIRT4 and SIRT5 are primarily localized in the mitochondria.

Objective: Previously we showed that SIRT4 ADP-ribosylates and down-regulates glutamate dehydrogenase (GDH), thereby regulating insulin secretion in the pancreas. We now aimed to identify novel functions of SIRT4 in the liver.

Procedure: We analyzed sirtuin expression levels during fasting in mouse liver using qRT-PCR. The metabolic phenotype of SIRT4 null mice and primary cells were characterized using metabolite profiling and microarrays. Furthermore, we studied the phenotype of SIRT4 null and wild-type mice fed a high fat diet for 16 weeks.

Results: We have uncovered a novel connection between a mitochondrial sirtuin, SIRT4, and the transcriptional regulation of fatty acid oxidation by PPAR α . We find that SIRT4 expression decreases in the liver during fasting, which is associated with a concomitant up-regulation of genes associated with fatty acid catabolism. As a result, SIRT4 null hepatocytes exhibit higher rates of fatty acid oxidation than wild-type hepatocytes. The effect of SIRT4 on fatty acid metabolism derives from its ability to negatively regulate the transcriptional activity of PPAR α by signaling through SIRT1. At the organismal level, the increase in fatty acid oxidation in SIRT4 null liver has a striking phenotypic effect. SIRT4 null animals are resistant to weight gain induced by a high fat diet.

Conclusion: Our findings suggest that SIRT4 is involved in inhibiting fatty acid oxidation, which could potentially impact aging and aging related diseases. Furthermore, our data demonstrate a unique example whereby mitochondria sense changes in nutrient availability (via SIRT4) and then transmit this information to the nucleus in order to alter gene expression and regulate lipid accumulation in vivo.

11.15 - 11.30

N-3 fatty acids as phospholipids are superior over triacylglycerols in ameliorating hepatic steatosis and other disorders induced by high-fat feeding

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Objective: n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), could prevent development of obesity and insulin resistance. In this study, metabolic consequences of dietary n-3 LC-PUFA supplemented as either phospholipid or triacylglycerol concentrates were characterized in obesity-prone C57BL/6J mice.

Procedure: In the *Prevention* study, mice were fed for 9 weeks either a corn oil-based high-fat diet (cHF; lipids ~35% wt/wt) or cHF-based experimental diets, matched for the total EPA and DHA content (3.15% wt/wt), in which part of lipids was replaced by the EPA and DHA concentrates from EPAX AS (Aalesund, Norway) either in the form of triacylglycerols (67% EPA+DHA; EPAX 1050 TG; TG-concentrate) or a novel phospholipid concentrate (27% EPA+DHA; PhL-concentrate). Markers of glucose and lipid homeostasis, glucose tolerance, adipocyte hypertrophy and adipose tissue inflammation were analyzed. In the *Reversal* study, the effects of both concentrates were also studied in dietary obese mice treated with metformin (2 g/kg diet).

Results: In the *Prevention* study, none of the concentrates affected either weight gain or adiposity, while both concentrates were equally effective in reducing plasma non-esterified fatty acid levels. PhL-concentrate was more effective than TG-concentrate in reducing triacylglycerols and increasing high-molecular weight adiponectin in plasma. As compared to TG-concentrate, PhL-concentrate significantly improved glucose tolerance and tended to reduce plasma insulin ($p = 0.066$), while markedly reducing hepatic steatosis. In the *Reversal* study, both concentrates reduced abdominal fat accumulation, plasma levels of triacylglycerols, non-esterified fatty acids and cholesterol, and induced adiponectin. However, hepatic steatosis was more effectively reduced by PhL-concentrate, and only PhL-concentrate significantly reduced adipocyte hypertrophy and plasma insulin.

Conclusion: n-3 LC-PUFA as phospholipids exert a number of superior effects as compared to triacylglycerols in both prevention and reversal of obesity-associated metabolic disorders. The PhL-concentrate is especially effective in reducing hepatic steatosis.

11.30 - 11.45

Hepatic de novo lipogenesis is promptly altered in the STZ-diabetic rat

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Background: Lipid synthesis is an important insulin-dependent anabolic process occurring in the liver after a meal. Type 1 insulinopenic Diabetes is characterized by the disruption of glucose as well as lipid metabolism. **Objective:** Follow alterations in sources to glucose production (GP) and de novo lipogenesis (DNL) secondary to Diabetes during the natural feeding cycle of the Rat. **Procedure:** Healthy and streptozocin(STZ)-diabetic were let to feed overnight under 2H₂O administration. Hepatic lipid content and blood glucose were analyzed by 2H-NMR. An external pyrazine standard was used to calculate the absolute 2H-enrichment in the triglyceride-methyl groups. The fractional contribution of DNL to hepatic triglyceride was estimated as the methyl 2H-enrichment relative to the 2H-enrichment of the body water. Glucose was extracted from the blood by ion-exchange chromatography and derivatized to monoacetone glucose (MAG). Gluconeogenesis contribution to GP was estimated as the 2H enrichment in position 5 of MAG relative to the one in position 2. **Results:** Four days after induction of diabetes, the gluconeogenesis contribution to GP was similar to healthy controls (65%) but DNL to hepatic triglyceride decreased from 16% to 7%. At days 10 and 20 of the diabetic condition gluconeogenesis contribution to GP increased to 90% and DNL was further reduced to 2%. **Conclusion:** Following Diabetes induction the loss of DNL capacity in the liver can be detected earlier than the alterations on sources to GP when rats are assessed after an overnight feeding. These observations may serve as valuable markers for evaluating alterations in glucose and lipid metabolism during the progress of STZ-induced Diabetes.

11.45 - 12.00

Tissue specific sex differences in the expression of genes involved in docosahexaenoic acid biosynthesis in rats

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Background: Increased levels of docosahexaenoic acid (DHA) have been observed in females as compared with males. **Objective:** To investigate sex differences in the expression of genes and transcription factors involved in DHA production in liver, heart, and brain.

Procedure: Male and female rats (n=6 per group) fed a standard chow diet were sacrificed at 14 weeks of age after an overnight fast. Liver, brain, and heart were collected for the determination of the mRNA (by RT-PCR) of delta-6 desaturase

(D6D), delta-5 desaturase (D5D), very long chain elongase (ELOVL2), acyl-CoA oxidase (AOX), sterol-response element binding protein 1-c (SREBP1-c), peroxisome proliferator activated receptor α (PPAR α), and estrogen receptor α (ER α). The fatty acid composition of plasma, erythrocytes, liver, brain, and heart was also determined.

Results: The hepatic mRNA content of D6D, D5D and ELOVL2 were 2-2.5 fold higher in females as compared with males, while no difference was observed in AOX. Additionally, hepatic expression of ER α and SREBP1-c was 1.6 and 1.8 fold higher, respectively, while hepatic PPAR α was 2-fold lower in females. No sex differences were observed in mRNA expression of heart or brain. DHA was increased in the total lipids of females as compared with males in all tissues examined except brain. In plasma and liver, the DHA content of phospholipid was 64% and 80% higher, respectively in females as compared with males.

Conclusion: These results suggest that the higher DHA in females may involve elevated hepatic expression of D6D, D5D and ELOVL2. This difference is specific to the liver, as no difference in mRNA content was seen in heart and brain. The transcription factors SREBP1-c and PPAR α may participate, and differences in ER α expression in liver but not heart or brain suggest that estrogen signaling may be involved.

Concurrent Session - 21

Lipids and Nutrition: Novel topics

10.30 - 11.00

Invited contribution

Interactions between dietary fat and inflammatory genes on the metabolic syndrome - Insights from the LIPGENE Study

Helen M Roche

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Background: Dietary fats and obesity represent metabolic stressors that often interact with inflammatory stressors to augment the risk of insulin resistance the key metabolic perturbation in the Metabolic Syndrome (MetS) and Type II Diabetes Mellitus.

Objective: This presentation will focus on key findings from LIPGENE (www.ucd.ie/LIPGENE) a large pan European study that investigated the effect of modifying dietary fat intake and genes on the risk of the MetS.

Results: A cross-sectional approach showed clear links between inflammatory genes and saturated fatty acids in the development of insulin resistance. Key inflammatory genes included STAT3, TNF α , IL-6, LTA all of which interacted with fatty acid composition to augment the risk of developing the MetS. Furthermore a dietary intervention study investigated the metabolic effects of substituting dietary SFA by replacement with monounsaturated fatty acids (MUFA) or as part of a low-fat diet in subjects with the MetS. Interestingly there were clear examples wherein the effect of dietary fat modification interacts with different inflammatory genes, for example the adiponectin gene (ADIPOQ) and its two receptors (ADIPOR1 and ADIPOR2) on risk factors associated with the MetS. IL-1RI mediated inflammation represents another interesting target that is modulated by dietary fat intake - functional studies detailing the interaction between obesity and IL-1R mediated inflammation will also be discussed.

Conclusion: Overall this data suggests that a personalized approach based on an individual's metabolic or inflammatory status may be more effective in providing more successful dietary based prevention strategies and treatments.

This research was supported by EU Sixth Framework Food Safety & Quality Programme, Contract Number (FOOD-2003-CT-505944) and Science Foundation Ireland PI Programme (06/IM.1/B105).

11.00 - 11.15

A myristic acid enriched cream improves the metabolic syndrome: the SEMYRAMIS study

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Objective: Previous studies have shown that low intakes of Myristic acid (MA) (3-4 g/d) increase HDL-cholesterol, LCAT activity and membrane fluidity in healthy volunteers. In the SEMYRAMIS study we evaluate such intakes in the Metabolic Syndrome (MS).

Procedure: One hundred and twenty obese subjects (mean BMI: 37.2) were enrolled in a 6-month double blind trial. Half of them had a MS. They were randomized to consume each day a MA enriched cream in an hypocaloric diet with MA intake of 3.0, 3.5 or 4.0 g/d for 3 months (Period 1). After Period 1, all subjects fed an usual hypocaloric diet (MA intake of 1 g/d) without MA enriched cream for 3 months (Period 2). Intakes of other fatty acids were at recommended levels, mainly for alpha-linolenic acid (2 g/d). Anthropometric parameters and blood samples were performed at T0, T30, T90 and T180.

Results: During Period 1, reduction of weight, BMI and waist were more important in the MS. MA intake of 3.0 g/d in the MS led to a greater reduction of weight, BMI and waist than higher intakes (-7.0 kgs, -2.5 of BMI and -7.5 cms of waist). Glycemia, insulinemia, HOMA ratio, triglycerides and TG/HDL ratio were significantly decreased from 10, 20, 25 and 30%, respectively. HDL-C was increased of 10% only with MA intake of 4.0 g/d in the MS. Neither total cholesterol, LDL, total/LDL ratio nor Lp(a) were increased. Leptin was decreased of 40% and adiponectin was increased of 100%. Period 2 was not associated with further beneficial effects within each group.

Conclusion: Influence of MA on parameters of the MS appears as a U-shaped curve with the more favourable effects for the lower intake. A MA enriched cream with a total daily MA intake of 3.0 g dramatically improves the MS, reduces leptin and increases adiponectin.

11.15 - 11.30

Krill oil improves metabolic syndrome by modifying endocannabinoid biosynthesis

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Background: Disruption of peripheral endocannabinoid system in obese subjects contributes to impairment of lipid deposition and metabolism which may lead to metabolic syndrome. It is emerging that changes in phospholipid fatty acid composition may affect endocannabinoid biosynthesis. In fact, the two major endocannabinoids, anandamide (AEA) and 2 arachidonoyl-glycerol (2-AG) derive from phospholipid arachidonic acid in position sn-1 and sn-2 respectively. n-3 PUFAs seem particularly efficient in modifying endocannabinoid tissue levels.

Krill oil is characterized by the high content of n-3 PUFAs mainly incorporated into phospholipids (PLs), similarly to the natural form of fish products.

Objective: Our study aimed to investigate whether dietary krill oil, by modifying tissue PLs, is able to ameliorate metabolic syndrome by affecting endocannabinoid biosynthesis in a rodent model of obesity, as well as in overweight and obese patients.

Procedure: As rodent model of metabolic syndrome we used obese Zucker rats. Animals were fed krill oil to get about 0.8% en as EPA+DHA in a ratio ~ 2/1. Krill oil intake in humans was 2g daily for 4 weeks.

Results: Our data show that krill oil was able to modify tissue phospholipid fatty acid profile by significantly increasing the ratio n-3/n-6, and greatly improve some metabolic syndrome parameters such as reduction in ectopic fat and improved cholesterol metabolism. These effects were associated with a reduction of endocannabinoid content in visceral adipose tissue of both AEA and 2-AG. In humans, krill oil significantly decreased plasma 2-AG levels in obese subjects. Moreover, there was an inverse correlation between plasma 2-AG levels and n-3/n-6 ratio.

Conclusions: Our results suggest that low doses of krill oil modify arachidonic acid tissue phospholipids, decrease endocannabinoid levels and elicit a physiological re-equilibration of lipid metabolism and deposition in obesity-related metabolic syndrome.

11.30 - 11.45

Vegetarian source of omega-3 fatty acids by metabolic engineering of *Yarrowia lipolytica*

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Background: Omega-3 fatty acids, eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), mainly found in fish oil, have proven health benefits for both human and animals. Concerns over the quality and sustainability of the fish oil supply have generated interest in alternative sources for production of EPA and DHA.

Objective and Procedure: We report development of a clean and renewable source of omega-3 fatty acids by fermentation, using a metabolically engineered strain of the oleaginous yeast, *Yarrowia lipolytica*.

Results: While certain strains of *Y. lipolytica* can accumulate oil up to 40% of the dry cell weight, the only PUFA normally synthesized by the organism is linoleic acid (C18:2n-6). Coordinate expression of desaturase genes and elongase genes comprising omega-3 fatty acid biosynthesis pathways were sufficient to demonstrate the synthesis of EPA. However, only an integrated strategy based on use of strong promoters, increase in gene copy numbers, push and pull of carbon into the engineered pathway, enhanced acyl-exchange and use of improved fermentation process conditions to increase oil production resulted in the generation of a high EPA production strain for commercialization. We will also describe the importance of the peroxisome in regulating fatty acid metabolism to achieve high EPA titer in engineered *Yarrowia* strains.

Conclusion: The yeast triacylglyceride oil has a unique fatty acid profile with greater than 55% as EPA, and less than 8% as saturated fatty acid. The safety of this oil for human nutrition has been demonstrated by clinical and toxicology studies. The metabolic engineering of *Yarrowia* generates a platform technology to produce tailored omega-3 or omega-6 fatty acid compositions. Our land-based production of EPA, provides a superior source of this vital nutrient for applications in nutritional supplements, functional foods, pharmaceuticals, and animal feeds.

11.45 - 12.00

Strategies for production and structural assessment of modern omega-3 based nutraceuticals and medicinals

JA Kralovec, E Reyes-Suarez, W Wang
Ocean Nutrition Canada, Dartmouth, Canada

Background: There have been a large number of studies demonstrating the health benefits of omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Not surprisingly, there is a big demand for EPA and DHA concentrates and considerable efforts are being made to develop omega-3 based metabolites into medicines.

Objective: To establish robust enzymatic methods of producing EPA/DHA concentrates, quantify EPA/DHA regioselectivity within a triglyceride framework, and to apply the relevant know-how to oxylipins and other omega-3 derived molecules with medicinal potential.

Procedure: The first strategy to generate triglyceride forms of EPA/DHA concentrates was based on dismantling the starting triglyceride molecules by converting them into the corresponding free fatty acids or ethyl esters, concentrating these to boost EPA and DHA levels, and finally reassembling them back into triglyceride forms. The second strategy was based on the selective removal of unwanted fatty acid residues of triglyceride oils and replacing them with EPA/DHA. The structural determination of these products along with the evaluation of the positional distribution of fatty acid residues in glycerol moieties was conducted using a specifically developed quantitative ¹³C NMR method.

Results: We have produced EPA/DHA concentrates using the above mentioned methodologies and demonstrated that the disintegration of the triglyceride framework lead to a significant rearrangement of fatty acid residues, whereas the "selective removal" approach largely maintained the structural integrity.

Conclusion: ¹³C NMR was shown to be an effective and elegant tool to answer important questions about the stereochemistry of EPA/DHA containing triglycerides. The enzymatic methodologies constitute gentle approaches towards chemical manipulation of sensitive omega-3 structures. Similar strategies have great potential for obtaining omega-3 oxylipins while other omega-3 metabolites can potentially be useful as medicinal products.

Plenary: Award Session

Early Career Award

13.00 - 13.30

Emerging opportunities for an integrated systems approach to the study of nutrient-disease relationships: Technical application and findings from investigations on the impact of highly unsaturated fatty acids in health and disease of the retina

JP SanGiovanni

Clinical Trials Branch of the U.S. National Eye Institute, Bethesda, United States of America

The existence of high quality public-access bioinformatic data on gene-disease relationships provides a rich opportunity to examine aspects of diet-disease relationships in the context of gene sets, pathways, and biological systems. I will discuss our approach to combining such data with those from model systems and comment on the promise that work of this nature holds for informing research applied to develop effective screening tools, predictive prognostic indicators, and potential drug targets. Examples will be given from our work in the human retina ranging from model cell systems to a phase III randomized clinical trial in 4000 people.

New Investigator Awards

The abstracts of the nine nominees are scheduled for poster presentation (Sunday through Tuesday). Additionally, the winner of each track will give an oral presentation.

13.30 - 13.45

Track 1: Lipids and Health

M. Bernabe (Mexico) - Evidence of beneficial effects of enteral docosahexaenoic acid on cytokine production and clinical outcomes in surgical neonates

P. Jackson (UK) - DHA-rich fish oil modulates cerebral haemodynamics in a dose response manner in healthy young adults

K. Su (Taiwan) - The effects of polymorphisms in phospholipase a2 and cyclo-oxygenase 2 genes on interferon-alpha-induced depression and polyunsaturated fatty acids levels

13.45 - 14.00

Track 2: Biochemistry of Lipids

Giuseppe Astarita (USA) - Deficient liver biosynthesis of docosahexaenoic acid correlates with cognitive impairment in alzheimer's disease

M. Gregory (Australia) - Cloning and functional characterisation of the fatty acyl elovl2 and elovl5 from rat

C. Coomans (Netherlands) - Brain insulin signaling promotes fatty acid uptake by adipose tissue

14.00 - 14.15

Track 3: Lipids and Nutrition

J. Lambert (Canada) - Islet transplant patients do not respond to hypolipidemic dietary intervention role of hepatic de novo lipogenesis

C. Childs (UK) - The DHA content of rat liver phospholipids are significantly higher when an alpha-linolenic acid rich diet is provided during pregnancy

R. Kuipers (Netherlands) - The relation between DHA and AA is synergistic at low DHA status and antagonistic at high DHA status

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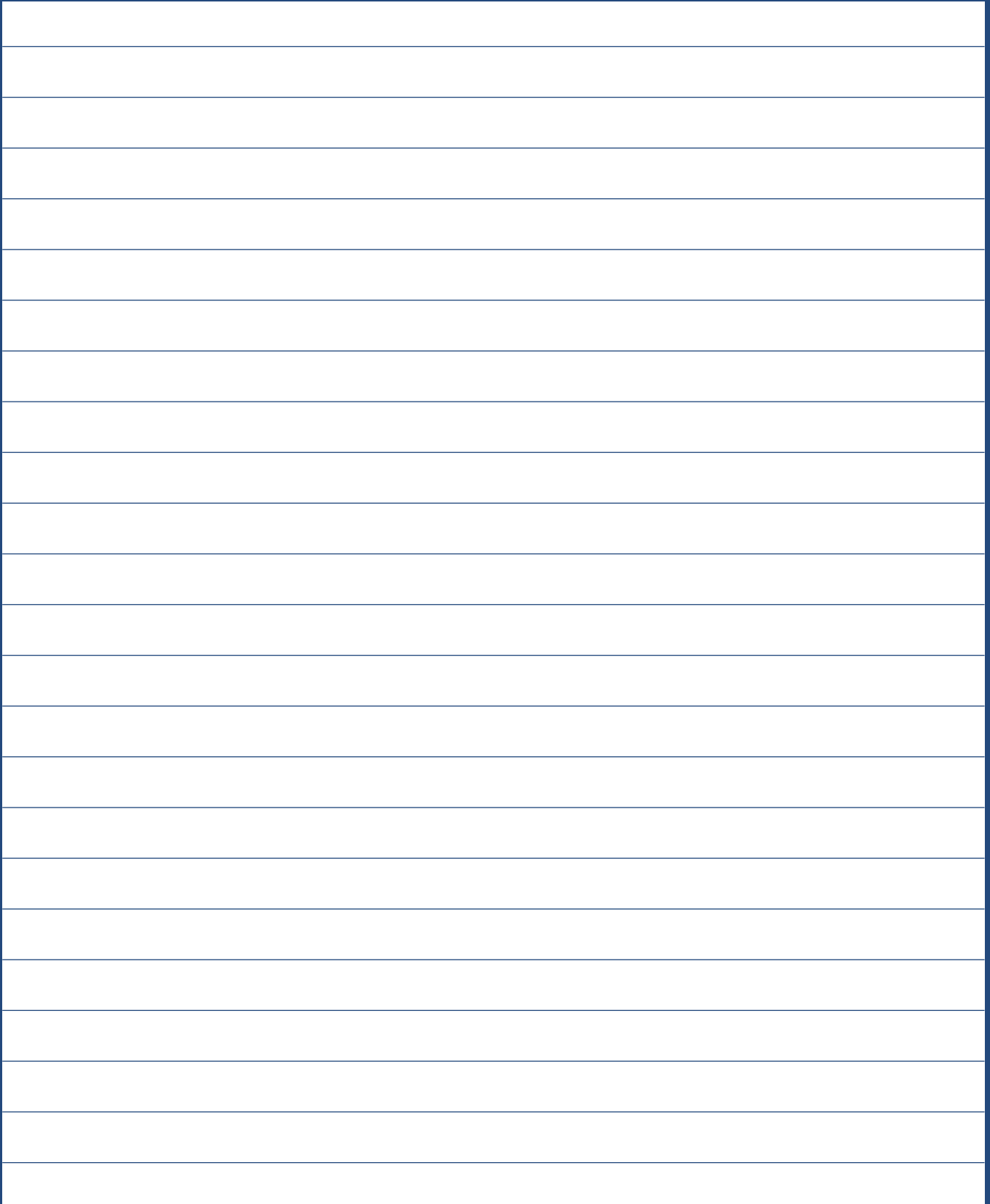
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