POSTER SESSIONS

The LIPID MAPS meeting features two poster sessions with 60 posters. Of these, seven were selected for short talks this year (see program) by the poster committee (H. Alex Brown, Edward A. Dennis, Alfred H. Merrill, Jr., Robert C. Murphy, and David W. Russell). We received so many high quality, relevant abstracts that making these selections was very difficult. The poster presenters are also cross referenced in the attendee list.

1. **Development of a versatile GPCR assay and its application for ligand identification of orphan GPCRs**
   Asuka Inoue, Jun Ishiguro, Kumiko Makide, Junken Aoki (Tohoku University, Japan).
2. **Phospholipid metabolism and autophagy**
   Keita Nakanaga, Asuka Inoue, Junken Aoki (Tohoku University, Japan).
3. **Identification of 17,18-diHEPE as a novel EPA-derived anti-inflammatory lipid mediator ***
   Yosuke Isobe, Makoto Arita, Shinnosuke Matsueda, Hiroki Nakanishi, Ryo Taguchi, Daisuke Urabe, Masayuki Inoue, Hiroyuki Arai (University of Tokyo, Japan).
4. **Increased saturation of membrane phospholipids activates unfolded protein response**
   Nozomu Kono, Hiroyuki Ariyama, Rikuto Tanaka, Shinji Matsuda, Takao Inoue, Hiroyuki Arai (University of Tokyo, Japan).
5. **Prostaglandin E2-dependent blockade of fibroblast-to-myofibroblast transition is mediated through S1379 phosphorylation of ROCK2: A phosphoproteomic analysis**
   Casimiro Gerarduzzi, John A. Di Battista (McGill University Health Center, Canada).
6. **Metalloproteinase processing of HBEGF is a proximal event in the response of human aortic endothelial cells to oxidized phospholipids**
   Sangderk Lee, James R. Springstead, Brian Parks, Casey E. Romanoski, Aldons J. Lusis, Judith A. Berliner (University of California, Los Angeles).
7. **Oxidized phospholipid interaction with cysteines is important in the regulation of human endothelial cell function ***
   James R. Springstead, B. Gabriel Gugiu, Sangderk Lee, Seung Cha, Andrew D. Watson, Judith A. Berliner (University of California, Los Angeles).
8. **Novel endogenous N-acyl amides activate TRPV receptors and are regulated in an acute model of inflammation ***
   Heather B. Bradshaw, Siham Raboune, Jordyn M. Stuart (Indiana University).
9. **Computational analysis of metabolic remodeling specificities of choline and ethanolamine glycerophospholipids**
   Kourosh Zarringhalam, Lu Zhang, Michael A. Kiebish, Kui Yang, Xianlin Han, Richard W. Gross, Jeffrey Chuang (Boston College).
10. **Dynamics of the ethanolamine glycerophospholipid lipid remodeling network**
    Lu Zhang, Norberto Diaz-Diaz, Kourosh Zarringhalam, Martin Hermansson, Pentti Somerharju, Jeffrey H. Chuang (Boston College).
11. **Shotgun sphingolipidomics of an early-divergent protozoan, *Giardia lamblia* reveals life-cycle dependent sphingolipid metabolism**
    Trevor T. Duarte, Igor C. Almeida, Siddhartha Das (University of Texas at El Paso).
12. **A comparison of the total lipid and fatty acid profiles of abdominal blubber from Indian Ocean bottlenose (*Tursiops aduncus*) and common (*Delphinus sp.*) dolphins**
    Bruce C. Davidson, Geremy Cliff, Martine Blumenau, Amanda Axcel (Saint James School of Medicine, Caribisch Nederlands).

Presenting author is underlined. # indicates equal effort contributed by first authors.
** indicates short talk selection*

13. **Changes in lipid and fatty acid profiles in female spotted raggedtooth (*Carcharias taurus*) sharks with season and ovulation**
Bruce C. Davidson, Geremy Cliff (Saint James School of Medicine, Caribisch Nederlands).

14. **Comparison of the fatty acid profiles of liver and fat from five Indian Ocean loggerhead turtles (*Caretta caretta*)**
Bruce C. Davidson, Anet Ayvazyan, Sumedha Evani, Geremy Cliff (Saint James School of Medicine, Caribisch Nederlands).

15. **A lipidomics perspective of macrophage eicosanoid signaling: Relating metabolism to phenotype and phospholipid PUFA composition**
Paul C. Norris, Donna Reichart, Darren S. Dumlao, Christopher K. Glass, Edward A. Dennis (University of California, San Diego).

16. **Lipidomic analysis of primary astrocytes, microglia, and oligodendrocytes reveals origin of hepoxilin and prostacyclin function in neuroinflammation and nociception**
Darren S. Dumlao, Ann M. Gregus, Paul C. Norris, Bethany Fitzsimmons, Hua Xiao-Ying, Tony L. Yaksh, Edward A. Dennis (University of California, San Diego).

17. **Spinal 12-Lipoxygenases (12-LOX) contribute to the development of inflammatory hyperalgesia via activation of TRPV1 and TRPA1 receptors**
Ann M. Gregus[#], Suzanne Doolen[#], Darren S. Dumlao[#], Matthew W. Buczynski, Toshifumi Takasusuki, Bethany L. Fitzsimmons, Xiao-Ying Hua, Bradley K. Taylor, Tony L. Yaksh, Edward A. Dennis (University of California, San Diego).

18. **Two-step extraction protocol for lipidomic analysis in human skeletal muscle samples**
Donato A. Rivas, Mauricio da S. Morais, Evan P. Morris, Roger A. Fielding (Tufts University).

19. **Broad substrate specificity of the *Arabidopsis thaliana* lyso-glycerophospholipid acyltransferase At1g78690**
Teresa A. Garrett (Vassar College).

20. **Lipid oxidation in foods: Global methods, specific markers or lipidomic approach?**
Michèle Viau, Anne Meynier, Lucie Ribourg, Claude Genot (INRA, France).

21. **Coupling of 25 hydroxycholesterol (25-HC) to Interferon antiviral response**
Mathieu Blanc, Kevin A Robertson, Wayne Hsieh, Thorsten Forster, Nathan Spann, Christopher Glass, William Griffiths, Peter Ghazal (University of Edinburgh Medical School, UK).

22. **Lipidomics and phase behaviour: the surface chemistry of oil bodies**
Samuel Furse, Catharine Ortori, David Barrett, David A. Gray (University of Nottingham, UK).

23. **The third and last cardiolipin synthase of *Escherichia coli* ***
Brandon Tan, Mikhail Bogdanov, Jinshi Zhao, Willam Dowhan, Christian R. H. Raetz, Ziqiang Guan (Duke University Medical Center & University of Texas Medical School).

24. **Genetic deletion of nitric oxide synthases in a lupus mouse increases deposition of oxidized lipoprotein immune complexes in aortic lesions and elevates plasma sphingosine 1-phosphate levels**
Mohammed M. Al Gadban, Jashalynn German, Jean-Philip Truman, Farzan Soodavar, Ellen C. Riemer, Waleed O. Twal, Kent J. Smith, Demarcus Heller, Ann F. Hofbauer, Jim C. Oates, Samar M. Hammad (Medical University of South Carolina).

25. **Sphingolipid metabolism: A novel link between obesity and cardiomyopathy**
Stanley Walls, Steve Attle, Deron Herr, Gregory Brulte, Marlena Walls, Dale Chatfield, Anthony Cammarato, Karen Occor, Rolf Bodmer, Greg Harris (San Diego State University).

26. **DHA supplementation in mice with Alzheimer's amyloid pathology: Effects on brain phospholipids**
Cécile Bascoul-Colombo, Ben H. Maskrey, Valerie B. O'Donnell, Mark Good, John L. Harwood (Cardiff University, UK).

27. **Phospolipases C cause a decrease in phosphatidylinositol (4,5)-bisphosphate levels, potentially causing insulin resistance**
Alexander J. Ryan, Katherine A. Hinchliffe (University of Manchester, UK).

28. **Impact of 6 month cigarette smoke exposure on the plasma, liver and aorta lipidome of ApoE-/- mice**
Stefan Lebrun, Stephanie Boue, Kirill Tarasov, Minna Jänis, Reini Hurme, Walter Schlage, Manuel C. Peitsch, Reijo Laaksonen, Julia Hoeng (Phillip Morris International R&D, Switzerland).

29. **Saturated fatty acid-induced activation of TLR-mediated pro-inflammatory signaling pathways is not due to contaminants in BSA or fatty acid preparation**
Shurong Huang, Jennifer M. Rutkowsky, Ryan G. Snodgrass, Kikumi D. Ono-Moore, Dina A. Schneider, John W. Newman, Sean H. Adams, Daniel H. Hwang (USDA & University of California, Davis).

30. **Diet macronutrient composition: A determinant of metabolic inflammation, insulin sensitivity and longevity?**
Berit Johansen, Hans-Richard Brattbakk, Ingerid Arbo, Thuy Than, Martin Kuiper (Norwegian University of Science and Technology, Norway).

31. **Quantification of platelet activating factors in human plasma and serum utilizing liquid chromatography with isotopic dilution tandem mass spectrometry**
William Bragg, Beth Hamelin, Rebecca Shaner, Jerry Thomas, Rudolph Johnson (National Center for Environmental Health, CDC).

32. **IRE1a-XBP1s induces PDI expression to regulate MTP activity for hepatic VLDL assembly and lipid homeostasis**
Shiyu Wang, Zhouji Chen, Vivian Lam, Randal J. Kaufman (Sanford-Burnham Medical Research Institute).

33. **In mouse dendritic leucocytes, are lipid droplets the organelles which move lipids to the membrane of the parasitophorous vacuoles hosting live *Leishmania* amastigotes?**
Hervé Lecoeur, Emilie de la Llave, Marie-Christine Prévost, Geneviève Milon, Thierry Lang (Institut Pasteur, France).

34. **Lipid profiling of serum samples from calcific coronary artery disease patients using ultra performance LC and ion mobility TOF MS**
Giorgis Isaac, Panagiotis A. Vorkas, Elizabeth J. Want, Stephen McDonald, Ulf Naslund, Anders Holmgren, Michael Y Henein, Alan Millar, Elaine Holmes, John P. Shockcor, Jeremy Nicholson, James Langridge (Waters Corporation, MA).

35. **Mapping lipidomes by coupling HILIC and ion mobility MSE**
Giuseppe Astarita, Marc V Gorenstein, Giorgis Isaac, John P. Shockcor, Mark Ritchie, James Langridge (Waters Corporation, MA).

36. **Tocotrienol attenuates triglyceride accumulation in HepG2 cells and F344 rats**
Gregor Carpentero Burdeos, Kiyotaka Nakagawa, Fumiko Kimura, Teruo Miyazawa (Tohoku University, Japan).

37. **A software tool for automated structural characterization of glycerophospholipids using raw mass spectra from nLC-ESI-MS-MS**
Sangsoo Lim, Seul Kee Byeon, Ju Yong Lee, Myeong Hee Moon (Yonsei University, Korea).

38. **Effect of ionization modifiers on the simultaneous analysis of all classes of phospholipids by nanoflow liquid chromatography/tandem mass spectrometry in negative ion mode**
Dae Young Bang, Myeong Hee Moon (Yonsei University, Korea).

39. **Top-down phospholipid analysis for low density lipoproteins with on-line chip-type flow field-flow fractionation/ESI-MS-MS**
Ki Hun Kim, Myeong Hee Moon (Yonsei University, Korea).

40. **A chemical biology/mass spectrometry based approach to study isoprenoid diphosphate metabolism in live cells ***
Andrew J. Morris, Thangiah Subramanian, Fredrick Onono, Manjula Sunkara, H. Peter Spielmann (University of Kentucky College of Medicine).

41. **Structural determination of estradiol product ions**
Kerry M. Wooding, Chris A. Johnson, Joseph A. Hankin, Andrew P. Bradford, Nanette Santoro, Robert C. Murphy (University of Colorado Denver).

42. **Lung cancer progression results in a complex pattern of eicosanoids which is dependent on cPLA$_2$ expression in the tumor microenvironment**
Joanna M. Poczobutt, Miguel A. Gijon, Howard Li, Teresa Joyal, Mary C.M. Weiser-Evans, Robert C. Murphy, Raphael A. Nemenoff (University of Colorado).

43. **Microwave-assisted: Fast and accurate sample preparation technique for high-throughput lipidomics**
Sakda Khoomrung, Pramote Chumnanpuen, Intawat Nookaew, Jens Nielsen (Chalmers University of Technology, Sweden).

44. **Investigating the effects of cholesterol and bupivacaine on the degree of lipid peroxidation in the phospholipid bilayer membrane system**
Stewart Gruey, Nisreen Nusair (Walsh University, OH).

45. **Quantitative yeast lipidomics via LC-MS profiling using the Q Exactive Orbitrap mass spectrometer**
David A. Peake, Jessica Wang, Pengxiang Huang, Adam Jochem, Alan Higbee, David J. Pagliarini (Thermo Fisher Scientific, CA).

46. **Metabolite profiling of bone marrow and peripheral biofluids from acute lymphoblastic leukemia patients before and after induction therapy**
Stefano Tiziani, Yunyi Kang, Jacob Feala, Ricky Harjanto, William Roberts, Giovanni Paternostro (Sanford-Burnham Medical Research Institute).

47. **Plasma phosphatidylcholine turnover is increased in preterm human infants ***
Kevin Goss, Howard Clark, Victoria Goss, Tony Postle (University of Southampton, UK).

48. **Targeted serum lipidomics by ultra performance liquid chromatography coupled with tandem mass spectrometer**
Jun Feng Xiao, Lihua Zhang, Yue Luo, Amrita Cheema, Habtom W. Ressom (Georgetown University Medical Center).

49. **Genetic determinants of 24(S)-hydroxycholesterol levels**
Ashlee R. Stiles, Jeffrey G. McDonald, David W. Russell (UT Southwestern Medical Center).

50. **A simple method for isolation and identification of bioactive lipids having monoester type phosphate using phosphate capture molecule, Phos-tag**
Jun-ichi Morishige, Mai Urikura, Tamotsu Tanaka, Kiyoshi Satouchi (Fukuyama University).

51. **Assessing metabolite production capabilities in adipose tissue**
Alex Thomas, Daniel Zielinski, Bernhard Palsson, Dorothy Sears (University of California, San Diego).

52. **Oxysterols direct immune cell migration via EBI2**
Andreas W. Sailer, Juan Zhang, Tangsheng Yi, Birgit U. Baumgarten, Silvio Roggo, Francois Gessier, Thomas Suply, Jason G. Cyster, Klaus Seuwen (Novartis Institutes for Biomedical Research, Switzerland).

53. **Fiber type specific distribution of lipid droplets in skeletal muscles of inbred Berlin fat mice (BFMI) lines**
Ozlem Bozkurt, Christopher S. Shaw, Sebastian Heise, Gudrun A. Brockmann, Feride Severcan (Middle East Technical University, Turkey).

54. **Obesity induced alterations on structure and content of lipids in adipose and skeletal muscle tissues in inbred obese mouse models: a novel ATR-FTIR study**
Ilke Sen, Ozlem Bozkurt, Sebastian Heise, Gudrun Brockmann, Feride Severcan (Middle East Technical University, Turkey).

55. **Alternative arachidonic acid cascade in the brain ***
Yoshihiro Kita, Asami Tanimura, Maya Yamazaki, Kenji Sakimura, Masanobu Kano, Takao Shimizu (University of Tokyo, Japan).

56. **Role of lysophosphatidic acid signaling during liver regeneration and hepatocellular carcinoma (HCC) progression**
Ashley M. Lakner, Kerri A. Simo, Erin M. Hanna, David A. Iannitti, Iain H. McKillop, Eugene Sokolov (Carolinas Medical Center).

57. **Transcriptomic and lipidomic data analysis of macrophages stimulated with KLA/ATP reveals enhanced role of STATs in apoptosis, immunity and lipid metabolism**
Ashok Reddy Dinasarapu[#], Shakti Gupta[#], Mano Ram Maurya, Eoin Fahy, SungJun Min, Manish Sud, Merril J. Gersten, Shankar Subramaniam (University of California, San Diego).

58. **Lysophospholipid-based strategies for mitigation of high-dose radiation injury *in vivo***
Gabor Tigyi, Wenlin Deng, Veeresh Gududuru, Shannon McCool, Duane D. Miller, Leonard Johnson, Karen Emmons-Thompson, C. Ryan Yates, Louisa Balazs, Yongzhi Geng, Koen Van Rompay (University of Tennessee Health Science Center).

59. **An LC/MS/MS Method That Resolves Glucosyl- and Galactosylceramide, and Tracks Other Related Sphingolipids**
Lindsay Sweet, Jennifer McCullough, Patrick Starremans, Lingyun Li, Eva Budman, Aharon Cohen, John Leonard, Bing Wang (Genzyme Corporation, MA).

60. **C60+-SIMS imaging: The in-situ structural characterization of phosphatidylcholines**
Anita Durairaj, Nicholas Winograd (The Pennsylvania State University).

1. Development of a versatile GPCR assay and its application for ligand identification of orphan GPCRs

Asuka Inoue, Jun Ishiguro, Kumiko Makide, Junken Aoki

Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai City 980-8578, Japan.

Actions of bioactive lipids are mainly mediated by G protein-coupled receptors (GPCRs). Activation of GPCRs induces G protein-dependent secondary signaling such as intracellular Ca^{2+} efflux (Gq) and cAMP accumulation/inhibition (Gs/Gi), which are detectable by conventional assays. However, there are no single-format methods to detect a wide range of GPCRs, especially those coupled with G12/13. In the present study, we developed a novel GPCR assay, which we designate as a transforming growth factor-α (TGFα) shedding assay, and evaluated usefulness of the method for lipid-recognizing GPCRs. In this assay, activation of GPCRs induced TACE-dependent ectodomain shedding of a membrane-bound proform of alkaline phosphatase (AP)-tagged TGFα (AP-TGFα), a reporter fusion protein, followed by colorimetric quantification of soluble AP-TGFα in conditioned media. We found that TGFα shedding responses occurred downstream of Gq and G12/13 signaling. Among 41 GPCRs that recognize lipids (fatty acids, leukotrienes, lysophospholipids, prostaglandins, *etc.*) examined, 28 GPCRs (68%) were detectable in the TGFα shedding assay and, remarkably, 36 GPCRs (88%) were detectable by co-transfecting chimeric Gα and/or promiscuous G16 proteins. Furthermore, we screened orphan GPCRs with various lipids and identified two GPCRs as G12/13-coupled lysophosphatidylserine (LysoPS) receptors. Thus, the TGFα shedding assay will be a useful, powerful tool for GPCR studies as well as ligand searching of orphan GPCRs.

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2. Phospholipid metabolism and autophagy

Keita Nakanaga, Asuka Inoue, Junken Aoki

Department of Molecular and Cellular Biochemistry, Graduate School of Pharmaceutical Science, Tohoku University, Sendai City 980-8578, Japan.

Autophagy is a pivotal physiological process for survival during starvation. During autophagy, dynamic membrane rearrangements are observed, suggesting active phospholipid metabolism. In this study, we developed a rapid and simple method for detection of various species of phospholipids, lysophospholipids, and sphingolipid, and performed lipid profiling in the course of autophagy. Lipids were extracted with methanol and separated by C18 reverse-phase columns using a gradient of solvent A (5 mM ammonium formate in water) and solvent B (5 mM ammonium formate in 95% (v/v) acetonitrile). Formic acid was added to prepare them to pH 4.0. MS/MS was carried out on a Quantum Ultra triple quadrupole mass spectrometer. MS/MS analysis was performed using selected reaction monitoring (SRM). Using the system we established, more than 85 phospholipids, 35 lysophospholipids, and 10 sphingolipids were indentified in HeLa cells. Furthermore, LPA, PA and PS species, which were eluted as broad peaks in the previous reverse-phase LC conditions, were detected in sharp peaks in our present methods. We starved HeLa cells to induce autophagy and measured their phospholipid levels. We found that LPE species increased after 0.5-hour starvation, and then decreased to the basal levels. Unsaturated LPG increased after induction of starvation. S1P and sphingosine decreased dramatically under starvation conditions. It should be noted that the levels of diacyl phospholipid species were changed. These results suggest that deacylation of phospholipids and sphingolipid metabolisms are activated in the process of autophagy.

⋆ *indicates short talk selection*

3. Identification of 17,18-diHEPE as a novel EPA-derived anti-inflammatory lipid mediator *

Yosuke Isobe[1], Makoto Arita[1,4], Shinnosuke Matsueda[1], Hiroki Nakanishi[2], Ryo Taguchi[2,5], Daisuke Urabe[3], Masayuki Inoue[3], Hiroyuki Arai[1,5]

[1]Department of Health Chemistry, Graduate School of Pharmaceutical Science; [2]Department of Metabolomics, Graduate School of Medicine; [3]Department of Integrated Analytical Chemistry, Graduate School of Pharmaceutical Science, University of Tokyo, Tokyo 113-0033, Japan; [4]PRESTO, JST, Japan; [5]CREST, JST, Japan.

Dietary supplementation of eicosapentaenoic acid (EPA) is well appreciated to have beneficial impacts in many inflammatory disorders. The bioactive EPA-derived mediators including E-series resolvins elicit potent anti-inflammatory actions in controlling inflammation. Resolvin E1 (RvE1) and RvE2 are biosynthesized via the 5-lipoxygenase (5-LOX) pathway from a common precursor of E series resolvins, namely 18-hydroxyeicosapentaenoic acid (18-HEPE). In this study, we set up lipidomics employing LC-MS/MS based analysis designed to search for other 18-HEPE-derived mediators present in vivo. We found that human peripheral blood leukocytes produced a novel series of EPA metabolites including 8,18-dihydroxy-eicosapentaenoic acid (8,18-diHEPE), 11,18-diHEPE, 12,18-diHEPE, and 17,18-diHEPE via the 12/15-LOX pathway upon incubation with 18-HEPE. Among them, two stereoisomers of 17,18-diHEPE, collectively termed resolvin E3 (RvE3), displayed a potent anti-inflammatory action by limiting neutrophil infiltration in zymosan-induced peritonitis. The planar structure of RvE3 was unambiguously determined to be 17,18-dihydroxy-5*Z*,8*Z*,11*Z*,13*E*,15*E*-EPE by high-resolution NMR, and the two stereoisomers were assigned to have 17,18*R*- and 17,18*S*-dihydroxy groups, respectively, using chemically synthesized 18*R*- and 18*S*-HEPE as precursors. Both 18*R*- and 18*S*-RvE3 inhibited neutrophil chemotaxis *in vitro* at low nanomolar concentrations. Moreover, endogenous production of RvE3 and their precursor 18-HEPE was observed in inflammatory exudates of murine peritonitis and was increased with EPA-supplemented. These findings suggest that RvE3 contributes to beneficial actions of EPA in controlling inflammation and related diseases.

Isobe Y, et al. (2012) Identification and structure determination of a novel anti-inflammatory mediator resolvin E3: 17,18-dihydroxy-eicosapentaenoic acid. J Biol Chem. in press.

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4. Increased saturation of membrane phospholipids activates unfolded protein response

Nozomu Kono, Hiroyuki Ariyama, Rikuto Tanaka, Shinji Matsuda, Takao Inoue, Hiroyuki Arai

Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo 113-0033, Japan.

The degree of fatty acid saturation in membrane phospholipids affects many membrane-associated functions and can be influenced by diet and by altered activities of lipid-metabolizing enzymes such as fatty acid desaturases. However, little is known about how mammalian cells respond to changes in the saturation degree of phospholipid acyl chains. Stearoyl-CoA desaturase 1 (SCD1), which catalyzes a desaturation of saturated fatty acids to monounsaturated fatty acids, plays an important role in determining the saturation degree of membrane phospholipids. In this study, we examined the effect of SCD1 knockdown on phospholipid fatty acid composition and cellular signaling in HeLa cells. SCD1 knockdown increased the SFA/MUFA ratio in membrane phospholipids without affecting the free fatty acids. Gene expression analysis revealed that markers of unfolded protein response (UPR) were up-regulated in SCD1 knockdown cells. Consistent with increased expression of UPR target genes, activation of the UPR sensor proteins, inositol requiring-1 (IRE1) and PKR-like ER kinase (PERK), occurred in SCD1 knockdown cells. The SCD1 knockdown-induced UPR was rescued by various unsaturated fatty acids and was enhanced by saturated fatty acid. Furthermore, knockdown of lysophosphatidylcholine acyltransferase 3 (LPCAT3), which incorporates preferentially polyunsaturated fatty acids into phosphatidylcholine, synergistically enhanced UPR with SCD1 knockdown or saturated fatty acid. Taken together, these results suggest that increased saturation of membrane phospholipids activates UPR.

5. Prostaglandin E2-dependent blockade of fibroblast-to-myofibroblast transition is mediated through S1379 phosphorylation of ROCK2: A phosphoproteomic analysis

Casimiro Gerarduzzi, John A. Di Battista

Department of Experimental Medicine, McGill University & Division of Rheumatology, Royal Victoria Hospital, McGill University Health Center, Montreal, Canada, H3A 1A1.

One of the more complex GPCR signaling pathways that remain ill-defined is that activated by Prostaglandin E2 (PGE2). To better understand this, we have devised a strategy to identify and quantify the numerous phosphorylated sequences for a number of key kinases activated by PGE2 in Human Fibroblast (HF) samples. In doing so, our study has revealed by Western Blot that PGE2 predominantly induced a RXX(Phospho-Ser/Thr) specific pathway through GPCR/cAMP/PKA. In a movement to identify these substrates, we describe a draft of a complete PGE2-dependent phosphoproteome, which include proteins associated with cytoskeletal structure and cellular differentiation. As such, we have precise data about novel phosphorylation sites through amino acid sequencing data, providing vital information about a target protein's activity. Immunocytochemistry and Western Blot confirmed changes in cytoskeletal structures by PGE2 such as showing the inhibition of myosin light chain (MLC, a ROCK2 substrate) phosphorylation, which regulates the assembly of stress fibers. Our observed cytoskeletal remodeling has led us to the differentiation effect that PGE2 influences between normal fibroblasts and stress fiber-rich myofibroblasts. As effector cells of wound repair, myofibroblast persistency can lead to the formation of excess fibrous connective tissue known as fibrosis. Prominent signaling pathways that support myofibroblastic formation include those from our PGE2-dependent phosphoproteome, such as ROCK2. Giving weight to our strategy, mutation of our identified ROCK2 phosphosite prevented PGE2 inhibition of MLC phosphorylation by ROCK2. In addition, the pro-fibrotic cytokine TGF-β is known to differentiate normal fibroblasts to myofibroblasts by synthesizing stress fibers and α-smooth muscle actin. We show that PGE2 reverted this TGF-β phenotypic change by inhibiting the synthesis of these myofibroblast hallmark proteins. In general, we have developed a strategy to identify activated kinases and their phosphorylated substrates in an initial step of profiling the PGE2 signaling network, beneficial in understanding downstream functions such as fibrosis.

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6. Metalloproteinase processing of HBEGF is a proximal event in the response of human aortic endothelial cells to oxidized phospholipids

Sangderk Lee[1], James R. Springstead[3], Brian Parks[2], Casey E. Romanoski[2], Aldons J. Lusis[2,3], Judith A. Berliner[1,3]

[1]Department of Pathology; [2]Department of Human Genetics; [3]Division of Cardiology, Department of Medicine, University of California, Los Angeles, Los Angeles, CA 90095.

Atherosclerosis is a chronic inflammatory disease initiated by monocyte recruitment and retention in the vessel wall. An important mediator of monocyte endothelial interaction is the chemokine interleukin-8 (IL-8). The oxidation products of phospholipids, including Ox-PAPC, accumulate in atherosclerotic lesions and strongly induce IL-8 in human aortic endothelial cells (HAECs). The goal of this study was to identify the proximal events leading to induction of IL-8 by Ox-PAPC in vascular endothelial cells. In a systems genetics analysis of HAECs isolated from 96 different human donors, we showed that HBEGF transcript levels are strongly correlated to IL-8 induction by Ox-PAPC. The silencing and overexpression of HBEGF in HAECs confirmed the role of HBEGF in regulating IL-8 expression. HBEGF has been shown to be stored in an inactive form and activation is dependent on processing by a dysintegrin and metalloproteinases (ADAM) to a form that binds and activates EGF receptor (EGFR). Ox-PAPC was shown to rapidly induce HBEGF processing and EGFR activation in HAECs. Ox-PAPC increases ADAM activity in the cells by substrate cleavage assay. We provide evidence for one mechanism of Ox-PAPC activation of ADAM involving covalent binding of Ox-PAPC to cysteine on ADAM. This study is the first report demonstrating a role for the ADAM-HBEGF-EGFR axis in Ox-PAPC induction of IL-8 in HAECs.

7. Oxidized phospholipid interaction with cysteines is important in the regulation of human endothelial cell function *

James R. Springstead[1], B. Gabriel Gugiu[1], Sangderk Lee[2], Seung Cha[1], Andrew D. Watson[1], Judith A. Berliner[1,2]

[1]Department of Medicine, Cardiology, [2]Department of Pathology, University of California, Los Angeles, CA 90095.

Oxidation products of 1-palmitoyl-2-arachidonoyl-*sn*-glycerol-2-phosphatidylcholine (PAPC), referred to as OxPAPC, and an active component, 1-palmitoyl-2-(5,6-epoxyisoprostanoyl E_2)-*sn*-glycero-3-phosphatidylcholine (PEIPC), accumulate in atherosclerotic lesions and other sites of chronic inflammation and regulate over 1000 genes in human aortic endothelial cells (HAEC). We previously demonstrated that OxPAPC covalently binds to a number of proteins in HAEC. The goal of these studies was to gain insight into the binding mechanism and determine if binding regulates activity. In whole cells *N*-acetylcysteine inhibited gene regulation by OxPAPC, and blocking cell cysteines with *N*-ethylmaleimide strongly inhibited OxPAPC binding. Using MS, we demonstrate that most of the binding of OxPAPC to cysteine is mediated by PEIPC. Furthermore, with MS we show that PEIPC covalently binds to a model cysteine-containing peptide. We also demonstrate that OxPAPC and PEIPC bound to a model protein, H-Ras, at cysteines previously shown to regulate activity in response to $15dPGJ_2$. This binding was observed with recombinant protein and in cells overexpressing H-Ras. $15dPGJ_2$ and OxPAPC increased H-Ras activity at comparable concentrations. Using microarray analysis, we demonstrate a considerable overlap of gene regulation by OxPAPC, PEIPC, and $15dPGJ_2$ in HAEC, suggesting that some effects attributed to $15dPGJ_2$ may also be regulated by PEIPC since both molecules accumulate in inflammatory sites. Overall, we provide evidence for the importance of OxPAPC-cysteine interactions in regulating HAEC function.

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8. Novel endogenous N-acyl amides activate TRPV receptors and are regulated in an acute model of inflammation *

Heather B. Bradshaw, Siham Raboune, Jordyn M. Stuart

Department of Psychological and Brain Sciences at Indiana University, Bloomington, IN 47405.

During the last decade, a novel family of endogenous lipids, structurally analogous to the endogenous cannabinoid, *N*-arachidonoyl ethanolamine (Anandamide), called *N*-acyl amides has emerged as a family of biologically active compounds. Examples are *N*-arachidonoyl dopamine and *N*-oleoyl dopamine, structurally similar to the exogenous TRPV1 agonist capsaicin that are potent endogenous ligand at TRPV1 receptors; however, their distribution is limited to only a few brain areas (striatum and hippocampus) and they not produced where TRPV1 receptors are more highly expressed in the periphery. Similarly, other endogenous TRPV agonists such as Anandamide and the *N*-acyl taurines have either relatively low abundance or lower efficacy at the receptors, suggesting that the full complement of endogenous TRPV activation is not fully recognized. We have ongoing studies aimed at isolating and characterizing additional members of the family of *N*-acyl amides in both central and peripheral tissues in mammalian systems. Here, we have screened over 80 *N*-acyl amide molecules for activity at TRPV1-4 using Fura2AM calcium imaging and have identified 12 novel *N*-acyl amides that collectively activate TRPV1, TRPV2 and TRPV4. Using lipid extraction and HPLC coupled to tandem mass spectrometry we show that levels of many of these *N*-acyl amides that activate TRPVs are regulated in paw skin and brain after intraplantar vehicle or carrageenan injection. Together these data provide new insight into the family of *N*-acyl amides and their roles as signaling molecules at TRPV receptors in the context of inflammation.
This work was supported by NIH DA032150.

9. Computational analysis of metabolic remodeling specificities of choline and ethanolamine glycerophospholipids

Kourosh Zarringhalam[1], Lu Zhang[1], Michael A. Kiebish[2], Kui Yang[2], Xialin Han[3], Richard W. Gross[2], Jeffery Chuang[1]

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The regulation and maintenance of the cellular lipidome through biosynthetic, remodeling, and catabolic mechanisms are critical for biological homeostasis during development, health and disease. These complex mechanisms control the molecular architectures of lipid molecular species, which have diverse yet highly regulated fatty acid chains at both the sn1 and sn2 positions. The distribution of fatty acid chains at the sn1 and the sn2 positions of these molecules is determined by regulated processes essential for the biological function of the molecules, but the nature of these processes is not well understood. In this work, we present a rigorous statistical analysis of the extent of cooperative interactions among acyl chains at the sn1 and the sn2 positions of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), the most abundant phospholipids in the vast majority of tissues and membranes. We show that in PC and PE, sn1 and sn2 positions are largely independent, though for low abundance species remodeling processes can interact with both the sn1 and sn2 chain simultaneously, leading to cooperative effects. Chains with similar biochemical properties appear to be remodeled similarly. We also see that sn2 positions are more regulated than sn1, and that PC exhibits stronger cooperative effects than PE. A key aspect of our work is a novel statistically rigorous approach to determine cooperativity based on a modified Fisher's exact test using Markov Chain Monte Carlo sampling. This computational approach provides a novel tool for developing mechanistic insight into lipidomic regulation.

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10. Dynamics of the ethanolamine glycerophospholipid lipid remodeling network

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Acyl chain remodeling in lipids is a critical biochemical process that may play a central role in disease. However, remodeling remains poorly understood, despite massive increases in lipidomic data. In this work, we determine the novel dynamic network of ethanolamine glycerophospholipid (PE) remodeling, using data from six independent pulse-chase experiments and a novel bioinformatic network inference approach. The model uses a set of ODE functions based on the assumptions that (1) sn1 and sn2 acyl positions are independently remodeled; (2) remodeling reaction rates are constant over time; and (3) acyl donor concentrations are constant. We developed a fast and accurate two-step algorithm to automatically infer reaction parameters. Our model closely fits experimental observations and shows strong cross-validation across experiments using distinct deuterium-labeled precursors. We are able to determine the deacylation and reacylation rates of different acyl chain types, as well as explain the enrichment of saturated acyl chains at the sn1 position and unsaturated acyl chains at the sn2 position. Our computational framework allows us to precisely and robustly determine phospholipid remodeling mechanisms and demonstrates that dynamic lipid remodeling processes can be effectively identified from lipidomic pulse-chase data.

11. Shotgun sphingolipidomics of an early-divergent protozoan, *Giardia lamblia* reveals life- cycle dependent sphingolipid metabolism

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Giardia lamblia is a waterborne enteric parasite and the causative agent of giardiasis. This evolutionarily conserved organism has a two stage lifecycle consisting of an infectious cyst form and a replicative trophozoite form. The position of giardia as a basal eukaryote makes it an ideal model for studying rudimentary metabolic pathways. Previously we have identified putative enzymes in the giardial sphingolipid metabolic pathway (Hernandez, 2008). Interestingly many of the key enzymes of *de novo* sphingolipid synthesis appear to be absent from the giardial genome. To better understand how sphingolipids are synthesized and metabolized, we analyzed sphingolipids from *Giardia* trophozoites, differentiating (encysting) stages and cysts as well as the culture medium that was used to cultivate *Giardia* in the laboratory. The samples were analyzed by ESI- MS/MS on a linear triple- quadrupole mass spectrometer (LTQ XL Thermo Scientific). To identify molecules, we employed neutral loss- and precursor ion- scanning. Putative sphingolipids were then subjected to MS(n) analyses to fully characterize both long chain base (LCB) and fatty acyl moiety. More than ninety percent of sphingolipids in *Giardia lamblia* were sphingomyelins which appeared to be scavenged from the culture media. The rest were a mixture of ceramides and glycosphingolipids (GSLs) including mono-, di- and tri-hexosylceramides as well as gangliosides. Interestingly, the abundance of di- and tri-hexosylceramides was substantially increased in cysts when concentration was calculated relative to trophozoite and cyst volumes. Furthermore, differences between fatty-acyl chain lengths of ceramides and glycosphingolipids indicated that this primitive protozoan has the ability to re-model these fatty acyl chains, a phenomenon known to exist in multicellular organisms. In conclusion, our study shows that ceramide is scavenged from the extracellular environment then heavily glycosylated in *Giardia* and the pattern of glycosylation appeared to depend upon the lifecycle stage under current growth conditions.

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12. A comparison of the total lipid and fatty acid profiles of abdominal blubber from Indian Ocean bottlenose (*Tursiops aduncus*) and common (*Delphinus sp.*) dolphins

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Blubber samples from resident, sub-tropical Indian Ocean dolphins, *Tursiops aduncus*, and migratory dolphins from colder waters, *Delphinus sp.*, were obtained from the abdominal region of animals caught in the shark nets that protect the popular swimming beaches off the coast of KwaZuluNatal in South Africa. Analysis of these samples showed several significant differences between both total lipid and individual fatty acids, depending on age, sex, season and species. The bottlenose dolphins showed reduced total fat, saturated fatty acids, and some polyunsaturated fatty acids, while some monounsaturated fatty acids were increased, compared to the common dolphins. While there were some changes dependent on age, gender, geographical location and season there was no consistent pattern. However, none of these samples were examined for blubber stratification, while a second batch of bottlenose dolphin samples were. Contrary to other reports, predominantly from animals in colder waters, they did not show visible stratification. Also, there were no differences in fatty acid profiles between the inner and outer sections of the blubber samples, or between abdominal and thoracic samples.

13. Changes in lipid and fatty acid profiles in female spotted raggedtooth (*Carcharias taurus*) sharks with season and ovulation.

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Several shark species are caught in the beach-protecting nets off the South African Indian Ocean coast, including the spotted raggedtooth (*Carcharias taurus*). Female raggedtooth sharks migrate from off the eastern Cape, past KwaZulu-Natal and up to southern Mozambique, and then back on an annual basis. They mate off the KwaZulu-Natal coast, gestate the pups off Mozambique, then deliver same off the eastern Cape. Prior to mating they hypertrophise their livers and store large amounts of lipid, then towards the end of gestation subsist on this stored lipid as well as using it to feed their pups *in utero*. Raggedtooth sharks are aplacental and hepatic lipids provide nutrients to the pups via continued ovulation throughout pregnancy. The fact of the liver hypertrophy was well documented, but whether the nature of the stored lipid or the amount of lipid per Kg of liver changed with season was unknown. Samples from raggedtooth females caught throughout the year were analysed for their lipid and fatty acid contents and significant differences noted between lipid, but not fatty acid, concentration with certain seasons. Liver mass decreased from Spring to Winter as did lipid concentration. In parallel, samples of both liver and ova in different stages of development were obtained from several females and their lipid and fatty acid profiles quantitated. During the development of the oocytes the total lipid content increased, and mature oocytes showed approximately double the amount as liver. The fatty acid profiles remained fairly constant throughout oocyte development, but were different to those from liver, with greater amounts of the polyunsaturated fatty acids, less monounsaturates and relatively constant saturates. As the continued ovulation throughout pregnancy is a mechanism to provide nutrients to the embryos, this may reflect a requirement for these polyunsaturates for development, especially neural, in the embryos.

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14. Comparison of the fatty acid profiles of liver and fat from five Indian Ocean loggerhead turtles (*Caretta caretta*)

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Many sea turtle species are threatened or endangered according to the IUCN Red List. Loggerhead turtles are protected in South African waters, but are migratory, so may not be throughout their range. Five individual turtles were caught in the beach-protecting nets off the Indian Ocean coast and provided liver and adipose samples for lipid and fatty acid analysis. Comparing adipose with liver there was little variation in the saturated, monounsaturated and n6 polyunsaturated fatty acids, but increased n3 polyunsaturated fatty acids in adipose compared to liver. These results compared well with data published elsewhere on loggerhead turtles, as well as green turtles, but not other turtle species raised in captivity or other aquatic reptilian species.

15. A lipidomics perspective of macrophage eicosanoid signaling: Relating metabolism to phenotype and phospholipid PUFA composition

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Many eicosanoid pathway enzymes and metabolites have been identified and the full network is now believed to be mostly complete. Great focus is now placed on understanding how global metabolism occurs in cells after inflammatory receptor stimulations. Here, we demonstrate how different macrophage phenotypes can be used to quantitatively relate eicosanoid production to drastically different enzyme expression scenarios via targeted lipidomics with LC-MS/MS coupled with protein and mRNA analysis. From this, we have revealed a unique disproportion between very low PGIS transcript expression despite robust PGI_2 production and selective competition between PGIS and mPGES-1. Additionally, different macrophage phenotypes produced total levels of COX metabolites after TLR4 stimulation that was proportional to the maximal level of upregulated COX-2. In a separate study involving supplementation of omega-3 EPA and DHA to RAW264.7 cells, major changes in phospholipid PUFA composition led to drastic changes in TLR4 and $P2X_7$ stimulated eicosanoid signaling. EPA was increased in phospholipids, yet about half of the increased phospholipid EPA was elongated to DPA. After supplementation, COX pathways were inhibited and caused shunting of arachidonic acid (AA) substrate to 5-LOX with $P2X_7$ stimulation. 5-LOX was ultimately uninhibited by EPA, DPA, or DHA, and metabolized these substrates at a similar rate as with AA. Supplementation with different 22-carbon PUFAs AdA (22:4 ω6), DPA (22:5 ω3), and DHA (22:6 ω3) revealed increasing degrees of COX-1 and COX-2 inhibition that correlated positively with increasing degree of unsaturation. Finally, we observed in resident peritoneal macrophages that about 75% of the increased EPA incorporated in phospholipids was elongated to DPA and was the major omega-3 fatty acid released that could compete with AA and inhibited COX metabolism, indicating that DPA may actually be the primary inhibiting fatty acid in some cells after EPA supplementation.

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16. Lipidomic analysis of primary astrocytes, microglia, and oligodendrocytes reveals origin of hepoxilin and prostacyclin function in neuroinflammation and nociception

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Hyperalgesia, an increased sensitivity to pain, is one of the primary causes of chronic pain and the associated behavioral effects have been well characterized using the carrageenan model of inflammation in rats. Using high-throughput mass spectrometry methodology, we have previously shown the contribution of centrally derived bioactive lipid species to carrageenan-induced hyperalgesia (Buczynski *et al*, 2010). Interestingly, 12-lipoxygenase (12-LOX) metabolites were observed to increase in the spinal cord of carrageenan treated rats. Therefore, we examined involvement of spinal LOX enzymes in inflammatory hyperalgesia. In the current work, we found that intrathecal (IT) delivery of the LOX inhibitor NDGA prevented the carrageenan-evoked increase in spinal HXB_3 at doses that attenuated the associated hyperalgesia. Further, IT delivery of inhibitors targeting 12-LOX (CDC, Baicalein), but not 5-LOX (Zileuton) dose-dependently attenuated carrageenan-induced tactile allodynia. Likewise, IT delivery of 12-LOX metabolites of arachidonic acid 12(S)-HpETE, 12(S)-HETE, HXA_3 or HXB_3 evoked profound, persistent tactile allodynia while 12(S)-HpETE and HXA_3 produced relatively modest, transient thermal hyperalgesia. To determine the cellular origin of these spinally-derived 12-LOX metabolites, eicosanoid profiling was conducted on stimulated primary glial cell cultures (microglia, astrocytes, oligodendrocytes) from neonatal rat pubs. Treatment with KLA or BZATP, but not Substance P caused the production of eicosanoids in all cell types, although microglia and astrocytes were the main contributors to the eicosanoid pool. We observed that HXB_3 is produced from microglia, but not astrocytes or oligodendrocytes.

17. Spinal 12-Lipoxygenases (12-LOX) contribute to the development of inflammatory hyperalgesia via activation of TRPV1 and TRPA1 receptors

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Peripheral inflammation initiates changes in spinal nociceptive processing mediated in part by eicosanoids, ultimately leading to hyperalgesia. Previously we demonstrated that among 102 lipid species detected by LC-MS/MS analysis in rat spinal cord, the most notable increases that occur after intraplantar (IPLT) carrageenan are metabolites of 12-lipoxygenases (12-LOX), particularly hepoxilins (HXA_3 and HXB_3). Thus, we examined involvement of spinal LOX enzymes in inflammatory hyperalgesia. In the current work, we found that intrathecal (IT) delivery of the LOX inhibitor NDGA prevented the carrageenan-evoked increase in spinal HXB_3 at doses that attenuated the associated hyperalgesia. Further, IT delivery of inhibitors targeting 12-LOX (CDC, Baicalein), but not 5-LOX (Zileuton) dose-dependently attenuated tactile allodynia. Likewise, IT delivery of 12-LOX metabolites of arachidonic acid 12(S)-HpETE, 12(S)-HETE, HXA_3 or HXB_3 evoked profound, persistent tactile allodynia while 12(S)-HpETE and HXA_3 produced relatively modest, transient heat hyperalgesia. The pronociceptive effect of HXA_3 correlated with enhanced release of Substance P (SP) from primary sensory afferents. Importantly, HXA_3 triggered sustained mobilization of calcium in cells stably overexpressing TRPV1 or TRPA1 receptors and in acutely dissociated rodent sensory neurons. Constitutive deletion or antagonists of TRPV1 (AMG9810) or TRPA1 (HC030031) attenuated this action. Furthermore, pretreatment with antihyperalgesic doses of AMG9810 or HC030031 reduced spinal HXA_3-evoked allodynia. These data indicate that spinal HXA_3 is increased by peripheral inflammation and promotes initiation of facilitated nociceptive processing through direct activation of TRPV1 and TRPA1 at central terminals. Ongoing studies are focused on examination of the expression, distribution and lipidomic profiles of 12-LOX enzymes as well as further investigation of their roles in facilitated nociceptive processing at the spinal level. *Supported by NIH NS16541 and DA02110 (TLY), NIH GM064611 (EAD) and NIH NS62306 (BKT).*

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18. Two-step extraction protocol for lipidomic analysis in human skeletal muscle samples

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The measurement of novel bioactive lipid metabolites (eg. ceramides and diacylglycerols) within skeletal muscle has recently emerged as an important approach for understanding the dysregulation whole-body energy homeostasis with aging and chronic disease. Recently, multiple researchers have reported an increased accumulation of these bioactive lipids in multiple models of metabolic syndrome. The importance of these classes of lipid molecules has led to the development and/or use of several techniques for lipid analysis. Of these the diglyceride kinase (DGK) assay has become the leading method for the rapid and convenient determination of total ceramide and diacylglycerol mass levels. However, despite its quantitative utility, this assay is unable to detect individual lipid species. In light of this evidence the ability to improve and more sensitively measure bioactive lipids is imperative to further lipidomic research. Using high-performance liquid chromatography tandem mass spectrometry (LC/MS^2) we analyzed skeletal muscle from 3 young healthy males (~22 yrs/old) using two separate extraction (2-step vs. 1-step) protocols for a comparison of optimum results. We show using the 2-step extraction we are able to determine more specific peaks and larger concentrations compared to the 1-step protocol. In conclusion, we believe using the 2-step extraction with LC/MS^2 will improve the sensitivity of lipidomic analysis compared to traditional 1-step extraction and DGK methods.

19. Broad substrate specificity of the *Arabidopsis thaliana* lyso-glycerophospholipid acyltransferase At1g78690

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The *Arabidopsis thaliana* enzyme At1g78690 shares significant homology with tafazzin, a cardiolipin transacylase defective in patients with Barth syndrome. We have recently shown that At1g78690 is a lyso-glycerophospholipid (GPL) acyltransferase that catalyzes the transfer of an acyl chain from acyl-CoA to the *sn*-2 position of 1-acyl GPLs such as 1-acyl phosphatidylethanolamine and 1-acyl phosphatidylglycerol to generate phosphatidylethanolamine and phosphatidylglycerol, respectively. Despite this *in vitro* activity, expression of At1g78690 in *E. coli* leads to the *in vivo* accumulation of acylphosphatidylglycerol (acyl-PG), phosphatidylglycerol with an acyl chain added to the glycerol headgroup. To begin to understand why the overexpression of a lyso-glycerophospholipid (GPL) acyltransferase leads to the accumulation of a headgroup acylated GPL in *E. coli* we investigated the substrate specificity of At1g78690. Using solubilized membranes prepared from *E. coli* overexpressing At1g78690, we assessed the ability of At1g78690 to catalyze the transfer of eicosapentaenoate from eicosapentaenoyl coenzyme A to a variety of GPL acyl acceptors including monolysocardiolipin (MLCL), dilysocardiolipin (DLCL) and three stereoisoforms of *bis*-monoacylglycerophosphate (BMP). Low levels of the predicted products were formed using MLCL and DLCL as the lipid acceptors when solubilized membranes containing At1g78690 were used as the enzyme source but not when control solubilized membranes were used. In contrast, At1g78690 robustly acylates two BMP isoforms with *sn*-2 and/or *sn*-2' hydroxyls in the *R*-stereoconfiguration, as compared to the BMP isoform with the *sn*-2 and *sn*-2' hydroxyls in the *S*-stereoconfiguration suggesting that At1g78690 is stereoselective for hydroxyls with *R*-stereochemistry. In addition, this robust acylation of BMPs by At1g78690, which yields acyl-PG like molecules, may explain the accumulation of acyl-PG in *E. coli* when At1g78690 is overexpressed in cells. Experiments to access the ability of At1g78690 to use other lyso-GPLs acyl-acceptors and acyl-donors are underway to completely elucidate the mechanism by which At1g78690 so strikingly alters the lipid composition of *E. coli*.

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20. Lipid oxidation in foods: global methods, specific markers or lipidomic approach?

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Lipid oxidation in foods is a main concern for their nutritional, sensory and functional properties. Recent works evidence that among the large array of products, arising directly from lipids or indirectly through interactions with other food components such as protein, some of them could have deleterious effects when daily consumed, even at very low level. However, the current methods used by the industry, such as peroxide value, anisidine value and TBA-reactive substances to evaluate oxidation in food products are generally global, non-specific and not always quantitative. Consequently, there is a lack of quantitative information about the deleterious oxidation products present in the food or in nutritional supplements. We have developed in our laboratory a range of methods to characterize both lipids and their oxidation products in foods and food model systems. They include detailed analysis of lipid composition (from fatty acid composition by GC, tocopherols and lipid classes by HPLC to molecular species by MALDI-TOF MS approach), micro method to quantify hydroperoxydes, evaluation of protein alteration (protein carbonyls, front face fluorescence, SDS-PAGE) and methods to quantify specific markers of oxidation, among them volatile compounds, malondialdehyde (MDA) and hydroxyl alkenals (4-hydroxy-2 nonenal and 4-hydroxy-2-hexenal). Other methods for the quantification of hydroperoxides molecular species are in development. These methods, when combined, give an overview of the oxidative fate of the food matrices and information on the amounts of potentially toxic compounds, However, a deeper understanding of the oxidative phenomena in foods or biological systems would require a more integrated lipidomic approach especially as regard to the identification of relevant and robust markers.

21. Coupling of 25 hydroxycholesterol (25-HC) to Interferon antiviral response

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Little is known about inflammatory mediators in modulating lipid metabolism in infection. In particular, the interrelation between sterol metabolic genes, infection and subsequent immune reactions that alter cellular metabolism on a functional level are poorly understood. We have recently reported that the coordinate down-regulation of multiple sterol metabolic genes upon infection by viruses is mediated by pattern recognition receptor activation of Interferon. Drug and interference RNA Inhibition of the sterol pathway confers protection, indicating that the down-regulation of the sterol network has a cellular countermeasure role to infection. Genetic knock-out studies elucidate an inflammatory regulatory loop mechanism linking the innate immune response activation of type 1 interferon and signalling of its receptor to the down-regulation of the pathway upon infection. Coordinate transcriptional control of the sterol pathway is governed by the SREBP2 transcription factor, which is reduced at both the protein and gene transcription level upon infection or interferon action in a type 1 receptor dependent manner. We show that 25-HC is markedly enhanced upon infection with submicromolar levels secreted by an infected macrophage. This production of 25-HC is strictly interferon-dependent and evidence for direct coupling to interferon signalling is shown by rapid Stat1 recruitment to the hydroxylase promoter. This work underscores a new physiological role for 25-HC as part of a host protection pathway that acts against infection and it highlights the potential for pharmacological targeting of host metabolic regulatory networks as an anti-viral strategy.

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22. Lipidomics and phase behaviour: The surface chemistry of oil bodies

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Despite mounting commercial interest, the structure of plant Oil Bodies (OBs) remains unclear. Some work has been done to elucidate the structure[1-3], however more recent work in lipidomics suggests that the reported lipid profile for the surface may be incomplete. Additionally, the importance of minor components of the lipidome with respect to the physical behaviour of the lipid fraction, cannot be under-estimated[4, 5] and thus an exhaustive search is required in order to understand the physical behaviour of the lipid fraction. Additionally, emerging evidence for the presence of phytochemical species being present in the surface *mono*-layer of OBs may yet be important to the physical behaviour of the surface[6]. In order to gain an understanding of the physical behaviour of the surface *mono*-layer of OBs, the triglyceride storage organelles in many desiccating seeds, a full lipidome is undoubtedly required. The work in this project is based upon not only determining the lipidome but in building an understanding of its phase behaviour. The lipidome was determined using ^{31}P NMR and MS techniques. A picture of the contribution of the lipid fraction of OBs can be made both from the understood behaviour of individual lipids present, and from physical studies on the mixtures isolated. An understanding of the physical contribution of the lipid fraction, in turn, suggests the possible physical role(s) played by the proteins found on the surface of Oil Bodies.

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23. The third and last cardiolipin synthase of *Escherichia coli* *

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Cardiolipin (CL) is a major phospholipid in found in many prokaryotes and in all eukayotes. In *Escherichia coli*, CL is found in addition to phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). Depending on growth phase and culture conditions, CL makes up 5% to 35% of the cellular phospholipids in the wild type cells. It is generally accepted that in bacteria, cardiolipin synthase (cls) catalyzes a reversible phosphatidyl group transfer from one phosphatidylglycerol (PG) molecule to another PG to form CL and glycerol. Two cls genes in *E. coli*, *cls* and *ybhO* (now renamed as *clsA* and *clsB*, respectively), have been identified. However, double deletion mutants lacking *clsA* and *clsB* are viable and still make CL in the stationary phase, indicating the existence of additional CL synthase (s) in *E. coli.* In this study, we identified the third and last CL synthase in *E. coli*, *ymdC* (renamed as *clsC1*). *ClsC1* shares sequence homology with *clsA* and *clsB* in the phospholipase D superfamily, with each containing two HKD motifs. Triple deletions of *clsA, clsB* and *clsC1* caused the complete depletion of CL in *E. coli* cells regardless cell culture growth phase, even when the mutant cells are cultured to the stationary phase. CL can be restored to the wild type levels in the triple mutant cells by expressing either *clsA* or *clsB*, but not by *clsC1*. To restore the CL biosynthesis by *clsC1* requires the co-expression of its neighboring gene, *ymdB* (renamed as *clsC2*). The mechanism of CL synthesis by *clsC1C2* remains to be elucidated. Unlike *clsA* and *clsB,* which synthesize CL by condensing two PG molecules, *clsC1C2* seems to utilize PG and another unidentified substrate as shown from our unique biological system.

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24. Genetic deletion of nitric oxide synthases in a lupus mouse increases deposition of oxidized lipoprotein immune complexes in aortic lesions and elevates plasma sphingosine 1-phosphate levels

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Systemic lupus erythematosus (SLE) patients display impaired endothelial nitric oxide synthase (eNOS) function required for normal vasodilatation. SLE patients express increased compensatory activity of inducible nitric oxide synthase (iNOS) generating excess nitric oxide that may result in inflammation. We examined the effects of genetic deletion of *NOS2* and *NOS3,* encoding iNOS and eNOS respectively, on accelerated vascular disease in MRL/lpr lupus mouse model. *NOS2* and *NOS3* knockout (KO) MRL/lpr mice had higher plasma levels of triglycerides (23% and 35%, respectively), ceramide (45% and 21%, respectively), and sphingosine 1-phosphate (S1P) (21%) compared to counterpart MRL/lpr controls. Plasma levels of the anti-inflammatory cytokine interleukin 10 (IL-10) in *NOS2* and *NOS3* KO MRL/lpr mice were lower (53% and 80%, respectively) than counterpart controls. Nodule-like lesions in the adventitia were detected in aortas from both *NOS2* and *NOS3* KO MRL/lpr mice. Immunohistochemical evaluation of the lesions revealed activated endothelial cells and lipid-laden macrophages (foam cells), elevated sphingosine kinase 1 expression, and oxidized low-density lipoprotein immune complexes (oxLDL-IC). The findings suggest that advanced vascular disease in *NOS2* and *NOS3* KO MRL/lpr mice maybe mediated by increased plasma triglycerides, ceramide and S1P; decreased plasma IL-10; and accumulation of oxLDL-IC in the vessel wall. The results expose possible new targets to mitigate lupus-associated complications.

25. Sphingolipid metabolism: A novel link between obesity and cardiomyopathy

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Ceramide, the metabolic hub of sphingolipid (SL) metabolism, has recently been associated with metabolic syndrome in various model systems. We use *Drosophila* as a model organism to determine whether *direct* perturbation of SL metabolism is sufficient to induce metabolic syndrome. Specifically, our strategy centers on using genetic manipulations to cause accumulation or depletion of various SL intermediates, including ceramide. Using electrospray ionization liquid chromatography tandem mass spectrometry (ESI/LC/MS/MS), we profiled the sphingolipidome of 10 SL mutant fly lines. Our measurements included ceramide, sphingoid bases and their derivatives. Obesity phenotypes were characterized using classic hallmarks of the human disease: increases in body weight, fat storage, adiposity and fat cell hypertrophy. Cardiac phenotypes were characterized using high speed video recordings to generate M-mode traces, which were used to determine multiple heart function parameters. One gene of interest, *infertile crescent* (*ifc*) encodes for a sphingosine Δ-4 desaturase, which catalyzes the conversion of dihydroceramide into ceramide. While mutant ifc^4 flies exhibit a decrease in total ceramides, certain ceramide subspecies were increased. This distribution was sufficient to induce obesity via adipokinetic hormone-producing (Akhp) cell death. These neuroendocrine cells produce Akh, which induces fat mobilization. Akh shares functional homology with both glucagon and β-adrenergic signaling. Akh cell death was rescued by Akhp-cell specific ectopic expression of *inhibitor of apoptosis 1* (*dIAP1*). This suggests that Akhp cell death is occurring through the intrinsic apoptotic pathway. In conjunction with obesity phenotypes, heart function analysis showed that ifc^4 mutants exhibit dilated heart chambers, arrhythmias, dysfunctional diastolic intervals, and loss of fractional shortening: all classic hallmarks of cardiomyopathy. Together, these data show that ceramide accumulation is sufficient to induce classic hallmarks of obesity and cardiomyopathy, establishing an active role for SLs in the onset and/or progression of metabolic syndrome. Furthermore, this establishes a link between multiple aspects of metabolic syndrome via SL metabolism.

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26. DHA supplementation in mice with Alzheimer's amyloid pathology: Effects on brain phospholipids

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Evidence from both epidemiological and animal studies has suggested that dietary docosahexaenoic acid (DHA) may reduce the incidence of Alzheimer's disease (AD). However, the exact mechanisms of action by which DHA may affect the onset of AD remain unclear. As part of a study as to how dietary DHA supplementation might alleviate amyloid pathology and cognitive abnormalities, we examined the incorporation of DHA into phospholipids of different brain regions in a transgenic mouse model of AD (Tg2576). A mouse cohort composed of transgenic Tg2576 mice and their wild type littermates was fed normal chow enriched with either 5% DHA-rich oil or 5% blend oil (as control), from the age of 4 months, which was prior to the onset of AD symptoms. Animals were then sacrificed after 8 and 12 months on the diets. The hippocampus, the cortex and the cerebellum were dissected and the compositions of individual phospholipids in each of these regions examined. Separation by thin-layer chromatography followed by fatty acid analysis by gas liquid chromatography was used to measure changes in fatty acid distribution. In addition, the phospholipid molecular species were analysed using reverse phase liquid chromatography followed by electrospray ionization tandem mass spectrometry (ESI-MS-MS). A significant increase in the amount of DHA was observed in the brains of animals fed with the DHA supplemented diet in all the three brain regions. Although both phosphatidylserine and phosphatidylethanolamine (PE) were the main phospholipids containing DHA, the composition of PE was the most affected by the diets. Further experiments should determine the regulation of this selective metabolism and PE may present an interesting target in the prevention of AD using dietary DHA supplementation.

27. Phospolipases C cause a decrease in phosphatidylinositol (4,5)-bisphosphate levels, potentially causing insulin resistance

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Insulin resistance, a risk factor for type 2 diabetes, occurs when cells fail to respond effectively to insulin. In striated muscle and fat, this manifests as impaired insulin-stimulated glucose uptake due to reduced plasma membrane insertion of the glucose transporter GLUT4. In cell culture models, insulin resistance induced by chronic exposure to insulin is correlated with reduced immunoreactivity of the lipid phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) in plasma membrane sheets. However, the reason for this decrease, and whether other factors that induce insulin resistance affect PtdIns(4,5)P_2, is unknown. Using L6 skeletal muscle myotubes, we have investigated whether PtdIns(4,5)P_2 levels are perturbed in insulin resistance induced by several factors, including exposure to insulin, oxidative stress, and treatment with tumour necrosis factor alpha, endothelin-1 or angiotensin II. All these pre-treatments abolish insulin-stimulated ^{3}H 2-deoxy-glucose uptake, and significantly decrease PtdIns(4,5)P_2 levels, measured in cell extracts by quantitative blotting using a PtdIns(4,5)P_2-specific probe, developed from the PH domain of phospholipase C (PLC) δ. Importantly the ability of insulin to stimulate glucose uptake can be restored by replenishing PtdIns(4,5)P_2. PtdIns(4,5)P_2 levels are regulated by three families of proteins; PIP kinases, which synthesise it, phosphatases, which degrade it, and PLCs, which hydrolyse it. Membrane preparations from insulin resistant cells showed no differences in PtdIns(4,5)P_2 production or dephosphorylation. However a significant increase in PLC activity was detected. Furthermore, studies using PLC inhibitors show a restoration of PtdIns(4,5)P_2 levels in insulin resistant cells. This study shows a causal link between decreased PtdIns(4,5)P_2 levels and insulin resistance in L6 myotubes, and that PLCs are the reason for the decrease. This work suggests that PLCs may be a novel target for combating insulin resistance, and preventing type 2 diabetes.

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28. Impact of 6 month cigarette smoke exposure on the plasma, liver and aorta lipidome of ApoE-/- mice

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Although the relationship between smoking and cardiovascular diseases on one side and lipids and cardiovascular disease on the other side are established, the direct impact of cigarette smoke on lipidomes remains elusive. We therefore investigated for the first time the effect of 6 month smoke exposure on ApoE$^{-/-}$ mouse molecular lipid profiles using an array of lipidomics platforms. Plasma, liver, and aorta samples obtained from ApoE$^{-/-}$ mice exposed either to mainstream smoke of the 3R4F reference research cigarette (CS) or to fresh air were extracted for lipids using a robotic-assisted method and analyzed on six mass spectrometric platforms. Targeted and non-targeted mass spectrometry methods allowed quantification of more than 400 lipid species. CS exposure for six months was shown to significantly elevate the plasma sphingosine-1-phosphate concentration. In addition, the levels of free cholesterol, lysophosphatidylcholine, ceramides, and cerebrosides were increased in plasma. In liver, CS exposure generally elevated several lipid species including free and esterified cholesterol, triacylglycerols, phospholipids, sphingomyelins, and ceramides. In aorta, more than 2-fold higher cholesteryl ester, lysophosphatidylcholine, and glucosyl/galactosylceramide levels could be detected in CS exposed mice compared to mice exposed to fresh air. In addition, the relative proportion of cholesterol esters in aorta increased by 32% out of all lipids quantified. Moreover, CS exposure induced a decrease in plasma concentration of 20:4 and 22:6 polyunsaturated fatty acids in cholesteryl ester and phospholipid species as they accumulated in the aorta. Cigarette smoke exposure was shown to result in generally elevated plasma, liver, and aorta lipid levels except for triacylglycerols. In addition, significant accumulation of specific lipids into aortic vessel wall could be observed. This detailed quantification of lipid molecular species in an animal model for atherosclerosis is a first step towards the identification of novel cardiovascular risk markers that could prove more discriminative than conventional lipid markers.

29. Saturated fatty acid-induced activation of TLR-mediated pro-inflammatory signaling pathways is not due to contaminants in BSA or fatty acid preparation

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Toll-like receptor 4 (TLR4) and TLR2 were shown to be activated by saturated fatty acids (SFAs) but inhibited by docosahexaenoic acid (DHA). However, one report (ATVB 11:1944, 2009) suggested that SFA-induced TLR activation in cell culture systems is due to contaminants in BSA used for conjugating fatty acids. This report casted doubt about proinflammatory effects of SFAs. Our studies herein demonstrate that sodium palmitate (C16:0) or laurate (C12:0) without BSA conjugation induced phosphorylation of IκBα, JNK, ERK, and NFκB p65 and TLR target gene expression in THP1 monocytes or RAW264.7 macrophages, respectively when cultured in low FBS (0.25%) medium. C12:0 induced NFκB activation through TLR2 dimerized with TLR1 or TLR6, and through TLR4. Since BSA was not used in these experiments, contaminants in BSA have no relevance. Unlike suspension cells (THP-1), BSA conjugation is required for C16:0 to induce TLR target gene expression in adherent cells (RAW264.7). BSA-conjugated C16:0 transactivated TLR2 dimerized with TLR1 or TLR6, and through TLR4 as seen with C12:0. These results and additional studies with LPS sequester polymixin B and MyD88-/- macrophages indicated that SFA-induced activation of TLR2 or TLR4 is a fatty acid-specific effect, but not due to contaminants in BSA or fatty acid preparations. *This work supported by USDA-ARS-WHNRC Program Funds and NIHDK 064007.*

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30. Diet macronutrient composition: A determinant of metabolic inflammation, insulin sensitivity and longevity?

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Obesity and type 2 diabetes are global public health crises that threaten the economics of all nations. For several decades, the diet-heart paradigm that high intake of fats increases the risk of atherosclerosis and ischemic heart disease (IHD) has been the driving force behind international dietary recommendations for prevention of IHD. This model, which promotes diets that are typically low in fat and high in carbohydrates, has led to a substantial decline in the percentage of energy intake from total and saturated fats. At the same time, it has spurred a compensatory increase in consumption of refined carbohydrates and added sugars - a dietary shift that may be contributing to the current twin epidemics of obesity and diabetes. The changed landscape in dietary patterns suggests a need to reassess the strategy of replacing total and saturated fats with carbohydrates. We performed a 28-day normocaloric diet intervention in obese men where each meal contained an approximately equal caloric load of proteins, carbohydrates and fats. Time series analyses of blood gene expression revealed a cluster of downregulated genes involved in immunological processes already after 24 hrs. Next, the intake of two normocaloric diets differing in relative macronutrient quantity, 65:15:20 (HC) and 27:30:43 (MC) energy percent (E %) of carbohydrates, proteins and fats, respectively, identified activation of genes for transcription factors NF-□B; STAT3/5; CTNN□, LEF/TCF; and CEBPA on the HC diet, and inhibition of transcription factor NF-□B, proatherogene genes and activation of FOXO3 during the MC diet in blood leukocytes. Moreover, the MC diet improved the atherogenic index, main blood cytokines/adipokines, and HOMA indices significantly. Monocyte mechanistic analyses identified cytokines and insulin as immediate downregulators of FOXO3a expression, a prominent pro-longevity factor with tumor suppression activity and responses to oxidative stress. In conclusion, a reduction in diet carbohydrate quantity, paralleled by increase in fat and protein seem to be important factors in reducing the lifestyle-associated diseases and decreased lifespan.

31. Quantification of platelet activating factors in human plasma and serum utilizing liquid chromatography with isotopic dilution tandem mass spectrometry

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Platelet activating factors (PAF) are bioregulators responsible for mediation of crucial biological systems including regulation of blood pressure, platelet aggregation and reproduction. In addition, disorders and life threatening conditions are in part associated with specific levels of PAF, including hypotension, thrombosis, bronchoconstriction, and systemic shock. When administered at high enough levels PAF has been shown to be lethal in animal models and may result in immediate symptoms with no apparent source. To differentiate between naturally occurring levels in a healthy individual from a person exposed to an external source (e.g. poisoning) of these compounds, a robust and reliable analytical method for quantification of PAF in human plasma and serum is needed. Toward this goal a method to quantitate levels of C_{16} and C_{18} forms of PAF, along with their inactivated lyso forms in human plasma and serum has been developed. A 96 well solid phase extraction high throughput protocol was developed for sample clean-up. After sample processing, optimized separations were carried out utilizing liquid chromatography with isotopic dilution tandem mass spectrometric detection. This method provides a means for monitoring PAF in patients who may have been exposed to dangerous levels of the compounds.

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32. IRE1a-XBP1s induces PDI expression to regulate MTP activity for hepatic VLDL assembly and lipid homeostasis

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The endoplasmic reticulum (ER) is a vital organelle to coordinate multiple synthetic and secretive pathways in the liver. Perturbation of ER homeostasis induces ER stress and causes lipid accumulation in the liver. To maintain ER homeostasis, a complex protective response termed the unfolded protein response (UPR) has evolved. The UPR has also emerged as an essential signaling pathway to regulate hepatic lipid metabolism. However, the physiological role of inositol-requiring transmembrane kinase and endonuclease 1 (IRE1), the most conserved arm in the UPR, is not well defined in hepatocytes. Therefore, to determine the function of IRE1α in hepatocytes, we generated a conditional deletion of *IRE1α* in hepatocytes by transgenic expression of Cre under the control of the albumin promoter. We found that the *IRE1α* hepatocyte-specific null animals were hypocholesterolemic and hypotriglyceridemic accompanied by marginally increased accumulation of triglyceride (TG) in the liver. We also found that *IRE1α* hepatocyte-specific null animals had impaired VLDL-TG secretion with unaltered lipogenesis. Further analysis on lipid contents of cytosol and smooth ER indicates that hepatocyte-specific deletion of *Ire1α* reduces lipid partitioning into the ER lumen to decrease the assembly of triglyceride (TG)-rich VLDL. Through metabolic labeling studies using primary hepatocytes, we further revealed a reduced sER-TG accretion as a key underlying cellular mechanism for defective TG-rich VLDL assembly in *Ire1α*-deleted hepatocytes. Finally, proteomic analysis and genetic complementation approaches demonstrated that this defect in TG-rich VLDL assembly is caused in large part by reduced MTP activity due to decreased expression of PDI. Collectively, our findings reveal a key role for the IRE1α-XBP1s-PDI axis in linking ER homeostasis with regulation of VLDL production and hepatic lipid homeostasis.

33. In mouse dendritic leucocytes, are lipid droplets the organelles which move lipids to the membrane of the parasitophorous vacuoles hosting live *Leishmania* amastigotes?

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While analyzing transcript profiles of mouse bone marrow-derived dendritic leucocytes hosting live *Leishmania (L) amazonensis* amastigotes we noticed unique modulations of genes involved in the metabolism of neutral lipids, *i.e.* the coordinated increase of : i) triacyl-*sn*-glycerol synthesis and storage, ii) long chain fatty acid uptake and transport, and iii) cholesterol uptake and esterification to cholesteryl esters.
Thus, we reasoned that such lipid metabolism modulation could result in lipid droplet/LD biogenesis and dynamics: indeed, though LDs have long been considered as inert organelles, originating from the endoplasmic reticulum/ER of eukaryotic cells to store fatty acids as esterified non-toxic lipid species, the latter have been recognized as a source of membrane building blocks. We showed that LDs were more abundant in dendritic leukocytes harbouring live *Leishmania* amastigotes than in amastigote-free dendritic cells. In addition, these LDs did display intimate contact with the membrane of parasitophorous vacuole/PV where amastigotes reside and may also induced changed PV membrane curvature. These observations lead us to discuss the issue of movement of LD-derived lipids to the amastigote bound to the PV membrane.

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34. Lipid profiling of serum samples from calcific coronary artery disease patients using ultra performance LC and ion mobility TOF MS

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Traditionally, lipids are only associated with cellular roles involving energy storage and used as structural building blocks. Recent developments in lipid research have identified the important role of lipids in modulating cellular signaling and cellular trafficking. Chromatographic separation was performed using Waters ACQUITY UPLC column with charged surface hybrid (CSH C18) chemistry coupled with ion mobility/time-of-flight MS (IMS-TOF/MS^E) for global lipid molecular species profiling from calcific coronary artery disease CCAD serum lipid extract (n= 44) and control (n=26). The chromatographic separation provided superior performance over traditional RP techniques, giving baseline separation of lipids based upon their acyl chain length, number of double bonds and the acidic/basic nature of the compounds. The use of UPLC coupled to ion mobility provides multiple degrees of orthogonal separation, required for the confident identification of lipid species. Data were collected in MS^E and HDMS^E acquisition mode, which allows obtaining parallel low and high collision energy information. The resulting raw data file contains the intact precursor ion at low energy and corresponding product ion information at elevated energy. The low energy accurate mass data is used for initial screening using Waters MarkerLynx application manager. Data processing consisted of peak-picking and grouping, multivariate statistics were applied and potential biomarkers were determined. The elevated energy product ions are used for lipid identification using SimLipid after the data was extracted using Waters MS^E Data Viewer, a software developed for visualization, processing, and interpretation of multi-dimensional MS^E or HDMS^E data. Preliminary principal component analysis results showed there is a separation between the two groups and potential biomarkers that discriminate between the two groups were identified. CCAD is a predictor of myocardial infarction (heart attack) and an early diagnosis using a panel of biomarkers may play an increasing role in cardiovascular disease risk stratification.

35. Mapping lipidomes by coupling HILIC and ion mobility MS^E

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One of the main challenges for a global lipid analysis – lipidomics – is the separation of the wide array of lipid species present in biological samples. The ability to perform multi-dimensional separations in one injection prior to MS analysis could improve our ability to map and measure complex lipid mixtures.
Here, we first applied hydrophilic interaction chromatography (HILIC; BEH HILIC 1.7 µm, 2.1x100 mm) with a reversed phase solvent system (organic/aqueous) characterized by high organic mobile phase (>80% acetonitrile). This UPLC method was highly compatible with ESI and allowed to separate lipids by classes, according to their polar properties. In addition to HILIC chromatography, we used an ion mobility TOF mass spectrometer to further discriminate lipids classes in their constituents based on the different cross collision sections. Lipid ions with different degree of unsaturation and acyl length migrate with characteristic mobility times due to their unique interactions with the nitrogen gas in the ion mobility cell. To gain more structural information, lipids were analyzed using parallel low and elevated collision energy (MS^E) to acquire both precursor and product ion information in a single chromatographic run, increasing the specificity of identification. Using this novel technological approach, we generated molecular maps of lipids present in various animal tissues and serum. After HILIC separation, ion mobility further separates lipid species according to their molecular shapes. The molecular landscape visualized using an unbiased 3D representation (drift time, m/z, intensity) for selected interval of retention times allows the detection of many low abundance molecular species that could go unnoticed otherwise. The comparison of biological samples is also facilitated. In conclusion, the combination of HILIC with ion mobility separation delivers a multi-dimensional separation of complex biological mixtures, enhancing the lipidomic profiling.

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36. Tocotrienol attenuates triglyceride accumulation in HepG2 cells and F344 rats

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Tocotrienol (T3) is an important phytonutrient found in rice bran and palm oil. T3 has gained much interest for lipid lowering effects, especially for cholesterol (Cho) by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR). Also, usefulness of T3 in improving triglyceride (TG) profiles has been suggested, but its efficacy and mechanism have been still unclear. We investigated how T3 decreases TG concentration in cultured cells and animals. In cell culture study, human hepatoma cells (HepG2) were incubated in control or fat (1 mM oleic acid)-loaded medium containing □-T3 for 24 h. We found that 10-15 □M□-T3 inhibited significantly cellular TG accumulation, especially in fat-loaded condition. This manifestation was supported by mRNA and protein expressions of fatty acid synthase (FASN), carnitine palmitoyltransferase 1 (CPT1A), and cytochrome P450 3A4 (CYP3A4). In concordance with these results, rice bran T3 supplementation to F344 rats (5 or 10 mg T3/day/rat) receiving high fat diet for 3 weeks significantly reduced TG and oxidative stress marker (phospholipid hydroperoxides, PLOOH) in the liver and blood plasma. T3 supplementation did not show changes in Cho level. These results provided new information and mechanism of TG-lowering effect of T3. The lipid lowering effects of dietary T3 might be mediated by the reduction of TG synthesis.

37. A software tool for automated structural characterization of glycerophospholipids using raw mass spectra from nLC-ESI-MS-MS

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Lipidomics is drawing a great attention with the development of mass spectrometry-related techniques. However, conventional analysis of CID spectra of lipid classes is manually processed and severely laborious. Although various sophisticated algorithms are currently used in proteomics field for facile comprehension of raw mass spectra, programmed analysis of mass spectra of lipidomes has a lot to be developed. A structural analysis tool (LiPilot) for the identification of phospholipids (PLs) including lysophospholipids (LPLs) and cardiolipins (CLs) from mass spectra is introduced. This algorithm utilize raw mass spectra generated from nanoflow liquid chromatography-electrospray ionization-tandem mass spectrometry (nLC-ESI-MS-MS) experiments accompanying both retention time and m/z values of precursor and fragment ions generated from data–dependent, collision-induced dissociation(CID). In addition, library files addressing the typical fragmentation patterns of PLs from an LTQ-Velos ion trap mass spectrometer to characterize PL or LPL molecular species by comparing the experimental fragment ions with theoretical fragment ions in the library file were implemented. Identification is processed by the calculation of a confidence score newly developed in our laboratory to maximize identification efficiency which encloses the effect of total peak intensities of matched and unmatched fragment ions, the difference in m/z values between observed and theoretical fragment ions, and a weighting factor to differentiate regioisomers. The software was validated using a mixture of 24 PL and LPL standards and was further evaluated with a human urinary PL mixture sample, which yielded the identification of 93 PLs and 22 LPLs and 3 CLs.

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38. Effect of ionization modifiers on the simultaneous analysis of all classes of phospholipids by nanoflow liquid chromatography/tandem mass spectrometry in negative ion mode

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In this study, the utility of a mixed modifier in nanoflow liquid chromatography-tandem mass spectrometry (nLC-ESI-MS^3) for the simultaneous analysis of all classes of phospholipids (PLs) at negative ion mode has been investigated. While MS analysis of most PL classes is carried out in negative ion mode, analysis of neutral polar (polar but electrically neutral) lipids like phosphatidylcholine (PC) and sphingomyelin (SM) is highly efficient in positive ion mode. Therefore, analysis of PL mixture samples often requires two separate runs in both positive and negative ion mode. In order to establish run conditions to carry out a single nLC-ESI-MS-MS for all PLs, the ionization efficiency of 13 different types of PL molecules in nLC-ESI-MS has been evaluated in negative ion mode by varying the modifiers and their concentrations. Experiments demonstrated that a mixed modifier (0.05% ammonium hydroxide plus 1 mM ammonium formate) was found to be effective in the analysis of PL mixtures without losing ionization efficiency. The optimized conditions were applied to the analysis of a phospholipid mixture extracted from a human urine sample, yielding the identification of a total of 85 PL species. Analysis of the same sample with dual nLC-ESI-MS^2 runs in both positive and negative ion mode confirmed that nLC-ESI-MS^3 with the mixed modifier run only in negative ion mode gave comparable results.

39. Top-down phospholipid analysis for low density lipoproteins with on-line chip-type flow field-flow fractionation/ESI-MS-MS

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A chip-type asymmetrical flow field-flow fractionation (AF4) channel has been hyphenated with electrospray ionization mass spectrometry (ESI-MS) for high speed separation of intact low-density lipoproteins (LDLs) and proteins in human serum and for the top-down analysis of phospholipids. AF4 is an elution technique for the separation of macromolecules or particulate species and has gained increasing interest as a size fractionation method for large biomolecules such as proteins, biomolecules, nano to micron sized biological particles, and cells. The new miniaturized AF4 channel was assembled by stacking multilayered thin stainless steel (SS, 1.5 mm each) plates embedded with an SS frit in such a way that the total thickness of the channel was about 6 mm. The use of volatile aqueous buffer and desalting or purification of LDL particles achieved during the AF4 operation by the action of a crossflow were useful for on-line coupling of the chip-type AF4 with ESI-MS. The direct analysis of LDL particles by AF4-ESI-MS exhibited reduced background signals and sodium adducts compared to that of direct infusion ESI-MS analysis. In this study, phospholipids (PLs), lyso-phospholipids (LPLs), cholesterol esters and triacylglycerols (TAGs) having various lengths of fatty acids were simultaneously analyzed from LDL particles with collision-induced dissociation (CID) experiments during AF4-ESI-MS-MS, resulting in identifications of a total of 13 PLs, 7 LPLs, 6 cholesterol esters and 13 TAGs.

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40. A chemical biology/mass spectrometry based approach to study isoprenoid diphosphate metabolism in live cells *

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Isoprenoid diphosphates are critical intermediates in the mevalonate pathway for synthesis of cholesterol and other essential metabolites. Two of these molecules, farnesol diphosphate (FPP) and geranylgeranyl diphosphate (GGPP) are substrates for enzymes that attach the respective prenyl groups to cysteine residues at the C-terminus of a wide variety of proteins. HMG CoA reductase inhibitors (statins) inhibit the synthesis of FPP and GGPP which may account for beneficial effects of these drugs in cardiovascular, neurological disease and cancer that are not directly related to their cholesterol lowering actions. Despite their biological and clinical importance, relatively little is known about mechanisms regulating intracellular levels of isoprenoid diphosphates. In large part, this is because their low levels and chemical properties impedes analysis by electrospray ionization tandem mass spectrometry. We present an approach using analogs of farnesol and geranylgeraniol with chemical properties that enable their detection by this technique to study isoprenoid diphosphate metabolism and protein isoprenylation in live cells. By using stable isotope labeled derivatives of the diphosphate and cysteine thioether metabolites of these probes as standards for quantitation of these intermediates we demonstrate that our isoprenol probes are variably effective precursors for protein isoprenylation in a variety of cell types. Although the mevalonate pathway synthesizes FPP and GGPP without producing the corresponding isoprenols, farnesol and geranylgeraniol are readily detectable in cells and biological fluids where they have been suggested to be formed by enzymatic dephosphorylation of FPP and GGPP. We have used our approach to demonstrate that certain members of the Phosphatidic Acid Phosphatase Domain Containing (PPAPDC) class of integral membrane lipid phosphatases are isoprenoid diphosphate-preferring enzymes that regulate production of mevaolonate pathway end products and protein isoprenylation. Taken together, our findings demonstrate an effective strategy to monitor isoprenoid phosphate metabolism and protein isoprenylation in live cells and identify a pathway for interconversion of isoprenols and isoprenoid diphosphates.

41. Structural determination of estradiol product ions

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Steroid hormones are important regulatory lipids in human physiology and disease; they are synthesized in various tissues, distributed throughout the entire body and finally metabolized into inactive molecules. Estradiol (E2) functions through estrogen receptors in cells as a growth hormone for female reproductive tissues as well as oocyte maintenance in ovaries and is required for optimal spermatogenesis. Several different approaches have been utilized in the quantitation of E2 and E2-metabolites but the sensitivity and specificity of such methods has been limited. Mass spectrometry based techniques (LC-MS/MS) would offer significant advantages in being able to measure E2, E2-metabolites as well as other estrogens in the same sample for a global analysis in biological fluids. Full scan mass spectrometry experiments revealed E2 ionizes with the production of an abundant $[M-H]^-$ ion m/z 271. Collision induced dissociation (MS^2) of m/z 271 resulted in several product ions including abundant m/z 145 and m/z 183 followed by m/z 143, m/z 171 and m/z 239, respectively. MS^3 and Precursor Ion experiments on a triple-quadrupole instrument were used to determine whether these ions were structurally related. Preliminary data indicates the two most abundant ions, m/z 145 and 183, are not structurally related in that m/z 183 did not fragment to yield m/z 145. High resolution mass spectrometry on a Fourier Transform Mass Spectrometer (FT-LTQ) was used to further investigate the m/z 183 product ion since it had been clearly misidentified in a previously reported study. ^{13}C and ^{2}H labeled E2 were employed to confirm the decomposition mechanisms we have hypothesized to account for the observed product ions. Understanding the product ions of E2 will facilitate the identification of unknown human metabolites and provide critical information to support translational studies of the role of steroid hormones in health and disease.

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42. Lung cancer progression results in a complex pattern of eicosanoids which is dependent on cPLA2 expression in the tumor microenvironment

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In cancer, cPLA2-derived eicosanoids play a complex role, either promoting or inhibiting cancer development. Eicosanoids are produced both by tumor cells, as well as cells of the tumor microenvironment (TME). Using an orthotopic immunocompetent mouse model, in which tumor cells are injected into lungs of mice, we found that loss of cPLA2 in the TME inhibited lung cancer metastasis. These effects are presumably due to altered production of specific eicosanoids critical for tumor progression and metastasis. In this study we used liquid chromatography/tandem mass spectrometry (LC/MS/MS) to define the spectrum of eicosanoids produced in tumor bearing lungs during progression, and to identify differences in tumors grown in cPLA2 knockout mice compared to controls. Lewis Lung Carcinoma cells, derived from a lung adenocarcinoma in a C57BL/6 mouse, were injected into the left lung lobes of syngeneic wild type (WT) or cPLA2 knockout (KO) mice. At different times after injection whole left lung lobes containing tumors were harvested, along with left lobes from uninjected WT and cPLA2-KO mice. Eicosanoids extracted from the harvested lungs were analyzed by LC/MS/MS. Eicosanoid levels in uninjected WT or KO mice were not significantly different. During tumor progression we found a time-dependent induction of multiple products of both the cyclooxygenase and lipoxygenase pathways. These included PGE2, LTC4, LTD4, LTE4, 12-HETE, 15-HETE and 5-HETE. For most of these products, the induction seen in tumors growing in $cPLA_2$ KO mice was inhibited. Since tumor cells were identical in the two groups of mice, we conclude that the TME makes a critical contribution to eicosanoid production. While individual eicosanoids have been previously implicated in cancer, this is the first systematic examination of the spectrum of these molecules produced during lung cancer progression. Defining the role of these compounds may lead to better strategies using pharmacological inhibitors of specific eicosanoids as therapeutic agents in lung cancer.

43. Microwave-assisted: Fast and accurate sample preparation technique for high-throughput lipidomics

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Lipids are a class of biomolecules that play important roles in cells such as energy storage, cellular support and signal transduction. The progress on lipid research is mainly driven by the development of analytical techniques by chromatography and mass spectrometric techniques. The work presented here is a new approach using modification of conventional microwave technique for rapid high-throughput sample preparation of lipid extraction and preparation of total fatty acid methyl esters. The work also describes several development/optimization of analytical techniques such as GC-MS, HPLC-CAD for global analysis of all lipid classes in mouse tissues. Sample preparation using microwave-assisted technique is significantly faster than the conventional method (at least 24 times) and shows comparable results with the conventional method. By using this new approach we are able to increase the number of sample preparations up to several hundreds per day and this facilitates large-scale studies.

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44. Investigating the effects of cholesterol and bupivacaine on the degree of lipid peroxidation in the phospholipid bilayer membrane system

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Membranes, in the form of phospholipid bilayers, are vital part of all forms of life. Lipid peroxidation in membranes plays a central role in many pathologic processes, including cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, type II diabetes, and other chronic inflammatory diseases. Lipid peroxidation is of great importance because it modifies the structural and dynamic properties of the membranes, which in turn, influences the membranes' function. One remarkable feature of all biological membranes is their dynamic properties or flexibility (fluidity). This study is focused on examining the dynamic properties of intact, as well as, oxidized model membrane system utilizing Fluorescence Polarization (FP) Spectroscopy. The study shows that lipid peroxidation decreases the fluidity of the phospholipid bilayers in the membrane system. The effects of cholesterol on the dynamic properties of phospholipid bilayers are examined. Cholesterol decreases the fluidity of the phospholipid bilayers in the liquid crystalline phase. In addition, the data suggests that a high concentration of cholesterol in the phospholipid bilayers decreases the degree of lipid peroxidation. The interaction of the local anesthetic (Bupivacaine) with the membrane system is also studied. The attained results indicate that the interaction of Bupivacaine with the phospholipid bilayers and its penetration across the membrane are decreased when the membrane is subjected to lipid peroxidation.

45. Quantitative yeast lipidomics via LC-MS profiling using the Q Exactive Orbitrap mass spectrometer

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Mitochondrial lipids from wild-type (WT) yeast (*S. cerevisiae*) and a knockout (KO) strain that does not produce coenzyme Q (CoQ) were profiled by liquid chromatography mass spectrometry (LC-MS) using a benchtop quadrupole – Orbitrap mass spectrometer (Q Exactive Orbitrap, 1). Mitochondria were isolated from WT and KO yeast, and mitochondrial lipids were extracted in triplicate with cold isopropanol. The LC-MS data was obtained at 70,000 mass resolving power in positive ion mode using a previously published LC method (2). Extracted ion chromatograms corresponding to high mass accuracy monoisotopic spectral components and the corresponding adducts, dimers, and isotopes were compared from aligned LC-MS chromatograms using SIEVE 2.0 software. Using the ChemSpider database search for metabolite entries from KEGG,HMDB, and LipidMaps), we tentatively assigned observed ions to known lipid species based on elemental composition at a 0.0015 m/z tolerance. Statistical analysis of differences in the abundance of individual lipids from WT and KO yeast revealed significant changes in multiple lipid species, including coenzyme Q as well as a variety of phospholipids, sphingolipids, and glycerolipids from this complex mixture. Our results also demonstrated that the novel configuration of the Q Exactive instrument using SIM mode analysis increased sensitivity for detection of the reduced form of coenzyme Q6 in a targeted assay.

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2. *Hu, C.; van Dommelen, J.; van der Heijden, R.; Spijksma, G.; Reijmers, T.H.; Wang, M.; Slee, E.; Lu, X.; Xu, G.; van der Greef, J.; Hankemeier, T.J. Proteome Res. 2008, 7, 4982–4991.*

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46. Metabolite profiling of bone marrow and peripheral biofluids from acute lymphoblastic leukemia patients before and after induction therapy

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Childhood cancer is the leading cause of disease-related death in children, with the most common childhood cancer being leukemia. Over the past decades, there has been an improvement in the survival of children affected by acute lymphoblastic leukemia (ALL); however, a substantial number of patients do not respond to therapy. Altered cellular metabolism is a hallmark of cancer, and it likely plays a key role in ALL by promoting tumorigenesis, sustaining tumor growth and favoring cancer drug resistance. Thus, monitoring cell metabolism at diagnosis and during treatment is extremely valuable to investigate cellular response, treatment efficacy and to design personalized therapy regimens for ALL patients. Bone marrow and peripheral serum are morphologically very different niches in which oxygen tension plays a pivotal role on the metabolism of both normal and cancer cells. For hematological malignancies like ALL, the difference between these two niches becomes even larger considering that, at the time of diagnosis the bone marrow is highly populated by tumor cells. Therefore, a metabolic characterization of bone marrow and peripheral serum samples might help to delineate mechanisms which regulate ALL cell metabolism. Here, we have employed two state-of-art analytical platforms, high- resolution magnetic resonance spectroscopy and gas chromatography-mass spectrometry to compare the metabolic differences between bone marrow aspirate and peripheral serum samples from the same ALL patients. The patients were studied prior to chemotherapy, during and at the end of the induction phase of treatment, in disease remission. Using an untargeted metabolic analysis, we have identified and quantified over 100 metabolites and obtained a significant metabolic signature of two different microenvironments. Hierarchical clustering analysis of Pearson's correlation coefficients for pairs of metabolites quantified in both individual biofluids was followed by analysis using a genome-scale, *in silico* model of the human metabolic network, revealed distinct metabolic signatures.

47. Plasma phosphatidylcholine turnover is increased in preterm human infants *

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The adaptation at birth from maternal supply of preformed PUFA to their synthesis from diet is arguably the greatest lifetime change in lipid metabolism. Infants delivered very preterm before the major period of PUFA supply via the placenta in late gestation are compromised in respect of lipid as well as other nutrition. How lipid metabolic activity in the livers of preterm infants adapts to and copes with this change is not known. Consequently, we measured plasma phospholipid compositions, synthesis and turnover within the first week of life in 27 preterm infants delivered <28 weeks gestation as a surrogate for liver PC metabolism. *Methyl*-9[^{2}H]choline (3.6mg/kg body wt) was infused over 3h and blood sampled at intervals over the following five days. Total plasma lipid extracts were analysed by flow injection on a Xevo triple quadruople mass spectrometer; endogenous phosphatidylcholine (PC) molecular species were quantified by P184 scans, incorporation by the CDP:choline pathway by P193 scans and incorporation by the phosphatidylethanolamine-*N*-methyltransferase (PEMT) pathway by P187 scans. Endogenous PC exhibited the expected progressive loss of PUFA-containing species. Rate of incorporation and turnover by the CDP:choline pathway was variable (0.022-0.195%/h total PC) and high compared to healthy adult volunteers, while degradation rate inversely correlated with gestational age ($r=-0.548$, $P<0.01$). PEMT activity was consistently low. The molecular specificity of characteristic of plasma PC synthesis in adults was different in these preterm infants, with the pattern of incorporation by CDP:choline reflecting endogenous composition at all time points while PEMT activity still generated PUFA –containing species. This study demonstrates for the first time that PC synthesis is highly active in livers of preterm infants at birth and that, in the first days of postnatal life and especially for the most preterm infants, lipid nutrition was inadequate to cope with their high lipid metabolic demand.

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48. Targeted serum lipidomics by ultra performance liquid chromatography coupled with tandem mass spectrometer

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Phospholipids, such as lysophosphotidylcholine (LPC), phosphotidylcholine (PC), Lysophosphatidyl-ethanolamine (LPE), phosphatidylethanolamine (PE), and sphingosine phospholipid are involved in a variety of human biological processes including cell proliferation, tumor cell invasiveness, and inflammation. Also, they have been identified as potential disease biomarkers in previous studies. The aim of our study is to establish a fast and accurate method for absolute quantitation of phospholipids using isotope dilution by selected reaction monitoring (SRM). The method will be useful for validation of phospholipids as disease biomarkers. Isotope labeled internal standard D31_LPC 16:0, D35_LPC 18:0, D54_PE 14:0, D7_S-1-P were spiked in 50 µL human serum during sample preparation. Waters ACQUITY UPLC was used to separate lipid extracts through both regular ACQUITY C18 and ACQUITY CSH C18 columns, and the results were compared. Chromatographic results showed that charged surface in CSH C18 column provides better peak resolution toward selected phospholipids than regular C18 column. Structural characterization and ID verification were performed by acquiring MS/MS of fragmentation patterns and comparing their retention time with those from authentic compounds. These were accomplished by enhanced product ion (EPI) scanning initiated by precursor ion (PI), neutral loss (NL), or SRM using the QTRAP 4000 mass spectrometer (AB Sciex). For example, PI-EPI scanning of LPC/PC can be performed based on fragment ion m/z 184 (+). Also, NL-EPI scanning of LPE/PE can be performed based on 141 Da. The concentrations of S-1-P, LPC 15:0, LPC 16:0, LPC 17:0, LPC 22:6, LPE 18:0, LPE 19:0, LPE 20:0, LPE 20:4 were determined by SRM on both QTRAP and Xevo TQ (Waters). Both mass spectrometers have dynamic range with four orders of magnitude (ranging from 10 fmol/µL to 100 pmol/µL) and the LODs are at fmol level. EPI feature in QTRAP has better sensitivity and reproducibility in acquiring MS/MS spectrum for target ID verification than Xevo TQ.

49. Genetic determinants of 24(*S*)-hydroxycholesterol levels

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Cholesterol metabolites are synthesized by mammalian cells and are known to play important physiological roles in the liver, brain, and immune system. Mutations in several genes encoding enzymes that metabolize cholesterol have been identified and clinical consequences of these mutations described. For example, the metabolite 24-hydroxycholesterol is produced in the brain by cholesterol 24-hydroxylase (encoded by the CYP46A1 gene). Disruption of this gene in the mouse causes severe learning defects, but it is not known whether mutations in the human gene cause the same phenotype. In order to investigate the phenotype arising from mutations in the human *CYP46A1* gene and to detect mutations in other cholesterol-specific genes, we developed mass-spectrometry based methods to measure >60 different sterol metabolites in small volumes (200 µl) of human serum. We applied these methods to quantify sterols in 3,230 serum samples derived from clinically well-characterized subjects participating in the Dallas Heart Study. Large intra-individual variation was detected in the serum levels of approximately 22 of the >60 sterols assessed. To identify the genes underlying the observed variation, we took both targeted and untargeted approaches. In the targeted approach we sequenced the corresponding genes in subjects with very high or low levels of the sterol in question. In the untargeted approach, subjects were genotyped for 9,229 non-synonymous (amino acid-changing) variations (SNPs) in multiple human genes. Direct sequencing of the oxysterol 7α-hydroxylase gene (*CYP39A1*), which uses 24-hydroxycholesterol as a substrate, identified several mutations that decrease enzymatic activity and thus lead to increased serum levels of this sterol substrate. In the genetic linkage analysis, a SNP in *CYP39A1* was strongly ($P=2.1x\ 10^{-27}$) correlated with serum 24-hydroxycholesterol levels. Expression analysis indicated that this SNP also decreased CYP39A1 enzyme activity. Together, these results illustrate that our approach has the ability to identify genetic determinants of serum sterol levels.

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50. A simple method for isolation and identification of bioactive lipids having monoester type phosphate using phosphate capture molecule, Phos-tag

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Generally, phospholipid, either glycero type or sphingo ones, has diester type of phosphoric acid occupied both sides with alcohol moieties, and constitutes cell membrane as major components. In contrast, phosphatidic acid (PA) and ceramide 1-phosphate (C1P), and their lyso types, lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P), all have monoester type of phosphoric acid. Although their amounts in a body are quite minute, all these monoester type of phospholipids are biologically active. Zinc coordinated complex, Phos-tag, was synthesized by referring the configuration of zinc metals in the active site of alkaline phosphatase. This reagent can capture phosphoric compounds in specific and dose-dependent manners. Using Phos-tag, we reported a simple method to isolate trace amounts of LPA and S1P by phase partition. In extension, we applied immobilized Phos-tag, which was chemically coupled with Sepharose resin, to isolate PA and C1P by solvent elution. MALDI-TOF MS is simple to handle and can analyze many samples in a short time, but a drawback of MALDI ionization is to use matrix. The advantage to use Phos-tag is that the adduct has high mass range over m/z 1000, where is low in background noise due to matrix. Furthermore, by using monoisotopic zinc, single molecular ion due to respective molecular species is detected in positive mode with no production of other cation adducts. Using Phos-tag both at isolation and identification steps, a simultaneous analysis of bioactive lipids having monoester type phosphate is being developed.

51. Assessing metabolite production capabilities in adipose tissue

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Models of the human metabolic network have been studied for years to provide a mechanistic background for different phenotypes and disease states. This study serves to use a constraints-based modeling framework to create tissue specific models pertaining to different patient cohorts in a thiazolidinedione (TZD) study. The previous study obtained adipose tissue samples from patients who underwent TZD treatment in order to decrease their insulin resistance. Expression data was studied through differential analysis and pathway mapping. This study finds a mechanistic basis for insulin resistance through mapping expression data onto a metabolic network and assessing model capabilities for producing different metabolites. Insulin sensitive subjects are shown to be better capable of producing lipids, TCA cycle products, and triglyceride whereas insulin resistant subjects are shown to produce more inflammatory factors and reactive oxygen species. These literature-validated results correspond to specific flux states of adipose tissue metabolism that are explored in detail. In addition, metabolite production capabilities of the adipose tissue are explored after TZD drug treatment, presenting a mechanistic understanding of TZD effect.

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52. Oxysterols direct immune cell migration via EBI2

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We and others have recently identified 7α, 25-dihydroxycholesterol (7α, 25-OHC) as a potent and selective agonist of EBI2[1,2] (Epstein-Barr virus induced gene 2). The EBI2 receptor is a G protein-coupled receptor that is required for humoral immune responses and has been linked to autoimmune disease. Functional activation of EBI2 by 7α, 25-OHC and closely related oxysterols was verified by monitoring second messenger readouts and saturable, high affinity radioligand binding. Furthermore we find that 7α, 25-OHC and closely related oxysterols act as chemoattractants for immune cells expressing EBI2 by directing cell migration *in vitro* and *in vivo*. A key enzyme required for the generation of 7α, 25-OHC is cholesterol 25-hydroxylase (Ch25h)[1]. Similar to EBI2 receptor knockout mice, mice deficient in Ch25h fail to position activated B cells within the spleen to the outer follicle and mount a reduced plasma cell response after an immune challenge. In order to delineate the physiological function of the oxysterol / EBI2 pathway we try to determine the sites of oxysterol production. In addition, we have isolated tool compounds which can be used to address the role of the oxysterol/EBI2 pathway in health and disease.

1. Hannedouche et al. Nature 475(2011)524 / [2] Liu et al. Nature 475(2011)519

53. Fiber type specific distribution of lipid droplets in skeletal muscles of inbred Berlin fat mice (BFMI) lines

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Skeletal muscle plays an essential role in glucose homeostasis and elevated intramyocellular lipid (IMCL) accumulation directly correlates with insulin resistance in skeletal muscle in metabolic disorders. This study aims to quantify the lipid droplet (LD) content in the longissimus and quadriceps muscles of standard and high fat diet fed control (DBA/J2) and two inbred obese (BFMI860, BFMI861) mouse lines using confocal microscopy. Serial muscle cross sections were stained for myosin heavy chain type I, IIa and IMCL, and viewed with confocal microscopy. A new neutral lipid stain, namely LD540, was used for staining IMCL.
The results revealed a fiber type specific accumulation of LDs in both skeletal muscles, where more total lipid content was observed in IIa fibers. The lipid content expressed as area fraction was found to be higher in both BFMI lines in comparison to the control. Furthermore, LD content was increased in high fat diet fed groups in longissimus muscles, whereas no significant difference was observed between the groups in quadriceps muscle. BFMI860 line which has higher body fat content and a slower clearance of glucose from blood upon injection of insulin was found to has the highest lipid content in longissimus muscle.

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54. Obesity induced alterations on structure and content of lipids in adipose and skeletal muscle tissues in inbred obese mouse models: a novel ATR-FTIR study

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Obesity is a metabolic disorder resulting in elevated levels of free fatty acids and triglycerides in the blood circulation together with accumulation of lipids within various tissues. The Berlin fat mouse inbred line (BFMI) is an important new model for obesity which has a complex genetic background and generates spontaneous obesity, even on standard breeding diet. In this current study, attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy was used to characterize content and structure of lipids of control and BFMI mice lines (BFMI852, BFMI856, BFMI860,BFMI861) in inguinal (IF) and gonadal fat (GF) adipose tissues, and longissimus dorsi (LD) and quadriceps (Q) skeletal muscles. FTIR spectroscopy is a rapid and effective technique in the determination of macromolecular alterations in biological tissue components, simultaneously. Differences in saturated and unsaturated lipid content, in the amount of methyl groups in lipids and triglyceride were determined in between the lines. A significant decrease was observed in unsaturated lipid content and unsaturated/saturated, unsaturated/total lipid ratio in BFMI852, BFMI860, BFMI861 in IF, and in BFMI860, BFMI861 lines in Q and LD, indicating an increased lipid peroxidation. Moreover, there was a significant increase in CH_3 anti-symmetric band area and CH_3 anti-symmetric/total lipid ratio in BFMI860 line in Q muscle, which reveals an increase in the amount of shorter acyl chains. Although triglyceride content did not change significantly in adipose tissues of all BFMI lines in comparison to control, in LD skeletal muscle there was a significant increase in triglyceride content of all BFMI lines, which is the best predictor of insulin resistance during obesity. BFMI860 is thought to be the most affected line according to the changes in lipid structure and function. The amounts of major adipokines released from adipose tissues are under investigation in order to determine the presence of inflammation in these lines due to obesity.

55. Alternative arachidonic acid cascade in the brain *

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Studies using phospholipase $A_2\alpha$ ($cPLA_2\alpha$)-deficient mice have shown that $cPLA_2\alpha$ plays a central role in arachidonate lipid mediator production during inflammation, in immune systems, and also in physiologies including female reproduction and neuronal function. However, in the brain, bulk eicosanoid levels were not much affected in the absence of $cPLA_2\alpha$, suggesting an alternative arachidonic acid source. In this study, we demonstrate that 2-arachidonoylglecerol (2-AG) is the major arachidonic acid source for the brain eicosanoids. We discuss the property of this pathway and its physiological relevance.

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56. Role of lysophosphatidic acid signaling during liver regeneration and hepatocellular carcinoma (HCC) progression

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Previous reports identify a central role for guanine nucleotide regulatory proteins (G-proteins) during liver regeneration and hepatocellular carcinoma (HCC) progression. However, identification of putative G-protein coupled receptors (GPCRs) associated with this G-protein signaling remains elusive. Lysophosphatidic acid receptors (LPARs) are GPCRs implicated in a wide range of disease pathologies, including cancer. Novel LPAR isoforms have recently been characterized, the function of which in hepatic biology is unknown. The aims of the current study was to determine expression and function of LPARs in hepatocyte repopulation and/or HCC proliferation. Mouse models of PHx and HCC (neonatal diethylnitrosamine) were established from which hepatic RNA and protein was analyzed by qRT-PCR, Western blot, and immunohistochemistry (IHC). The effect of LPA on hepatoma cell (Huh7) proliferation and motility was analyzed in vitro using CyQuant and chamber slide assays. Analysis of resected liver showed virtually undetectable LPAR 1-5 mRNAs in normal hepatic tissue, but an abundance of LPAR6 mRNA. Post-PHx, LPAR1/6 mRNA expression was significantly upregulated. A biphasic pattern of mRNA expression was observed for LPAR1, initial increases occurring 6h post-PHx, followed by further induction at 24h, before returning to baseline (48h). LPAR6 mRNA was increased (20 fold vs. control) 24h post-PHx, with LPAR6 protein expression being significantly increased at 48h. Dramatic increases in LPAR1/6 were also measured in HCC vs. NTL tissue by qRT-PCR and IHC. Addition of LPA (0-10uM) significantly increased Huh7 proliferation and migration in a dose-dependent manner compared to untreated cells. Regenerating liver tissue is associated with significant, temporal increases in LPAR 1 and 6 expression, effects that are pronounced and sustained in HCC (compared to NTL). Addition of LPA to HCC cells in vitro stimulated cell proliferation and migration. These data imply a conserved role for LPA signaling in normal and transformed hepatocyte proliferation.

57. Transcriptomic and lipidomic data analysis of macrophages stimulated with KLA/ATP reveals enhanced role of STATs in apoptosis, immunity and lipid metabolism

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Macrophage activation with Lipopolysacharide (LPS) and Adenosine triphosphate (ATP) has long been studied because of its importance in bacterial infection and subsequent immune response. Earlier studies on macrophages elucidated the biological roles of caspase-1 in activation of interleukin (IL)-1β and IL-18 in inflammation. However, the results from these studies focused only on a small number of factors. In order to study macrophages' role in inflammation and immunity in a global scale, we have performed and analyzed the transcriptomic and lipidomic data of Kdo_2-Lipid A (KLA) primed macrophages stimulated with ATP. We have utilized statistical tools such as CyberT for paired/unpaired regularized t-tests and functional enrichment analysis using Ontology, Pathways and TRANSFAC. The analysis suggests that treating macrophages with KLA and ATP produced "synergistic" effects that were not seen with single treatment of KLA or ATP. The time-dependent synergistic regulation of different signaling pathways and biological processes in the KLA/ATP treatment was observed. In particular, both KLA and ATP influenced NFκB and AP1 to produce synergistic regulations at signaling level. At the transcriptional level, this synergistic effect was observed as early as from 1hr. As a result, target chemokines and cytokines of NFκB and AP1 showed synergistic regulation for most of the time points. The synergistic regulation of these cytokines and chemokines then induced a similar regulation on cytokine-cytokine receptor pathway from 30 minutes onwards and on JAK-STAT pathway from 1hr onwards. From the analysis, Signal Transducer and Activator of Transcription (STAT)s were shown to regulate many important genes that can lead to increased apoptosis and impaired cell cycle progression. Lipid metabolites such as eicosanoids, triglycerides, and ceramides had higher concentrations in the KLA/ATP treatment than in the KLA or the ATP treatment and were consistent with the transcriptional regulation. Many of the synergistically regulated genes that influence lipid metabolism were regulated by STATs.

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58. Lysophospholipid-based strategies for mitigation of high-dose radiation injury *in vivo*

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Rx100 is a small molecule metabolically-stabilized agonist of the lysophosphatidic acid receptors. To test the effect of Rx100 in radiation injury models the C57BL/6 Mouse abdominal (ABD) GI-ARS model with chest, head and legs shielded ($LD_{50/8\text{-}12}$ ~ 15 Gy, ^{137}Cs ~260 cGy/min) and C57BL/6 Mouse TBI model ($LD_{80/30}$ dose 6.53 Gy, ^{137}Cs at ~320 cGy/min) were applied. Efficacy testing was done using single dose Rx100 treatment administered at +24, 48, and 72 hour postirradiation. PK and efficacy was also determined in Macaca mulatta (+/- irradiation, LINAC, 11.5 – 12.5 Gy TBI with 5% BM shielding). Multiple biomarkers and histological parameters were analyzed. A single dose of Rx100 administered up to 72 h after irradiation increased survival by a ≥40% in C57/BL6 exposed to $LD_{80/30}$ TBI. Rx100 treatment improved white blood cell and platelet counts. In the ABD GI-ARS model, Rx100 increased survival by ≥40% and showed a DMF of 1.2. Rx100 treatment increased crypt survival, protected carrier-mediated glucose absorption, and prevented endotoxemia. Rx100 was non-toxic at doses that are 50-100-fold higher than the effective dose. In nonhuman primates (NHP), Rx100 exhibits linear pharmacokinetics following S.Q. administration at doses ranging from 0.3 mg/kg to 3 mg/kg. Rx100 systemic exposure and half-life following S.Q. administration in irradiated NHPs were significantly different when compared to unirradiated NHPs. In NHP Rx100 treatment improved crypt survival and increased survival from 33% in the controls to 87% in the treated group.

59. An LC/MS/MS Method That Resolves Glucosyl- and Galactosylceramide, and Tracks Other Related Sphingolipids

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Gaucher disease is the most common lysosomal storage disorder that is caused by mutations in the enzyme glucocerebrosidase. Enzyme deficiency results in the progressive accumulation of glucosylceramide (GL1) in cells of the monocyte-macrophage lineage that ultimately leads to a variety of clinical symptoms, including splenomegaly, anemia, thrombocytopenia and skeletal complications such as osteoporosis, fractures and bone pain. Enzyme replacement therapy (ERT) with intravenous infusions of glucocerebrosidase has been available for almost two decades and has been shown to address most of the clinical manifestations of Gaucher disease. Substrate reduction therapy (SRT) is an alternative treatment strategy that aims to prevent the accumulation of GL1 through targeted inhibition of the enzyme glucosylceramide synthase. In order to accurately assess the effects of SRT, it's imperative that analytical methods can distinguish glucosylceramide from the structurally related lipid galactosylceramide, which can be very abundant in certain cell types. Here we report the development of a LC/MS method employing two joined HILIC-Silica columns coupled with an API-4000 for MRM-based quantitation of these two molecules. Our results show that we have developed a reliable and quick method to separate galactosyl- from glucosylceramide, allowing us to specifically monitor the effects of SRT both in vitro and in vivo. In addition, the method can be utilized to monitor related sphingolipids in the pathway, such as sulfatide, allowing us an opportunity to study the selectivity of glucosylceramide synthase inhibition. Preliminary results from a variety of cell lines demonstrated a strong correlation between the levels galactosylceramide and sulfatide and transcript levels of their respective biosynthetic enzymes, thus supporting the method's reliability. Future studies will be spent on expanding the repertoire of lipid classes that can be monitored simultaneously.

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60. $C60^{+}$-SIMS imaging: The *in-situ* structural characterization of phosphatidylcholines

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Imaging mass spectrometry (IMS) has been shown to be useful in mapping the distribution of lipids such as phosphatidylcholines which are an essential and major component of biological samples including cells and tissues [1,2]. Secondary ion mass spectrometry (SIMS) – based imaging is particularly advantageous because the high lateral resolution and sensitivity attributed to SIMS allows for the detection of lipid molecules at the nanometer scale and at attomolar concentrations [3,4]. However, the *in situ* identification and structural characterization of phosphatidylcholines remains problematic. Tandem MS by itself does not provide useful structural information as phosphatidylcholines are generally ionized in positive ion mode and these protonated molecular ions do not generate structurally relevant fragment ions. Salt doping is one approach that is used to generate alkali adduct molecular ions [5]. Distinct fragmentation pathways are favored in the tandem MS spectra of these cationized lipid molecular ions. In particular, fragments generated from lithium-cationized species have proven to be informative [6]. In this study, lithiation experiments on lipids were performed in conjunction with secondary ion mass spectrometry (SIMS) in imaging mode. Mammalian tissue sections were treated with lithium trifluoroacetate salt and analyzed on two new high performance time-of-flight SIMS instruments, the $C60^{+}$-SIMS QSTAR XL mass spectrometer and the Ionoptika J105 3D Chemical Imager. Results show that unique fragment ions characteristic of lithium cationized phosphatidylcholine are present in the SIMS spectra and unambiguous peak assignments can be made. This approach enables the direct tissue probing of phosphatidycholines and obviates the requirement for tandem MS in positive ion mode SIMS. In addition, the sensitivity and detection of the intact cationized molecular ions are enhanced.

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