# POSTER SESSIONS

The LIPID MAPS meeting features two poster sessions with 66 posters. Of these, twelve were selected for lightning talks (see program) by the review committee, Susan A. Henry, David W. Russell, and Sarah Spiegel. We received so many high quality, relevant abstracts that making these selections was difficult. The poster presenters are also cross referenced in the attendee list.

1. **Effect of imatinib on nitrite levels in LPS/IFNγ activated RAW 264.7 macrophages**
   *A.Z. Karabay, A. Koc, T. Ozkan, Z. Buyukbingol, A. Sunguroglu, Fugen Aktan (Ankara University, Turkey).*

2. **Separation and quantification of 2-acyl-1-lysophospholipids and 1-acyl-2-lysophospholipids in biological samples by LC-MS/MS**
   *Michiyo Okudaira[#], Asuka Inoue[#], Akira Shuto[#], Keita Nakanaga, Kuniyuki Kano, Kumiko Makide, Daisuke Saigusa, Yoshihisa Tomioka, Junken Aoki (Tohoku University, Japan).*

3. **Impaired inflammatory response in fatty liver dystrophy mice ***
   *Clara Meana, Lucía Peña, Esperanza Esquinas, Carlos Guijas, Gema Lordén, Martín Valdearcos, Jesús Balsinde, María A. Balboa (CIBERDEM, Spain).*

4. **Synthesis of analogs of myo-inositol to enable chemical biology studies**
   *Tanei Ricks, Michael D. Best (University of Tennessee-Knoxville, TN)*.

5. **Integration of lipidomic data and computational modeling for studying sphingolipid metabolism in macrophages**
   *Nathan Chiappa, Edward Botchwey (Georgia Institute of Technology, GA).*

6. **Fatty acid heme dioxygenase and catalase-related hydroperoxide lyase cooperate in linoleate metabolism in *Nostoc punctiforme***
   *Alan R. Brash, Narayan P. Niraula, William E. Boeglin, Zahra Mashhadi (Vanderbilt University, TN).*

7. **Calorie restriction increases insulin sensitivity in skeletal muscle through sphingolipid metabolism**
   *Diana Obanda, Yongmei Yu, Zhong Wang, William Cefalu (Pennington Biomedical Research Center, LA).*

8. **Post-squalene cholesterol biosynthetic pathways ***
   *Matthew A. Mitsche, Jeffrey G. McDonald, Helen H. Hobbs, Jonathan C. Cohen (UT Southwestern Medical Center, TX).*

9. **ABHD4 regulates the metabolism of multiple classes of N-acyl phospholipids in mammalian central nervous systems**
   *Hyeon-Cheol Lee, Gabriel M. Simon, Benjamin F. Cravatt (The Scripps Research Institute, CA).*

10. **Elucidating the mechanism of endocannabinoid metabolism by CYP2J2 epoxygenase in nanodiscs ***
    *Daniel R. McDougle, Daryl D. Meling, Amogh Kambalyal, Aditi Das (University of Illinois Urbana-Champaign, IL).*

11. **Enhanced phospholipid and sphingolipid analysis using ion mobility and UPLC mass spectrometry**
    *Aaron M. Armando, Paul R.S. Baker, Oswald Quehenberger, Edward A. Dennis (University of California San Diego, CA).*

12. **G-protein coupled receptor-dependent phosphorylation of Thr10 induces lamin A disassembly from the nuclear lamina**
    *Lina Khairallah, Giovanni (John) A. Di Battista (McGill University, Montreal, Canada).*

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

13. **Untargeted metabolomics of human ex-plant lung associated with cystic fibrosis**
*Neha Garg, Yan Wei Lim, Douglas Conrad, Forest Rohwer, Pieter C. Dorrestein (University of California San Diego, CA).*

14. **Smith-Lemli-Opitz Syndrome fibroblasts reveal the importance of endogenous cholesterol biosynthesis to the innate immune response**
*Kristin A. Gabor, Christopher A. Wassif, Forbes D. Porter, Michael B. Fessler (National Institute of Environmental Health Sciences, NC).*

15. **Meclizine inhibits mitochondrial respiration through direct targeting of cytosolic phosphoethanolamine metabolism ***
*Vishal M. Gohil, Lin Zhu, Charli D. Baker, Valentin Cracan, Abbas Yaseen, Mohit Jain, Clary B. Clish, Paul S. Brookes, Marica Bakovic, Vamsi K. Mootha (Harvard Medical School, MA).*

16. **Novel mechanism for brain prostanoid level regulation through bile acid receptors**
*Stephan A. Brose, Kendra L. Puig, Svetlana A. Golovko, Colin K. Combs, Mikhail Y. Golovko (University of North Dakota, ND).*

17. **Mapping lipid-dependent functional changes in TRPV1 ion channels expressed in cultured cell lines**
*Marcus D. Collins, Sharona E. Gordon (University of Washington, WA).*

18. **PPAR-pan induced metabolic changes in the liver**
*Zsuzsanna Ament, James A West, Elizabeth Stanley, Julian L Griffin (University of Cambridge, UK).*

19. **Using metabolomics to investigate the induction of non-alcoholic fatty liver disease in a rat model of hepatocellular carcinogenesis ***
*Yajing Chu, Aalim M Weljie, Luigi Atzori, Julian L Griffin (University of Cambridge, UK).*

20. **Assessing de novo lipogenesis in ObOb mice using stable isotopes and lipidomics**
*Francis W.B. Sanders, Zsuzsanna Ament, Priya Singh, Leslie Bluck, Julian L Griffin (Cambridge University, UK).*

21. **Nrp-1+Foxp3- CD4 T cells are a novel subset of T lymphocytes that are induced in aorta during atherosclerosis development**
*Dalia E. Gaddis, Yury Miller, Mary Sorci-Thomas, Catherine C. Hedrick (University of California San Diego, CA).*

22. **Impact of adaptive thermogenesis on systemic lipid and cholesterol handling ***
*Clara John, Anna Worthmann, Alexander Bartelt, Philipp Werner, Julia Schmidt, Nicola Schaltenberg, Markus Fischer, Ludger Scheja, Joerg Heeren (University Medical Center Hamburg-Eppendorf, Germany).*

23. **Changes in inositol-containing sphingolipid metabolism regulates stress response signaling**
*Stephen A. Jesch, Maria L. Gaspar, Susan A. Henry (Cornell University, NY).*

24. **Increased lipid identification coverage for global lipidomics analyses using a benchtop quadrupole-Orbitrap LC-MS/MS system**
*Reiko Kiyonami, David Peake, Junhua Wang, Kevin J. McHale, Josef Ruzicka, Yingying Huang (Thermo Fisher Scientific, CA).*

25. **Novel inhibitors of cytosolic Group IVA phospholipase $A_2$ ($cPLA_2$) ameliorate collagen induced arthritis**
*Berit Johansen, Astrid J. Feuerherm, Mari Sæther (Norwegian University of Science and Technology, Norway).*

26. **Sphingosine 1-phosphate counteracts hepatic insulin-sigaling in vitro and in vivo**
*Susann Fayyaz, Lukasz Japtok, Burkhard Kleuser (University of Potsdam, Germany).*

27. **Nat1 ablation contributes to increased lipolysis and mitochondrial impairment in 3T3-L1 adipocytes**
*Indumathi Chennamsetty, Qi Huang, Ivan Carcamo-Orive, Thomas Quertermous, Joshua W. Knowles (Stanford University, CA).*

28. **Microfluidics lipidomics using a novel integrated mass spectrometry technology**
    *Giuseppe Astarita, Angela Doneanu, Jay Johnson, Jim Murphy, Robert Plumb, James Langridge (Waters Corporation, MA).*

29. **Protection against fatty acid induced lipotoxicity: Modeled microgravity as a tool for the identification of novel pathways**
    *Magda Latorre-Esteves (University of Puerto Rico, Puerto Rico).*

30. **Bromodomain and extraterminal (BET) family is a key mediator for Ox-PAPC induced inflammatory gene responses in the vascular endothelium**
    *Sangderk Lee, Lihua Yang, Seon-gu Kim, Rachel Newcomb, Judith A. Berliner (University of Kentucky and University of California Los Angeles, CA).*

31. **Effect of insulin resistance on plasma and tissue fatty acid profile in severely obese subjects**
    *Alain Veilleux, Alain Montoudis, Picard Marceau, André C. Carpentier, Denis Richard, Emile Levy (Université de Montréal, Canada).*

32. **The effects of ozone therapy on serum neopterin level and necrosis in a rat model of acetaminophen induced liver injury**
    *Husamettin Gul, Bulent Uysal, Erdinc Cakir, Halil Yaman, Enis Macit, Ali Osman Yildirim, Yusuf Emrah Eyi, Umit Kaldirim, Emin Aktas, Emin Ozgur Akgul, Tuncer Cayci, Mehmet Ozler, Turgut Topal, Sukru Oter, Ahmet Korkmaz, Mehmet Toygar, Suzi Demirbag (Gulhane Military Medical Academy, Turkey).*

33. **Eicosanomic profiling reveals dominance of the epoxygenase pathway in human amniotic fluid at term in spontaneous labor ***
    *Krishna Rao Maddipati, Roberto Romero, Tinnakorn Chaiworapongsa, Sen-Lin Zhou, Zhonghui Xu, Adi L. Tarca, Juan Pedro Kusanovic, Hernan Munoz, Kenneth V. Honn (Wayne State University, MI).*

34. **Lipidomics by infusion-based MS/MSALL reveals novel aspects of fatty liver disease**
    *Jeffrey McDonald (UT Southwestern Medical Center, TX).*

35. **Lipidomic analysis of mouse brain using supercritical fluid chromatography-ion mobility-mass spectrometry**
    *Libin Xu, J. Rafael Montenegro-Burke, Zeljka Korade, Ned A. Porter, John A. McLean (Vanderbilt University, TN).*

36. **LIQUID: Lipid Informed Quantitation and Identification**
    *Kevin L. Crowell, Jennifer E. Kyle, Sangtae Kim, Yoshihiro Kawaoka, Richard D. Smith, Samuel H. Payne, Thomas O. Metz (Pacific Northwest National Laboratory, WA).*

37. **Dietary 1-deoxy-sphingolipids: a new concept in the dietary impact of sphingolipids**
    *Jingjing Duan, Alfred H. Merrill, Jr. (Georgia Institute of Technology, GA).*

38. **Application of untargeted and targeted lipidomic/metabolomic study of mdr2+/- mouse**
    *Wujuan Zhang, Kenneth D.R. Setchell, Alexandra Menchise, Xueheng Zhao, Julia Simmons, Alexander Miethke (Cincinnati Children's Hospital Medical Center, OH).*

39. **Apoc2 knockout zebrafish model of hypertriglyceridemia ***
    *Chao Liu, Longhou Fang, Yury I. Miller (University of California San Diego, CA).*

40. **AIBP inhibits inflammation and reduces foam cell formation**
    *Longhou Fang, Ayelet Gonen, Soo-Ho Choi, Felicidad Almazan, Yury I. Miller (University of California San Diego, CA).*

41. **Apolipoprotein A-I Binding Protein deficiency promotes development of high-fat diet-induced metabolic syndrome**
*Dina A. Schneider, Longhou Fang, Dorothy D. Sears, Yury I. Miller (University of California San Diego, CA).*

42. **Alterations of lipid metabolism in preeclampsia: Lipid characterization in the maternal circulation and placenta**
*Simon H.J. Brown, Samuel R. Eather, Dilys J. Freeman, Barbara J. Meyer, Todd W. Mitchell (University of Wollongong, Australia).*

43. **Protection of mitochondrial function in mammalian cells by deuterated polyunsaturated fatty acids**
*Alexander Y. Andreyev, Vadim V. Shmanai, Andrei V. Bekish, Anne N. Murphy, Mikhail S. Shchepinov (University of California San Diego, CA).*

44. **Identification of oxidized phospholipids in bronchoalveolar lavage fluid exposed to ozone**
*Ann-Charlotte Almstrand, Robert C. Murphy (University of Colorado Denver, CO).*

45. **Characterization and quantification of hopanoids in *Burkholderia multivorans* and *Rhodopseudomonas palustris* TIE-1 ***
*Chia-Hung Wu, Rebecca J. Malott, Nathan F. Dalleska, David P. Speert, Dianne K. Newman (California Institute of Technology, CA).*

46. **Membrane homeostasis of *R. palustris* in the absence of hopanoids**
*Cajetan Neubauer, Nathan F. Dalleska, Chia-Hung Wu, Dianne K Newman (California Institute of Technology, CA).*

47. **Tight control of inwardly rectifying potassium channel activity through the balance between PI(4,5)P2 and other anionic phospholipids in the membranes**
*Sun-Joo Lee, Shizhen Wang, William Borschel, Jacob Gyore, Sarah Heyman, Colin G. Nichols (Washington University, MO).*

48. **Total lipid extraction made easy – the new BUME methods for rapid automated chloroform-free lipid extraction of biofluids and tissue samples**
*Lars Löfgren, Gun-Britt Forsberg, Ralf Nilsson, Göran I Hansson, Marcus Ståhlman (AstraZeneca, Sweden).*

49. **Analysis of sterols and sterol derivatives by SFC-APPI-MS/MS**
*Ralf Nilsson (AstraZeneca, Sweden).*

50. **The dual effects of vitamin E on the degree of lipid peroxidation in the membrane system**
*Regina Friedl, Nisreen Nusair (Walsh University, OH).*

51. **Aged related increase in secretory phospholipase A2 group II D is associated with an anti-inflammatory environment in the lungs**
*Rahul Vijay, Stanley Perlman (University of Iowa, IA).*

52. **Sphingosine kinase 1 regulates adipose proinflammatory responses and insulin resistance**
*Jing Wang, Leylla Badeanlou, Jacek Bielawski, Theodore P. Ciaraldi, Robert R. Henry, Fahumiya Samad (Torrey Pines Institute for Molecular Studies, CA).*

53. **Characterization of the lipoxygenase-allene oxide synthase pathway in the stress responses of coral**
*Tarvi Teder, Alan R. Brash, Helike Lõhelaid, Nigulas Samel (Vanderbilt University, TN).*

54. **Lipidomic profiles of human adipose tissue associated with insulin resistance**
*Nassim Ajami, Alex Thomas, Dorothy D. Sears (University of California San Diego, CA).*

55. **Effects of diet intervention on plasma eicosanoid profiles in obese human subjects**
*Anthony Aylward, Aaron Armando, Oswald Quehenberger, Dorothy D. Sears (University of California San Diego, CA).*

56. **LipoxinA4 and Benzo-LipoxinA4 attenuate adipose inflammation and rescue obesity-induced kidney disease ***
*Emma Börgeson, Catherine Godson, Kumar Sharma (University of California San Diego, CA).*

57. **Application of sequencing, fatty acid profiling, and metabolomics investigation to explore pathogenesis and treatment strategy for anorexia nervosa ***
*Pei-an (Betty) Shih, Jun Yang, Christophe Morisseau, Ashley Van Zeeland, Aaron M. Armando, Edward Dennis, Oswald Quehenberger, Andrew Bergen, Pierre Magistretti, Wade Berrettini, Nicholas Schork, Walter Kaye, Bruce D. Hammock (University of California San Diego, CA).*

58. **Monoacylglycerol lipase mediates fever via hypothalamic prostaglandin E2 production**
*Yoshihiro Kita, Kenji Yoshida, Suzumi M. Tokuoka, Fumie Hamano, Kenji Sakimura, Masanobu Kano, Takao Shimizu (University of Tokyo, Japan).*

59. **Development of glycerophospholipid profiling methods using ternary gradient liquid chromatography/ high-speed triple quadrupole mass spectrometry**
*Suzumi Tokuoka, Yoshihiro Kita, Masaki Yamada, Takao Shimizu (University of Tokyo, Japan).*

60. **Deficiency of monoacylglycerol lipase attenuates diet-induced obesity in an endocannabinoid system-independent manner**
*Kenji Yoshida, Yoshihiro Kita, Suzumi Tokuoka, Kenji Sakimura, Masanobu Kano, Takao Shimizu (University of Tokyo, Japan).*

61. **Expression of diacylglycerol kinase theta during the organogenesis of mouse embryos**
*Shuji Ueda, Becky Tu-Sekine, Minoru Yamanoue, Daniel M. Raben, Yasuhito Shirai (Kobe University, Japan).*

62. **Oxidative stress induces endothelial dysfunction: Role of sterol regulatory element binding protein 2 and microRNA-92a**
*Zhen Chen, Liang Wen, Marcy Martin, John Y-J. Shyy (University of California San Diego, CA).*

63. **Peroxisomal Atg37 binds Atg30 or palmitoyl-CoA to regulate phagophore formation during pexophagy**
*Taras Y. Nazarko, Katharine Ozeki, Andreas Till, Geetha Ramakrishnan, Pouya Lotfi, Mingda Yan, Suresh Subramani (University of California San Diego, CA).*

64. **Runx1-mediated hair follicle stem cell activation and skin tumorigenesis by regulation of lipid metabolism ***
*Tudorita (Doina) Tumbar, Song Eun Lee, Aiko Sada, Prachi Jain (Cornell University, NY).*

65. **Gender-specific prostaglandin production**
*Elena Mejia, Wendy L. Becker, Kelsey D. Jordan, Rita K. Upmacis (Pace University, NY).*

66. **Molecular structures of phospholipids with very long chain fatty acids in skin fibroblasts of Zellweger Syndrome**
*Kotaro Hama, Yuko Fujiwara, Toru Nagai, Kazutaka Ikeda, Ryo Taguchi, Masashi Morita, Tsuneo Imanaka, Nobuyuki Shimozawa, Keizo Inoue, Kazuaki Yokoyama (Teikyo University, Japan).*

## 1. Effect of imatinib on nitrite levels in LPS/IFNγ activated RAW 264.7 macrophages

A. Z. Karabay[1], A Koc[1], T. Ozkan[2], Z. Buyukbiongol[1], A. Sunguroglu[2], Fugen Aktan[1]

[1]Faculty of Pharmacy and [2]Faculty of Medicine, Ankara University, Ankara, Turkey.

Imatinib is a tyrosine-kinase inhibitor used in the treatment of different cancers and inhibits BCR-ABL oncoprotein which is the product of the Philadelphia chromosome fusion gene in chronic myeloid leukemia. It is reported that imatinib treatment may have the potential to inhibit various inflammatory markers. Therefore in this study, we investigated the effect of imatinib on nitric oxide production and viability in LPS-IFNγ stimulated RAW 264.7 macrophage cells. For this purpose, cells were incubated with various concentrations of imatinib(0,125-50μM) for 1 hour and then stimulated with LPS-IFNγ for 20 h. Nitrite levels were determined with griess reaction and cell viability was determined with MTT assay. Our results showed that imatinib pretreatment of RAW 264.7 macrophage cells before stimulation with LPS/IFN gamma didn't suppress nitric oxide production and iNOS protein levels. The effects of imatinib on different inflammatory markers in this cell model can be analysed for further studies.

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## 2. Separation and quantification of 2-acyl-1-lysophospholipids and 1-acyl-2-lysophospholipids in biological samples by LC-MS/MS

Michiyo Okudaira[1,#], Asuka Inoue[1,#], Akira Shuto[1,#], Keita Nakanaga[1], Kuniyuki Kano[1], Kumiko Makide[1], Daisuke Saigusa[1,2], Yoshihisa Tomioka[1], Junken Aoki[1,3]

[1]Graduate School of Pharmaceutical Sciences, and [2]Department of Integrative Genomics, Tohoku Medical Megabank, Tohoku University, Sendai, Miyagi, Japan, [3]Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO.

Lysophospholipids (LPLs) serve as important lipid mediators and also as precursors for synthesis of diacyl phospholipids (PLs). LPLs detected in cells have various acyl chains attached at either the *sn*-1 or *sn*-2 position of the glycerol backbone. In general acyl chains at the *sn*-2 position of 2-acyl-1-lyso-PLs readily move to the *sn*-1 position, generating 1-acyl-2-lyso isomers by non-enzymatic reaction called intra-molecular acyl migration, which has hampered the detection of 2-acyl-1-lyso-PLs in biological samples. In this study, we developed a simple and versatile method to separate and quantify 2-acyl-1- and 1-acyl-2-lyso-PLs in a condition that completely eliminated the intra-molecular acyl migration. We found that the acyl migration reaction was dependent on both temperature and pH of the solvent. At pH 4 and 4°C, the ratio of 2-acyl-1-lyso- and 1-acyl-2-lyso-LPLs including lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylinositol, lysophosphatidylserine, lysophosphatidylglycerol and lysophosphatidic acid, which was prepared by either phospholipase $A_1$ or $A_2$ reaction, respectively, did not change at least for 1 week. By contrast at neutral pH or basic buffer (pH 9), all these 2-acyl-1-lyso isomers were quickly converted to the corresponding 1-acyl-2-lyso isomers. When LPLs were extracted from various mouse tissues at pH 4, most of the saturated fatty acid (16:0 and 18:0)-containing LPLs were found to be 1-acyl-2-lyso isomers, while most of the polyunsatulated fatty acid (18:2, 20:4, 22:6)-containing LPLs were 2-acyl-1-lyso isomers. Distribution of oleic acid (18:1) in LPLs of the *sn*-1 and *sn*-2 position differed among tissues. When extracted in neutral condition, the uneven distributions of acyl chains were completely lost. We further showed that cells treated with phosphatidylserine (PS)-specific phospholipase $A_1$ contained 2-acyl-1-lyso-LysoPS, suggesting that 2-acyl-1-lyso-LPLs are stable on the cell membrane. On the other hand, LysoPS extracted from the cells by albumin was converted into 1-acyl-2-lysoPS, indicating that albumin accelerated the acyl migration reaction. The present method is definitely useful for elucidating *in vivo* role of 2-acyl-1-lyso-PLs.

*For all poster abstracts:*

*Presenting author is underlined*

[#] *indicates equal effort contributed by first authors*

** indicates lightning talk selection*

## 3. Impaired inflammatory response in fatty liver dystrophy mice *

Clara Meana[1,2], Lucía Peña[1,2], Esperanza Esquinas[1], Carlos Guijas[1,2], Gema Lordén[1,2], Martín Valdearcos[1], Jesús Balsinde[1,2], María A. Balboa[1,2]

[1]Instituto de Biología y Genética Molecular, Consejo Superior de Investigaciones Científicas (CSIC), Universidad de Valladolid, Valladolid, Spain, [2]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Madrid, Spain.

**Background**: Fatty liver dystrophy (*fld*) mice are metabolically well characterized. They have reduced white and brown fat pads, hyperglycemia, hyperinsulinemia, and diet-related atherosclerosis. The factor responsible for this phenotype is a mutation in the Lpin-1 gene, which encodes for Lipin-1, a $Mg^{2+}$-dependent phosphatidic acid phosphatase (PAP) involved in the de novo synthesis of phospholipids and triacylglycerol. Previous work in our laboratory has shown that this enzyme plays a role in macrophage biology by regulating the size and amount of lipid droplets, and also by regulating the activation of group IVA phospholipase $A_2$ and prostaglandin production.

**Objective**: To study the impact of lipin-1 on TLR-4-mediated signaling and inflammatory responses of macrophages.

**Methods**: Adult *fld* and wild type (wt) mice were sacrificed, peritoneal macrophages and bone-marrow were isolated, and incubated with 100ng/ml LPS. Measurement of mRNA levels, protein expression, cytokine release and lipid levels were conducted.

**Results**: Bone marrow and peritoneal macrophages from *fld* mice exhibit more moderate iNOS and COX-2 increases and IL-6, IL-12 and IL-23 cytokine generation after TLR-4 activation compared to wild type cells. This restrained response is due to a lower phosphorylation of mitogen-activated protein kinases (MAPK), which leads to reduced activation of the AP-1 transcription factor and, consequently, to lower levels of pro-inflammatory proteins. Contrary to wild type cells, diacylglycerol levels in *fld* macrophages decreases after LPS stimulation, which presumably accounts for the reduced MAPK activation.

**Conclusion**: Fatty liver dystrophy restrains the inflammatory response of macrophages due to alterations in lipid metabolism.

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## 4. Synthesis of analogs of myo-inositol to enable chemical biology studies

Tanei Ricks, Michael D. Best

University of Tennessee-Knoxville, Knoxville, TN.

*Myo*-inositol is an important biological molecule that plays a role in a variety of cellular signaling processes and acts as the biosynthetic precursor to a number of other key biomolecules. Great work has been put into synthesizing naturally occurring products of *myo*-inositol, such as the phosphatidylinositolpolyphosphates (PIPs), as well as numerous analogs thereof for use as probes. Prior research has shown that the C-2 and C-6 positions of *myo*-inositol are the least modified positions intercellularly, which suggests greater ability for functionalization while maintaining biological function. Here, we will describe progress towards the design and synthesis of various azide-functionalized *myo*-inositol derivatives as well as potential applications for these derivatives in studying cellular signaling events.

## 5. Integration of lipidomic data and computational modeling for studying sphingolipid metabolism in macrophages

Nathan Chiappa, Edward Botchwey

Georgia Institute of Technology, Atlanta, GA.

The advent of high-throughput lipidomic technology has allowed for the generation of vast amounts of information on the lipidomic state of a cell. However, this information can be difficult to interpret given the size of the data sets and the complexity of the underlying metabolic network. Computational modeling is a powerful tool for integrating and interpreting these data. We have constructed for the first time a computational model of sphingolipid metabolism in RAW264.7 macrophages that accounts for all of the sphingolipid species measured in the 2010 LIPID MAPS mouse macrophage lipidome study. Two variants of the model were constructed using the traditional Michaelis-Menten formalism as well as the Biochemical Systems Theory formalism for comparison. The model contains 57 lipids connected by 121 reactions. All model parameters were obtained from an extensive literature search.

The model is locally stable and returned to steady-state following small perturbations to lipid concentrations. Additionally, the model exhibited small sensitivities to changes in parameter values. Both of these indicate that the model is robust. Additionally we validated the model against the dynamic time-series data for each sphingolipid available on the LIPID MAPS database. The model showed good agreement with the experimental data suggesting that the model is a good representation of the metabolic network.

This model represents a powerful tool for understanding and predicting the behavior of sphingolipid metabolism in response to a wide array of perturbations. The systemic response to transient and sustained changes in enzyme activity as seen during inflammation can readily be investigated. Additionally, the systemic response to various sphingolipid enzyme-modulating drugs can be determined. Thus, this model can help advance our understanding of the sphingolipid metabolic network as an integrated whole as well as allow for optimization of therapeutic intervention into sphingolipid-mediated pathologies.

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## 6. Fatty acid heme dioxygenase and catalase-related hydroperoxide lyase cooperate in linoleate metabolism in *Nostoc punctiforme*

Alan R. Brash, Narayan P. Niraula, William E. Boeglin, Zahra Mashhadi

Department of Pharmacology and the Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN.

In the course of exploring the scope of catalase-related hemoproteins' reactivity towards fatty acid hydroperoxides, we detected a novel candidate in the cyanobacterium *Nostoc punctiforme* PCC 73102. The immediate neighboring upstream gene, annotated as "cyclooxygenase-2", appeared to be a potential fatty acid heme dioxygenase. We cloned both genes and expressed the cDNAs in *E. coli*, confirming their hemoprotein character. Oxygen electrode recordings demonstrated a rapid (>100 turnovers/s) reaction of the heme dioxygenase with oleic and linoleic acids. HPLC including chiral column analysis, UV, and GC-MS of the oxygenated products identified a novel 10*S*-dioxygenase activity. The catalase-related hemoprotein reacted rapidly and specifically with linoleate 10*S*-hydroperoxide (> 2,500 turnovers/s) with a hydroperoxide lyase activity specific for the 10*S*-hydroperoxy enantiomer. The products were identified by NMR as (8*E*) 10-oxo-decenoic acid and the C8 fragments, 1-octen-3-ol and 2*Z*-octen-1-ol in ~ 3:1 ratio. Chiral HPLC analysis established strict enzymatic control in formation of the 3*R* alcohol configuration (99% ee) and contrasted with racemic 1-octen-3-ol formed in reaction of linoleate 10*S*-hydroperoxide with hematin or ferrous ions. The *Nostoc* linoleate 10*S*-dioxygenase, the sequence of which contains the signature catalytic sequence of cyclooxygenases and fungal linoleate dioxygenases (YRWH) appears to be a heme dioxygenase ancestor. The novel activity of the lyase expands the known reactions of catalase-related proteins and functions in *Nostoc* in specific transformation of the 10*S*-hydroperoxylinoleate and suggests that related enzymes may be harbored in other bacterial genomes. Supported by NIH grant GM-74888.

## 7. Calorie restriction increases insulin sensitivity in skeletal muscle through sphingolipid metabolism

Diana Obanda, Yongmei Yu, Zhong Wang, William Cefalu

Pennington Biomedical Research Center, Baton Rouge, LA.

The sphingolipid pathway exerts control over insulin signaling in insulin sensitive tissues. An improvement in insulin sensitivity is one of the most consistent features of calorie restriction (CR) as observed in rodent, primate and human models. The cellular mechanism(s) by which CR enhances insulin action are not precisely known. We sought to examine the effect of calorie restriction on sphingolipid metabolism in relation to insulin sensitivity in skeletal muscle of rats over 6 months. At baseline, male Fischer-344 rats (n=29) were randomized to an ad libitum (AL) diet or to 30% CR. Dietary intake, body weight, and insulin sensitivity were monitored routinely. At the end of the study, skeletal muscle (vastus lateralis) was obtained in the basal and insulin-stimulated states for insulin signaling and sphingolipid profiling.Compared to the AL diet, CR significantly increased whole-body insulin–mediated glucose disposal and significantly lowered the index of homeostasis model assessment of insulin resistance (HOMA-IR) ($p<0.05$; n=29). Western blotting showed that Akt 2 and IRS protein abundance and Akt phosphorylation in skeletal muscle was significantly upregulated in CR animals ($p<0.05$; n=29). CR altered the expression of proteins involved in sphingolipid formation and metabolism. CR significantly downregulated the expression of glucosylceramide synthase, lactosylceramide synthase and GM3 synthase, enzymes involved in glycosphingolipid formation ($p<0.05$; n=29). Quantification of metabolically stable sphingolipids showed that the quantities of ceramides were increased in CR while hexosylceramides and lactosylceramides were significantly lowered in CR ($p<0.01$; n=29). Ceramide phosphates, sphingomyelins, sphingosine and sphingosine phosphate were not significantly different between AL and CR groups. Lactosylceramide quantities correlated significantly with insulin resistance (HOMA-IR ($R=0.72$; $p<0.005$).We concluded that the increase in insulin sensitivity in CR is associated with modulation of proteins involved in sphingolipid formation and metabolism. While ceramides unexpectedly increased in CR, glycosphingolipids: hexosylceramides and lactosylceramides were significantly reduced in calorie restriction.

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## 8. Post-squalene cholesterol biosynthetic pathways *

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The final stage of cholesterol biosynthesis involves the conversion of lanosterol to cholesterol. Two alternative pathways, known as the Bloch and Kandutsch-Russell (K-R) pathways, have been proposed for this conversion. To assess the relative utilization of these pathways by different tissues we used LC-MS/MS methods developed by the Lipid MAPS consortium, deuterium water ($D_2O$) incorporation, and isotopic spectral analysis to measure flux of post-squalene cholesterol biosynthetic intermediates in cultured cells and mouse tissues. Flux through the Bloch pathway relative to the K-R pathway differed substantially among different cultured cell lines, ranging from >95 % in mouse adrenal cells (Y1-BS1), 60% in human liver cells (HuH7 cells) to < 10% in human skin fibroblasts (SV-589). We did not find any cell type in which the K-R pathway, as it was originally described, was operative. The initial K-R pathway intermediates (dihydrolanosterol, dihydro-ff-MAS, and dihydro-t-MAS) were present but did not turnover. Moreover, cholesterol biosynthesis proceeded through a hybrid pathway: lanosterol→zymosterol→ 24-25 side chain double bond desaturated→7-dehydrocholesterol →cholesterol. To determine if the patterns of pathway utilization in the cell lines reflected the tissues of origin *in vivo*, we performed labeling studies using $D_2O$ in 12-week old male mice. The Bloch pathway predominated in adrenals, spleen and testes, whereas the hybrid pathway was preferentially used in skin, brain and preputial glands. The liver and kidney were intermediate between these extremes. Thus, the cholesterol biosynthetic pathways utilized by cultured cells mirror those used by the tissue from which they are derived and a hybrid pathway between the Bloch and K-R pathway appears to be the alternative pathway to the Bloch pathway. There is no fully distinct alternative to the Bloch pathway as originally proposed.

## 9. ABHD4 regulates the metabolism of multiple classes of N-acyl phospholipids in mammalian central nervous systems

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*N*-acyl phospholipids are minor components of membrane lipids and potential precursors of lipid mediators such as anandamide. Here, we show that ABHD4, a member of serine hydrolase superfamily, regulates metabolic pathways of multiple *N*-acyl phospholipids in the mammalian central nervous systems. Brains from *Abhd4*$^{-/-}$ mice exhibited the significant decreases in the levels of glycerophospho-NAEs (GP-NAEs) and *N*-acyl lysophosphatidylethanolamines (lysoNAPEs). Moreover, *N*-acyl lyso ethanolamine plasmalogens (lyso pNAPEs) were remarkably decreased in *Abhd4*$^{-/-}$ brains. Interestingly, an untargeted metabolomics approach revealed that *N*-acyl lysophosphatidylserines (lysoNAPSs), a hitherto unidentified novel class of lipid, were dramatically decreased in brains from *Abhd4*$^{-/-}$ mice. Enzyme assays confirmed that *N*-acyl ethanolamine plasmalogen (pNAPE) and *N*-acyl phosphatidylserine (NAPS) are direct substrates of ABHD4. Together, our study indicates that ABHD4 is a key enzyme for the degradation of both glycerophospho-*N*-acyl ethanolamine and glycerophospho-*N*-acyl serine lipids.

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## 10. Elucidating the mechanism of endocannabinoid metabolism by CYP2J2 epoxygenase in nanodiscs *

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Endocannabinoids activate the cannabinoid receptors and produce similar psychoactive effects to Cannabis. Anandamide (AEA) and 2 -arachidonoylglycerol (2-AG) are the two most characterized endocannabinoids. Herein we demonstrate the direct metabolism of 2-AG and AEA by human heart CYP2J2 epoxygenase. CYP2J2 synthesizes epoxyeicosatrienoic acids (EET) from arachidonic acid (AA) that are implicated in inflammation, cardiovascular disease and cancer. In this work, we elucidate the nuances of ligand-protein interactions using spectral titrations, small molecule ligand egress, molecular modeling and LC-MS. We use Nanodiscs (nanoscale lipid bilayers) to solubilize and stabilize membrane bound CYP2J2 for these studies. The binding study shows that simply by changing the residue at the carboxylic acid end of AA to ester (in 2-AG) or amide (in AEA) changes the binding interaction of these lipids with CYP2J2 active site. Furthermore molecular modeling confirmed that this carboxylic acid site indeed plays an important role in docking of the substrate molecule to the active site. We further studied the metabolism and kinetics of product formation using LC-MS. Reactions of CYP2J2 with AEA formed four AEA-epoxide products (EET-EA). Interestingly, incubations of 2-AG with CYP2J2 yielded detectable levels of only two 2-AG epoxides (2EG) as shown to be present in tissues samples from kidney and spleen. The incubation of CYP2J2 with 2-AG also produces considerable amount of free AA, glycerol and EETs in solution, that are products of oxidative ester hydrolysis of 2-AG. This shows that CYP2J2 not only metabolizes 2-AG but also provides an alternative pathway for its hydrolysis. In summary, we successfully identified a cardiovascular CYP that binds and metabolizes both AEA and 2-AG as well as oxidatively cleaves the 2-AG ester, effectively attenuating 2-AG activity. Our investigation of the interactions of the lipids with CYP2J2 suggests potential cross talk between the endocannabinoind metabolism and lipid metabolism pathways in the heart.

## 11. Enhanced phospholipid and sphingolipid analysis using ion mobility and UPLC mass spectrometry

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We have developed and optimized protocols for separating phospholipid and sphingolipid classes from a phospholipid mixture and human serum using Hydrophilic Interaction Liquid Chromatography (HILIC), Reversed Phase (RP) Chromatography, and Differential Ion Mobility Spectrometry (DMS). This exploration was carried out on an AB SCIEC 6500 QTRAP (MS) equipped with an AB SCIEX Selexion DMS and Waters Acquity Ultra Performance liquid Chromatography (UPLC). Besides optimizing each approach separately, we also explored advantages of combining HILIC-UPLC with DMS as well as RP-UPLC with DMS and new results will be described and discussed. [Supported by LIPID MAPS Glue Grant U54 GM069338]

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## 12. G-protein coupled receptor-dependent phosphorylation of Thr10 induces lamin A disassembly from the nuclear lamina

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The nuclear lamina is a mesh-like layer of type V intermediate filaments that is tightly associated to the inner nuclear membrane and is required for the proper function of the cell. A well-known function is the provision of structural scaffolding for the cell nucleus, however it also plays an important role in regulation of gene transcription, cell-cycle progression, DNA-damage response, nuclear envelope breakdown and nuclear pore complex organization as well as other functions. The main protein isoforms constituting the nuclear lamina are lamins A, C, B1, B2 and B3. Post-transcriptional phosphorylation of lamin proteins on either serine or threonine sites have been shown to cause turnover of the protein. Gerarduzzi et al. performed phosphoproteomic analysis (phosphopeptide enrichment by affinity chromatography, LC-MS/MS phosphopeptide analysis (LTQ-Obitrap-CID), and SEQUEST bioinformatics analysis of spectra) of GPCR activated (prostaglandin E2 stimulation) human fibroblast cells. This study revealed PGE2/GPCR/PKA phosphosubstrates, one of which was a heavily phosphorylated (> 10 fold over control: control, $1.09 \times 10^6$ vs ligand, $13 \times 10^6$, relative peak intensities; $p < 0.0001$) peptide containing phosphothreonine (T)10 of lamin A. To study the function of this phosphorylation site, we developed rabbit polyclonal antibodies against the 20-mer N-terminal region of the wild-type and phosphoT-10 peptides as well as GFP-lamin A (wt and T10->A10; T10->E10 mutations) fusion expression plasmids. Subsequent analysis involved immunoprecipitation, western blot, transfection and confocal microscopy, disclosed that phosphorylation of T10 maybe a signal for lamin A disassembly from the nuclear lamina leading to its degradation.

## 13. Untargeted metabolomics of human ex-plant lung associated with cystic fibrosis

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Direct measurement of metabolites from diseased tissue remains a challenge owing to structural diversity of the large number of primary and secondary metabolites present. These metabolites represent the climax of all physiological responses and reflect ecological relationships between pathogenic microbes and human response. Strategies aiming at direct measurement of metabolites will enhance our understanding of the roles that these molecules play and may result in emergence of new molecules and novel biosynthetic pathways. Untargeted metabolomics employed for such studies generates large volume of datasets that are rich in information hampering comprehensive analysis. Recent advancements in mass spectrometry and bioinformatics tools have reinvigorated the field of untargeted metabolomics of complex samples. Herein, we employ high-scan-speed QTOF coupled with ultra high performance liquid chromatography for data collection combined with molecular networking as organizational tool, cytoscape as visualization tool, and automated database search for rapid identification of metabolites present in a human lung. A suite of bioactive lipids, sterols, fatty acids, and drugs were identified from direct tissue extractions of ex-plant human lung associated with cystic fibrosis.

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## 14. Smith-Lemli-Opitz Syndrome fibroblasts reveal the importance of endogenous cholesterol biosynthesis to the innate immune response

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Disorders of cholesterol metabolism contribute significantly to the burden of human disease. Smith-Lemli-Opitz Syndrome (SLOS) is a rare disorder caused by a defect in cholesterol biosynthesis that presents in infancy with multiple developmental abnormalities. SLOS results from mutation of 7-dehydrocholesterol reductase (DHCR7), the gene encoding the final enzyme in the Kandutsch-Russell cholesterol biosynthetic pathway. This results in reduced cholesterol and a coordinate increase in the precursor, 7-dehydrocholesterol. Lipid rafts are cholesterol-enriched membrane microdomains that serve as signaling platforms in multiple prototypical pathways, including that of the Toll like Receptors (TLRs). Raft-dependent signaling is sensitive to raft cholesterol levels. We hypothesized that SLOS cells would display abnormal TLR signaling, thus offering a unique opportunity to define the importance of endogenous cholesterol biosynthesis to the innate immune response. Primary dermal fibroblast lines from SLOS patients were confirmed for the SLOS metabolic phenotype by GC/MS. The SLOS fibroblasts produced significantly lower IL-6 and IL-8 in response to lipopolysaccharide (LPS) stimulation compared to controls. Intriguingly, an inverse correlation was observed between clinical SLOS severity scores of the source patients and IL-6 response to LPS, and a direct correlation between residual DHCR7 enzyme activity and IL-6 production. Further, SLOS cells displayed a decrease in NFkB p65 activation upon LPS stimulation in comparison with WT controls. Using DHCR7 inhibitors on primary human macrophages to recapitulate the SLOS phenotype, preliminary studies indicate that TNFα production is attenuated in response to LPS stimulation compared to vehicle controls. Taken together, these findings indicate that the SLOS mutation confers abnormal innate immune responses. The correlation between clinical severity and LPS responsiveness of SLOS cells may suggest a clinically relevant role for altered innate immunity in SLOS. Understanding mechanisms by which cholesterol impacts innate immune function may provide novel insight into pathogenesis and therapy of a wide range of immune-mediated diseases.

## 15. Meclizine inhibits mitochondrial respiration through direct targeting of cytosolic phosphoethanolamine metabolism *

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We recently identified meclizine, an over-the-counter anti-nausea drug, as an inhibitor of mitochondrial respiration. Curiously, meclizine attenuated respiration in intact cells but not in isolated mitochondria, suggesting an unorthodox mechanism. Using a metabolic profiling approach, we now show that treatment with meclizine leads to a sharp elevation of cellular phosphoethanolamine, an intermediate in the ethanolamine branch of the Kennedy pathway of phosphatidylethanolamine biosynthesis. Metabolic labeling and in vitro enzyme assays confirmed direct inhibition of the cytosolic enzyme CTP:phosphoethanolamine cytidylyltransferase (PCYT2). Inhibition of PCYT2 by meclizine led to rapid accumulation of its substrate, phosphoethanolamine, which is itself an inhibitor of mitochondrial respiration. Our work identifies the first pharmacologic inhibitor of the Kennedy pathway, demonstrates that its biosynthetic intermediate is an endogenous inhibitor of respiration, and provides key mechanistic insights that may facilitate repurposing meclizine for disorders of energy metabolism.

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## 16. Novel mechanism for brain prostanoid level regulation through bile acid receptors

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To develop an alternative strategy to specifically down-regulate prostanoid (PG) levels upon inflammatory conditions without affecting basal levels in brain cells to avoid multiple side effects of NSAID, we addressed the role for bile acid (BA) receptors in PG and proinflammatory cytokine level regulation in primary brain astrocytes and microglia. BA receptor ligands did not affect PG production under basal conditions in brain primary astrocytes in a wide range of bile acids concentrations. However, upon stimulation with LPS or β-amyloid, they dramatically decreased $PGE_2$, $PGD_2$, and 6keto-$PGF_{1\alpha}$ levels to basal levels when physiologically relevant bile acid concentrations were used. Consistent with these results, BA also decreased proinflammatory cytokine levels in microglia and astrocytes upon stimulation, including TNF$\alpha$ and IL6. Importantly, the viability of brain primary astrocytes, microglia, and neurons was not affected by BA treatment. The evaluation of BA receptor signaling pathways revealed an important role of FXR signaling mechanism in both BA effects and BA synthesis regulation in glia cells. Intriguingly, BA levels were altered in both human and mouse AD brains. In summary, our data demonstrate, for the first time, the regulatory role for bile acid receptors in PG regulation upon treatment with AD and infection relevant stimuli without affecting basal PG levels, and highlight a novel approach for PG level regulation. *Supported by NIH Grant 5R01 AG042819-02, and NIH funded COBRE Mass Spec Core Facility Grant 5P30 GM103329-02*

## 17. Mapping lipid-dependent functional changes in TRPV1 ion channels expressed in cultured cell lines

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Many ion channels are known to be regulated by specific lipids, such as the important signaling lipid PI(4,5)P2. The TRPV1 ion channel is activated by heat and capsaicin among other stimuli, and is potentiated by PI(4,5)P2. We have recently used calorimetry and electrophysiology to show that this potentiation requires only extremely low mole fractions of PI(4,5)P2 in the plasma membrane cytosolic leaflet. However we also found that PI(4,5)P2 applied to the extracellular side of the plasma membrane inhibits TRPV1 function. Other charged lipids appear to have similar effects of varying magnitude. Curiously, TRPV1 function also depends strongly on the cells in which it is expressed. For instance, ten times more capsaicin, and ten times more PI(4,5)P2, are required to open TRPV1 channels in F11 cells, a hybridoma of rat DRG cells and mouse neuroblastoma, than are required in HEK293T/17 cells. We hypothesize that these observations may result from differences between HEK293T/17 and F11 cells in the total amount of important lipids like PI(4,5)P2, other charged lipids, or even cholesterol in the plasma membrane. Our preliminary evidence suggests HEK293T/17 cells have a higher cholesterol:phospholipid ratio than F11 cells derived from dorsal root ganglia neurons. Alternatively, the observed functional differences may result from different transbilayer distribution of lipids in the plasma membrane, since we have shown that charged lipids in the extracellular leaflet inhibit TRPV1 function. Differences in post-translational modification of TRPV1 or in its interactions with other proteins could contribute to functional differences as well. We will outline our results characterizing the lipids of HEK293T/17 and F11 cells, and discuss our approach to future studies of lipid regulation of TRPV1.

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## 18. PPAR-pan induced metabolic changes in the liver

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The peroxisome proliferator-activated receptors (PPARs) are ligand activated nuclear receptors that regulate cellular homeostasis and metabolism. PPARs control the expression of genes involved in lipid metabolism and have emerged as potential targets for the treatment of a number of diseases mainly dyslipidaemias. However, PPAR ligands are associated with a variety of adverse pathological changes, including the induction of various cancers and skeletal muscle wasting, which have overshadowed their development as therapeutics. Given the important role of PPAR agonists in lipid metabolism, a metabolomic approach was used to investigate the effects of dietary treatment of male Sprague–Dawley rats with the PPAR-pan agonist GW625019 in order to develop a model of the liver pathophysiology associated with this agonist. The compound was administered by daily oral gavage at 30,100, 300, 1000 mg/kg/day for 13 weeks. A satellite group of animals were kept for a further 4 week treatment free period. The PPAR-pan agonist increased total phospholipid and decreased triacylglycerol (TG) concentrations. Acylcarnitine concentrations also increased including free- and acetyl-carnitine as well as stearoyl-, oleyl-, linoleyl- and palmitoyl-carnitines. The inverse correlation between the TGs and acylcarnitines reflect increased β-oxidation induced by the agonist treatment. The agonist caused an increase in the production of reactive oxygen species (ROS) such as oxomethionine, methyl cytosine and adenosyl methionine, associated with inflammation and DNA damage. Changes in the concentration of several eicosanoids were also increased in concentration including cyclooxygenase products, prostaglandins (PGs) 15d-PJG2 and PGB2. The effects observed in the present study were reversible indicating an adaptive rather than an adverse response. In summary, the results of clinical chemistry were in good agreement with high throughput metabolic profiling where increased liver weights correlated with mode of action specific effects associated with energy storage (TGs and acylcarnitines), cell and/or organelle proliferation (phospholipids) and inflammation (eicosanoids and ROS).

## 19. Using metabolomics to investigate the induction of non-alcoholic fatty liver disease in a rat model of Hepatocellular Carcinogenesis *

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Non-alcoholic steatohepatitis (NASH) is a progressive form of NAFLD associated with worsening cirrhosis and hepatocellular carcinoma (HCC). The pathogenic mechanisms underlying such hepatic pathologies remain to be fully elucidated. Improved metabolic characterisation of NASH may uncover diagnostic and prognostic disease markers or identify novel targets for treatment. In this study, a metabolomics approach was utilised to compare a rat model of NAFLD induced by a choline deficient (CD) diet with control animals. In addition rats were treated with a thyroid hormone analogue, GC-1, to assess the potential of this intervention as a therapeutic for NAFLD. A comprehensive metabolomics strategy combining $^{1}$H nuclear magnetic resonance (NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), ultra performance liquid chromatography-mass spectrometry (UPLC-MS) and direct infusion mass spectrometry was employed to investigate metabolite changes in the liver. CD diet significantly increased the total fatty acid concentration by increasing medium and long chain fatty acids. Consistent with the fatty acid profile, we observed marked accumulation of acylcarnitine species in the livers of animals on a CD diet which may be attributed to incomplete mitochondrial β-oxidation. Total lipid profiling revealed increased unsaturated (5-8 double bonds) of acyl chains within triacylglycerides (TAG) in the livers from the CD group. This liver tissue was also characterised by increased glycolysis and ketogenesis, and decreased gluconeogenesis. In addition, oxidative stress was increased as measured using the surrogate oxidised methionine. A 2 week co-feeding with GC-1 lowered the total fatty acid content and lipid accumulation in the CD diet livers. There was a dramatic increase in the concentration of betaine in the GC-1 treated liver, a reported lipotrope which may prevent or reduce accumulation of fat in the liver. Furthermore, positive correlations between the increase of betaine and several acylcarnitine species have been found.

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## 20. Assessing de novo lipogenesis in ObOb mice using stable isotopes and lipidomics

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The use of deuterium incorporation into fatty acids from deuterated water is a well-established method for assessing hepatic de novo lipogenesis (DNL). While it is a robust method, sample preparation is realtively time consuming and requires the subject's body water to be relatively highly enriched. Recent indications from various lipidomic-centred studies have shown that plasma triglycerides (TGs) containing saturated fatty acids of a low carbon number, for example palmitate (-C16:0), are associated with increased risk of insulin resistance (IR) or related disorders and may reflect increased DNL in the liver. Such a lipidomic marker of DNL would have widespread applicability in terms of following the contribution of DNL to IR in human epidemiology studies. To test the hypothesis that palmitate containing TGs are markers of DNL activity, ob/ob mice and wild type controls were fed for two weeks on either a regular chow diet (to allow overfeeding in the ob/ob mice to stimulate DNL) or a high fat diet (to inhibit DNL). Deuterium enriched water was consumed ad libitum for the two weeks to enrich the body water content to contain ~1% deuterated water, prior to an intraperitoneal injection of [U-13C] glucose. Mice were killed and blood plasma collected alongside liver tissue, both snap frozen after collection. Subsequently, the lipidomic and fatty acid profiles have been assessed using high resolution liquid chromatography- mass spectrometry (LC-MS), and gas chromatography- mass spectrometry (GC-MS) .The level of enrichment of the fatty acids with deuterium and 13-carbon isotopes calculated using the LC-MS data, GC-MS data and GC/C/IRMS. This has been performed for the hepatic tissue and blood of the mice and cross-correlated. The findings from this study are presented here.

## 21. Nrp-1+Foxp3- CD4 T cells are a novel subset of T lymphocytes that are induced in aorta during atherosclerosis development

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Neuropilin 1 (Nrp-1) is a type I transmembrane protein that plays an important role in axonal guidance and angiogenesis through interacting with Semaphorin-3A and vascular endothelial growth factor (VEGF), respectively. Recently, Nrp-1 was found on a subset of regulatory T cells (Treg) that migrated into VEGF producing tumors. Moreover, Nrp-$1^+$ CD4 T cells were protective in a murine model of autoimmune encephalomyelitis, suggesting that these cells may play a role in other inflammatory diseases. Since atherosclerosis is a chronic inflammatory disease where T cells, macrophages, and other immune cells orchestrate disease progression, ***we hypothesized that Nrp-$1^+$ CD4 T cells are induced in the aorta during atherosclerosis development and play a role during disease progression.*** We fed ApoE$^{-/-}$ mice a western diet for 15 weeks and found a 2-fold increase in Nrp-$1^+$Foxp$3^-$ CD4 T cells in the spleens, peri-aortic lymph nodes and aortas of western diet fed mice compared to chow-fed mice. In addition, these Nrp-$1^+$Foxp$3^-$ CD4 T cells expressed higher levels of the memory marker CD44 and produced more IFNγ when compared to Nrp-$1^-$ CD4 T cells, suggesting that these cells are more activated. Treatment of CD4 T cells with oxidized LDL (OxLDL) caused upregulation of Nrp-1 on CD4 T cells. Using a transwell migration assay, we found that Nrp$1^+$ CD4 T cells had a 2-fold higher migration index towards $VEGF_{165}$ than did Nrp$1^-$ CD4 T cells. Similarly, CD4 T cells from ApoE$^{-/-}$ mice fed a western diet, which expressed more Nrp-1, had a higher migration index towards $VEGF_{165}$ compared to cells from chow controls. In conclusion, we have identified a novel subset of Nrp-$1^+$CD$4^+$ T lymphocytes that is increased during atherosclerosis development, and is induced by OxLDL. Our data suggest that Nrp-1 may play a role in the migration of CD4 T cells to the aorta during atherosclerosis development.

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## 22. Impact of adaptive thermogenesis on systemic lipid and cholesterol handling *

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**Objective:** Brown Adipose Tissue (BAT) activation is a promising approach to treat obesity and associated disorders. Recently, it was shown that BAT activation lowers blood lipids by accelerating plasma triglyceride clearance into BAT. However, not only the amount of lipids but rather the complex composition of lipids controls cellular metabolic homeostasis and potential organ-specific lipotoxic effects. Here we investigate the impact of BAT activation on circulating lipoproteins and metabolically active organs in mice using targeted and non-targeted lipidomic approaches.

**Methods:** BAT was activated in wild-type mice by cold exposure or injection of the selective beta3-agonist CL316,243. High-resolution lipidomics of liver, BAT, white adipose tissue, faeces and isolated lipoprotein classes were performed. High density lipoprotein particle (HDL) function was assessed *in vivo* using a reverse cholesterol transport assay.

**Results:** BAT activation strongly lowered plasma glucose and triglyceride levels while HDL cholesterol was elevated. A significant increase in cholesterol efflux rate indicates improved HDL function after BAT activation. In accordance with this, we observed remodeling of main lipid classes in circulating HDL which may influence HDL properties and function. Furthermore activated BAT influences cholesterol as well as bile acid metabolism resulting in enhanced sterol excretion, especially of conjugated bile acids, into faeces.

**Conclusion:** BAT activation not only leads to an improved glucose and triglyceride disposal but also to an increased reverse cholesterol transport which might be linked to an altered lipid composition of circulating HDL as well as altered sterol metabolism. To summarize, brown fat activation initiates a systemic metabolic rewiring, improving whole body metabolic health.

## 23. Changes in inositol-containing sphingolipid metabolism regulates stress response signaling

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The phospholipid precursor inositol exerts profound effects on both the regulation of lipid metabolism and stress response signaling. In the present study, we show that inositol starvation in budding yeast results in a dramatic reorganization of sphingolipid metabolism, most notably the metabolic channeling of phosphatidylinositol into complex, inositol-containing sphingolipids. Following removal of inositol from the growth medium of dividing cells, a new steady-state lipid composition of the three inositol-containing sphingolipid species present in *S. cerevisiae* was established within 5-6 h. Inositol phosphoceramide (IPC) and mannosyl-di-IPC levels were reduced by 4-fold while mannosyl-IPC levels were elevated by almost 5-fold. To identify lipid-mediated signaling networks that respond to the reprogramming of inositol-sphingolipid metabolism, we carried out gene expression profiling following inositol starvation. These results revealed that genes controlled by three plasma membrane-localized stress response pathways, including PKC-MAPK, HOG signaling and calcineurin pathways, were initially highly up-regulated following inositol removal but were eventually down-regulated after long term inositol starvation. Consistent with these findings, mutants in each pathway exhibited inositol auxotrophy. Moreover, pharmacological inhibition of sphingolipid metabolism constitutively activated protein kinase C, HOG pathway, and calcineurin signaling and stimulated the enrichment of a PI4P biosensor on the plasma membrane. Together, these results demonstrate that plasma membrane localized inositol sphingolipids control stress response signaling and suggest a feedback mechanism for regulating sphingolipid metabolism.

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## 24. Increased lipid identification coverage for global lipidomics analyses using a benchtop quadrupole-Orbitrap LC-MS/MS system

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**Introduction:** Lipids play a key role in cell, tissue and organ physiology and with diseases such as cancer and diabetes which involve disruption of their metabolic enzymes and pathways. Identification of unique lipid biomarkers to distinguish healthy humans compared to those with a disease can have an impact on the early detection of diseases and personalized medicine. In this study, we present the number of lipid species identified in LC-MS experiments can be increased significantly by using optimized HPLC separation and HR/AM (high resolution accurate mass) MS and data-dependent $MS^2$ conditions. **Methods:** The whole lipid extract from Bovine liver (Avanti Polar Lipids) was separated via a 2.1 x 100 mm Ascentis C18 column using several different HPLC methods. Eluting compounds were detected in a bench-top quadrupole Orbitrap mass spectrometer (Q Exactive, Thermo Scientific) in data-dependant $MS^2$ acquisition mode under both positive and negative electrospray ionization conditions. Different Orbitrap resolutions (17.5K, 35K, 70K & 140K) were used. Acquired data were processed and compared using LipidSearch software (Thermo Scientific). **Preliminary Results:** For initial RPLC experiments, a 2.1 x 100 mm Ascentis C18 column was employed. Several gradient conditions were used to evaluate the separation efficiency. LipidSearch software was used for lipid identification through a database search of the accurate masses of precursors and the fragment ions predicted for each potential adduct form of the lipids in the database (> 1,500,000 entries). The number of lipid species identified in each different experiment were assessed at the sum composition (MS) and isomer (MS-MS) levels. The higher MS resolution enabled higher lipid identification coverage.

## 25. Novel inhibitors of cytosolic Group IVA phospholipase $A_2$ ($cPLA_2$) ameliorate collagen induced arthritis

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**Background:** Rheumatoid arthritis (RA) is an inflammatory disease of the joint characterized by chronic synovitis causing pain, swelling and loss of function due to destruction of cartilage and bone. The complex series of pathological events occurring in RA is largely regulated via excessive production of pro-inflammatory cytokines, the most prominent being tumor necrosis factor (TNF). TNF is a potent inducer of synoviocyte cPLA2$\alpha$[1]. **Objectives:** Determine if cPLA2$\alpha$ inhibitors AVX001 and AVX002[2] reduce parameters of arthritis progression and severity in prophylactic and therapeutic models of collagen-induced arthritis (CIA) in male DBA/1 mice. **Methods:** In the prophylactic study, treatment with AVX001 and AVX002 started one hour before the second challenge of type II collagen. In the therapeutic study, treatment started 7 days after the second collagen challenge. Methotrexate (MTX) and Enbrel were included as anti-rheumatic drug controls in the prophylactic and therapeutic studies, respectively. DMSO was included as vehicle control. Intraperitoneal injections of AVX001 and AVX002 were given daily for the first 4 days and then every second day, while MTX and Enbrel were administered daily and twice weekly, respectively. Disease activity and the effects of treatment were monitored by determining arthritis index (paw swelling), recording of histopathological scores and by measuring plasma level of PGE2. **Results:** Arthritis was evident in collagen-treated animals, and disease severity was significantly reduced with Enbrel or MTX treatment (reduction of AI by 42 % and 38 %, respectively). Treatment with cPLA2 inhibitors AVX001/2 significantly reduced AI by 54% in the prophylactic model and by 60% in the therapeutic model. Several parameters of joint damage were significantly reduced by between 75-90% in response to AVX001/2 treatment as evaluated by histopathology; articular cavity inflammatory cell infiltration, synovial hyperplasia, articular cartilage damage, and periosteal/endochondral ossification compared to ctr. mice, suggesting disease modifying properties of AVX compounds. The plasma PGE2 level was also sign. reduced. **Conclusion:** The cPLA2$\alpha$ inhibitors AVX001 and AVX002 significantly and potently improved the clinical course of arthritis and reduced the production of inflammatory and analgesic PGE2 in murine CIA. These data suggest that cPLA2$\alpha$ inhibitors, such as AVX001 and AVX002, are potent DMARDs and have the potential to be developed as novel, specific, small molecule, anti-rheumatic human therapeutics.

*[1]Sommerfelt, R. et al PLoS One 2013 Dec 12;8(12):e83555. [2]Huwiler, A. et al Br J Pharmacol 2012;* ***167:****1691*

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## 26. Sphingosine 1-phosphate counteracts hepatic insulin-sigaling *in vitro* and *in vivo*

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The liver plays a central role in glucose homeostasis and metabolism of lipids and amino acids. A chronic lipid oversupply of the liver is accompanied by excess lipid deposition called steatosis, an early form of nonalcoholic fatty liver disease (NAFLD). There is close correlation between hepatic steatosis and the development of insulin resistance.Recently, we have shown that palmitate induces an impressive formation of extra- and intracellular sphingosine 1-phosphate (S1P) in primary rat and human hepatocytes. In analogy to palmitate, S1P is able to counteract insulin signaling. Thus, insulin-mediated glucokinase expression as well as Akt-phosphorylation is significantly diminished in the presence of S1P. The inhibitory effect of S1P is receptor-dependent and is abolished in the presence of the $S1P_2$-receptor antagonist JTE-013 *in vitro.* Therefore, we examined the role of $S1P_2$-signaling on insulin resistance in a high fat diet (HFD) fed New Zealand Obese (NZO) mice model. As expected, there was a drastic increase of blood glucose levels in HFD- compared to standard diet (SD)-fed mice over a time period of 4 weeks. Most interestingly, after treatment of HFD-fed mice with JTE-013 glucose levels did not further increase compared to vehicle-treated HFD-fed mice. To proof whether JTE-013 mediates this action via improvement of hepatic insulin resistance, Akt-phosphorylation in the liver was measured after insulin injection. Indeed, Akt-phosphorylation was significantly decreased in HFD- compared to SD-fed mice. Treatment with JTE-013 partially restored insulin-mediated Akt-phosphorylation in HFD-fed mice. Moreover, S1P determination in the liver revealed a significant increase of the sphingolipid in HFD- compared to SD-fed mice. On the contrary $S1P_2$-receptor expression in all three groups were unchanged. These data indicate that modulation of $S1P_2$ is of potential interest as therapeutic target to prevent hepatic insulin resistance.

## 27. Nat1 ablation contributes to increased lipolysis and mitochondrial impairment in 3T3-L1 adipocytes

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Our recent genome-wide association studies (GWAS) have identified N-acetyltransferase 2 (*NAT2*) as a new candidate gene for insulin resistance. *NAT2* encodes a xenobiotic metabolizing enzyme which catalyses biotransformation of various drugs and carcinogens. The aim of this study was to evaluate the role of *NAT2* and its association with the development of insulin resistance. In this study, we demonstrated that silencing of *Nat1* (the mouse homolog to *NAT2)* in differentiated 3T3-L1 adipocytes resulted in increased basal and isoproterenol-stimulated lipolysis and reduced insulin-stimulated glucose uptake. In addition *Nat1* knockdown significantly decreased the expression of adipogenic marker genes and adipocyte differentiation. Furthermore, *Nat1* silencing led to increased production of intracellular of reactive oxygen species (ROS), decreased mitochondrial membrane potential, mitochondrial biogenesis and mitochondrial content in 3T3-L1 adipocytes. Collectively, our results suggest that Nat1 knockdown increased lipolysis and mitochondrial dysfunction in adipocytes, which might be responsible for the development of insulin resistance.

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## 28. Microfluidics lipidomics using a novel integrated mass spectrometry technology

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Lipidomics heavily relies on the development of new technologies for the comprehensive analysis of hundreds of lipid species in biological samples. The ability to measure the wide array of lipid species in biological samples could help our understanding of their roles in health and disease. The need for a fast, comprehensive and sensitive analysis of the hundreds of lipid species challenges both the chromatographic separation and mass spectrometry. Here we used a novel integrated microfluidics-mass spectrometry technology packed with C18 1.7 µm particles for fast and robust targeted lipid analysis. By integrating microscale LC components into a single platform design, the device avoids problems associated with capillary connections, including manual variability, leaks, and excessive dead volume. Mobile phases and analysis times were similar to regular LC methods using analytical-scale columns. Data was collected using MS systems (Q-Tof and triple quadrupoles) operated in both negative and positive mode in the data independent acquisition mode. Lipidomics analyses were conducted using small volumes of standards and organic extracts from typical biological samples including plasma, brain, heart and liver.We identified and quantified 215 lipid species belonging to various lipid classes including phosphatidylethanolamines (PE), lyso PE, phosphatidylcholines (PC), lyso PC, ceramides (Cer), sphingomyelins, hexosylceramides, lactosylceramides and cholesterol esters. Lipids were measured over approximately five orders of dynamic range. Lipids were separated according to acyl chain length and number of double bonds. The small column diameter (150 µm) of the microfluidic device allowed low injection volumes (0.2-0.5 µl) and low flow rates (2-5 µl/min) increasing up to 10x the sensitivity compared to regular analytical columns (e.g., 2.1 mm ID). Mobile phase consumption was reduced about 200X compared to 2.1 mm ID chromatography albeit maintaining comparable chromatographic resolution and analysis times. Potential applications include large-scale lipid profiling and low-abundance lipid analyses in biological materials.

## 29. Protection against fatty acid- induced lipotoxicity: modeled microgravity as a tool for the identification of novel pathways

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The NIH National Heart, Lung and Blood Institute defines the “metabolic syndrome” as a group of risk factors associated with an elevated risk for insulin resistance, diabetes and coronary heart disease. These diseases are projected to cost $48–66 billion/year in the USA by 2030. Metabolic syndrome is directly linked to an excess of free fatty acids in non-adipose tissues, such as pancreas, heart, and skeletal muscle. When these free fatty acids (FFAs) overwhelm the cell, it results in the formation of reactive lipid species that promote cellular dysfunction (lipotoxicity) and programmed cell-death (lipoapoptosis). One mechanism by which the cell handles excess FFAs is through their acylation, which leads to the formation of neutral lipids that can be stored in lipid droplets (LDs). Long considered to be central to the pathogenesis of metabolic diseases, there is now evidence that LDs can protect against lipotoxicity.Microgravity can be achieved during spaceflight or other modeled conditions, significantly alters numerous biological processes in organisms from bacteria to humans. This led us to observe that in microgravity conditions, cells and organisms *naturally adapt* to this environment by increasing LD generation. We have analyzed the expression of 113 genes associated with lipid droplet morphology under the Gene Ontology (GO) term “lipid particle morphology” in the Saccharomyces Genome Database Gene Ontology Database. Using microarray data obtained from yeast cells grown in modeled microgravity from the Gene Expression Omnibus (NIH) we confirmed that 26% of genes associated with “lipid particle morphology” show more than a 1.5 fold change in expression compared to cells grown in normal gravity.We hypothesize that enhancement of LD formation can be used as a mechanism of protection against FFA- induced lipotoxicity. We will explore this hypothesis by elucidating the molecular events that lead to increased LD formation in microgravity conditions. If these as yet unexplored mechanisms can be molecularly recreated in normal gravity conditions, we can potentially identify of pathways that can be used to create targets for the management FFA-induced lipotoxicity.

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## 30. Bromodomain and extraterminal (BET) family is a key mediator for Ox-PAPC induced inflammatory gene responses in the vascular endothelium

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Atherosclerosis is a chronic inflammatory disease initiated with monocyte recruitment into the vessel wall. Ox-PAPC (oxidized 1-palmitoyl-2-arachidonyl-sn-glycerol-3-phosphorylcholine) was identified as bioactive component of minimally modified low-density lipoprotein (MM-LDL) that accumulates in subendothelial space under hyperlipidemic condition. Ox-PAPC strongly activates vascular endothelial cells and regulates more than 1,500 genes including proinflammatory and prothrombotic genes IL-8, MCP1, and tissue factor (F3). Bromodomain and extraterminal (BET) family is a reader for histone acetylation signals written by endogenous and exogenous proinflammatory agents. Highly specific and potent BET inhibitors recently developed showed strong repression of inflammatory responses both in vitro and in vivo systems. In this study we tested whether BET family is a mediator for the inflammatory signals induced by Ox-PAPC in the vascular endothelial cells. First, in human aortic endothelial cells (HAECs) the BET inhibitors (JQ1(+) and negative control JQ1(-), I-BET, I-BET151) and siRNA targeting a member of BET family BRD4 showed vary potent inhibition of inflammation related gene expressions by Ox-PAPC. Next, we expanded this study to microarray analysis to obtain the full list of Ox-PAPC regulated genes inhibited by BET inhibitor JQ1(+) cotreatment. The gene list showed significant enrichment of genes in categories of inflammation, apoptosis, and cell death as determined by gene ontology (GO) and KEGG pathway analysis. In comparison of the role of BET family on Ox-PAPC versus LPS we found significant overlapping. In conclusion, in this study we showed that BET family, which is a reader of the histone acetylation, is a key mediator for the inflammatory responses induced by Ox-PAPC in the vascular endothelial cells with selectivity. Targeting of BET family could be an efficient approach to specifically modulate atherosclerosis and the other inflammatory vessel diseases correlated with hyperlipidemia.

## 31. Effect of insulin resistance on plasma and tissue fatty acid profile in severely obese subjects

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Growing evidence suggests that specific plasma fatty acid (FA) pattern may be indicative of insulin resistance (IR) as saturated, *trans* and omega (n)-3/n-6 FAs ratio enhance inflammation. However, the role FA composition of important metabolic tissues have not the focus of most of the investigations. **Objective:** To establish whether (i) IR is associated with FA composition in the intestine, liver and adipose tissue of obese subjects; (ii) the FA changes in tissues are related to those observed in plasma; and (iii) FA profile is related to specific desaturases. **Methods:** Duodenal, hepatic and fat specimens were obtained from 18 obese subjects undergoing bariatric surgery with either low or high IR. Plasma and tissue FA compositions were measured using gas chromatography following total lipid esterification. **Results:** Plasma of insulin-resistant subjects was mainly characterized by an increased amount of total FA, a higher proportion of saturated FA and a lower proportion of polyunsaturated FA. Similarly, we observed an increased amount of total FA in duodenum and liver of insulin-resistant subjects as compared to insulin-sensitive subjects. These increases were mainly explained by higher levels of saturated FA. The proportion of polyunsaturated FA, especially n-3, was reduced in liver and in subcutaneous adipose tissue, but not in duodenum, of insulin-resistant subjects. Importantly, eicosapentaenoic (EPA) and docosahexaenoic (DHA) as well as the n-3 to n-6 ratios were positively associated with IR in the liver but not in the plasma and other tissues. Finally, we noticed that the estimated delta 9-desaturase activity (product/precursor ratio) was increased only in the liver of insulin-resistant subjects. **Conclusion:** Several alterations in plasma and tissue FA composition were present in obese subjects with IR, but many disparities between tissues were noted. Plasma and hepatic saturated FA as well as hepatic n-3 FA were significant correlates of the Ir state in human.

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## 32. The effects of ozone therapy on serum neopterin level and necrosis in a rat model of acetaminophen induced liver injury

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Acetaminophen (APAP) is a widely used agent for its analgesic and antipyretic actions. Therapeutic dose of APAP is considered to be safe while its overdoses produce severe centrilobular liver injury that can lead to fatal fulminant hepatic failure. Elevated serum alanine aminotrasferase and aspartate aminotrasferase activities indicate liver damage. Although the elevated activities of these enzymes are indicators of hepatocellular damage, they are poor prognostic indicators for the severity of the liver injury or acute liver failure. It was shown that serum neopterin, a marker for immune system activation, levels were elevated in a rat model of acetaminophen induced liver injury. Increased neopterin levels were correlated with the dose of acetaminophen. Ozone therapy (OT) is shown to reduce inflammation and necrosis in several entities. This study is designed to evaluate the efficacy of ozone therapy in a rat model of APAP-induced liver injury. Serum neopterin levels were measured with HPLC-FD device. Phosphate buffer (0.015 M, pH 6.4) was used as mobile phase (isocratic elution) at a flow rate of 0.8 ml/min. APAP administration caused necrosis in the liver after 24 h. In the APAP group, serum neopterin concentrations (14.7±1.4 nmol/L) were significantly increased compared to other groups (in both, $p<0.05$). OT administration reduced both necrosis and neopterin levels significantly (7.5±2.4 nmol/L) ($p<0.05$) but still higher than the sham group (4.8±1.2 nmol/L) ($p<0.05$). Our results showed that ozone therapy prevented liver necrosis in rats and reduced neopterin levels. These findings suggest that the use of OT as an adjuvant therapy which might improve the outcome in APAP induced liver injury.

## 33. Eicosanomic profiling reveals dominance of the epoxygenase pathway in human amniotic fluid at term in spontaneous labor*

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Lipid mediators play an important role in reproductive biology, especially, in parturition. Enhanced biosynthesis of eicosanoids such as prostaglandin $E_2$ ($PGE_2$) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), precedes the onset of labor as a result of increased expression of the inducible cyclooxygenase (COX-2) in placental tissues. Metabolism of arachidonic acid results in bioactive lipid mediators beyond prostaglandins that could significantly influence myometrial activity. Therefore, an unbiased lipidomic approach was used to profile the arachidonic acid metabolome of amniotic fluid. Using liquid chromatography – mass spectrometry methods, this study, for the first time, quantitatively enumerates these metabolites in human amniotic fluid by comparing patients at 1) mid trimester , 2) term but not in labor, and 3) term in spontaneous labor. In addition to exposing novel aspects of cyclooxygenase pathway metabolism, this lipidomic study revealed a dramatic increase of epoxygenase and lipoxygenase pathway derived lipid mediators in spontaneous labor with remarkable product selectivity. Despite their recognition as anti-inflammatory lipid mediators and regulators of ion channels, little is known about the epoxygenase pathway in labor. Epoxygenase pathway metabolites are established regulators of vascular homeostasis in cardiovascular and renal physiology. Their presence as the dominant lipid mediators at term in spontaneous labor portends a yet undiscovered physiological function in parturition.

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## 34. Lipidomics by infusion-based MS/MSALL reveals novel aspects of fatty liver disease

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Lipidomics is a new and expanding field of research driven in part through advances in analytical measurements made by the LIPID MAPS Consortium. The mammalian lipidome is complex, requiring dedicated analytical techniques to measure each lipid class. This presents a challenge to researchers as few laboratories have the resources or capability to perform such analyses. Infusion-based $MS/MS^{ALL}$ is a novel mass spectrometry technique that yields comprehensive measurement of the lipidome in a single analysis. During infusion of a bulk lipid extract (Bligh-Dyer extraction) a high resolution TOF spectrum is acquired followed by a product-ion spectrum at each unit mass (150-1200 Da). This produces a complete lipidomic mass spectral dataset that can be interrogated post-acquisition for any lipid class or lipid feature using neutral loss and product/precursor-ion relationships. Recent work has identified Ezetimibe as a possible therapeutic target for FLD, but only global changes in cholesterol and triglycerides were described. Here we present lipidomic analysis of mouse models for FLD during treatment with Ezetimibe. Using $MS/MS^{ALL}$ workflows, we measured glycerolipids, cholesterol, cholesteryl esters, and profiled the entire lipidome in mouse liver and plasma during treatment with Ezetimibe. Initially, over 300 lipids were identified from all major classes by querying the dataset with a combination of neutral loss, precursor ion, and fragment ion analysis. Unique changes in several lipid species were identified relative to diet and treatment in both fatty acid composition and degree of saturation. Utilization of a second targeted extraction procedure for the isolation of neutral lipids resulted in the identification of sterols esterified with oxidized fatty acids. Oxidized fatty acids were also observed in glycerolipids. Additionally, we were able to identify steryl esters originating from biosynthetic precursors of cholesterol, oxysterols, and plant sterols.

## 35. Lipidomic analysis of mouse brain using supercritical fluid chromatography-ion mobility-mass spectrometry

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Supercritical fluids possess density and extraction ability similar to liquid, but diffusivities similar to gas, which allows high-speed separation of complex biological mixtures. Ion mobility spectrometry rapidly separates ions (on the scale of milliseconds) based on their three-dimensional structures in the gas phase (ion-neutral collision cross section; Ω). When ion mobility is coupled with mass spectrometry, a two-dimensional separation is achieved on the basis of the charge-to-collision cross section ($z/\Omega$) and the mass-to-charge ($m/z$), respectively. Here we report the first integration of supercritical fluid chromatography (SFC; ACQUITY UPC$^2$, Waters Corp.) with ion mobility-mass spectrometry (IM-MS; Synapt G2-S HDMS, Waters Corp.) and its application in studying the lipidome of mouse brains. Lipid extracts of mouse brains (3-week old) were re-constituted in chloroform and were subjected to SFC-IM-MS analysis in both positive and negative ion mode. The mobile phase used is a gradient of supercritical $CO_2$ and methanol (with 0.1% ammonium formate). Under the optimal SFC condition, separation of over 10 classes of lipids was achieved within 10 minutes. We found that ion mobility separation is particularly useful in removing the chemical noise MS signals from the desired lipid signals, which significantly improved the signal-to-noise ratios of the chromatographic peaks. The identified lipids include sub-classes of phospholipids, sphingolipids, and sterols. In the positive ion mode, post-mobility fragmentation provides the head-group information to confirm the lipid classes. In the negative ion mode, fragmentation provides information on the fatty acids composition of each class of lipids. Thus, we present a rapid lipidomics strategy that combines the chromatographic advantage of SFC and the structural separation advantage of ion mobility.

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## 36. LIQUID: Lipid Informed Quantitation and Identification

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LIQUID (Lipid Informed Quantitation and Identification) is a software program that has been developed to enable users to conduct both informed and high-throughput global liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based lipidomics analysis. This newly designed desktop application can quickly identify and quantify lipids from LC-MS/MS datasets while providing a friendly graphical user interface for users to fully explore the data. Informed data analysis simply involves the user specifying an electrospray ionization mode, lipid common name (i.e. PE(16:0/18:2)), and associated charge carrier. The software searches the loaded dataset for evidence of the specified lipid and returns the highest scored matches and associated evidence for the user to view. For global analysis of an entire dataset, the user must load in a set of lipid targets. The software iterates over each MS/MS spectrum in the loaded dataset to find the best lipid match(es) for each. For both informed and global analysis, the software allows the user to select a result in the output table, which triggers a graphical display of all observed and theoretical lines of evidence used for identification. The primary evidence shown is a stem plot of the MS/MS spectra including colors and labels for peaks that match to fragments of the identified lipid. A stem plot of the isotopic profile and a line plot of the extracted ion chromatogram are also provided to show the MS-level evidence of the identified lipid. In addition to plots, other information such as intensity, mass measurement error, and elution time are also provided. Typically, a global analysis for 15,000 lipid targets is executed in less than 5 seconds and evidence of each lipid is immediately displayed to the user.

## 37. Dietary 1-deoxy-sphingolipids: a new concept in the dietary impact of sphingolipids

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Sphingolipids have been classically defined as having sphingoid base backbones that are 1, 3-dihydroxy, 2-amino-alkanes and –alkenes; however, humans and other organisms also produce sphingoid bases without the 1-hydroxyl group (Merrill, A.H., Jr. Chem Rev 111, 6387-422, 2011). These are very interesting structural variants because such compounds have been found in screens of aquatic organisms for anti-cancer agents, and one (1-deoxysphinganine, a.k.a. “spisulosine” and ”ES-285,” from the arctic surf clam *Spisula polynyma*) has undergone human phase I clinical trials (Vilar, E. et al. Invest New Drugs 30, 299-305, 2012); likewise, a synthetic sphingoid base analog (Enigmol) that lacks the 1-hydroxyl-group has anti-tumor efficacy for colon and prostate cancer in animal models (Symolon, H. et al. Mol Cancer Ther 10, 648-57, 2011). However, 1-deoxy-sphingoid bases have also been associated with inherited peripheral neuropathies (Penno, A. et al. J Biol Chem 285, 11178-87, 2010; Murphy, S.M. et al. Neurology 80, 2106-11, 2013), so their occurrence and effect(s) on health still need to be determined. To aid in their analysis, we have developed methods for identification and quantitation of 1-deoxy-sphingoid bases and their N-acyl metabolites using liquid chromatography, electrospray ionization tandem mass spectrometry and have begun to survey their occurrence in cells, tissues, and food as well as factors that influence their metabolism. These methods and our findings from analysis of selected foods, as well as studies of the uptake and metabolites of 1-deoxy-sphingoid bases and a 1-deoxy-sphingolipid analog (1-deoxy-1-fluoro-sphingosine) will be shown.

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## 38. Application of untargeted and targeted lipidomic/metabolomic study of mdr2+/-

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Heterozygous mutations in ABCB4 encoding the canalicular phospholipid transporter mdr3 are implicated in causing fibrosing cholangiopathies in adults. We recently reported that decreased hepatic expression of ABCB4 is associated with an inflammatory genetic signature in infants with biliary atresia. In neonatal mice heterozygous for ABCB4 deficiency ($ABCB4^{+/-}$), challenge with rhesus rotavirus resulted in worse cholestasis and up-regulation of hepatic expression of the pro-inflammatory cytokines IFNγ and TNFα compared with age-matched, wild-type (WT) controls. Here, we examine mechanisms by which ABCB4 heterozygosity confers risk to neonatal liver injury in mice. By applying an untargeted metabolomics (UPLC-Q-TOF) platform to analyze liver and plasma samples from 10-day-old WT, $ABCB4^{+/-}$ and $ABCB4^{-/-}$ mice, we discovered distinct metabolic profiles separating the three groups from each other. For instance, several related phosphatidylethanolamine species were found to accumulate in livers of $ABCB4^{+/-}$ compared with WT mice, whereas several triacylglycerol (TAG) species were decreased in $ABCB4^{+/-}$ animals. Furthermore, targeted analysis revealed hepatic accumulation of phosphatidylcholine species in $ABCB4^{+/-}$ mice. Interestingly, total hepatic and plasma phosphatidylcholine concentrations differed only between neonatal (day 10), but not between 30-day-old adult $ABCB4^{+/-}$ and WT mice. Untargeted metabolomics study of neonatal plasma revealed low levels of two resolvin species in the $ABCB4^{+/-}$ compared with WT mouse. We conclude that subtle changes in expression of the canalicular phospholipid transporter, i.e., through heterozygous mutations in the encoding genes, may lead to significant changes in phospholipid homeostasis in the neonate, conferring temporarily restricted susceptibility to inflammatory hepatobiliary disease, like biliary atresia.

## 39. Apoc2 knockout zebrafish model of hypertriglyceridemia *

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APOC2 is a protein found on VLDL and chylomicrons and is obligatory for lipoprotein lipase activity to hydrolyze plasma triglycerides. *APOC2* is one of the most frequently mutated genes in patients with familial hypertriglyceridemia. Hypertriglyceridemia is associated with acute pancreatitis and is an independent risk factor for atherosclerosis. However, there is no genetic mouse model for APOC2 deficiency and hypertriglyceridemia. Here, we describe an *apoc2* knockout (KO) zebrafish model of hypertriglyceridemia, created with the transcription activator-like effector nuclease (TALEN) technique. The *apoc2* mutants survive to adulthood and are fertile. Homozygous *apoc2* embryos have retarded development and show increased triglyceride and cholesterol levels in blood as detected by Oil red O and BODIPY staining and colorimetric assays. Interestingly, the number of blood cells in *apoc2* mutants is decreased starting from the 3$^{rd}$ day post-fertilization and this phenomena is persistent over time. Our findings suggest that apoc2 and/or lipoprotein lipase may play an important role in hematopoietic stem cell (HSC) specification and/or maintenance. The *apoc2* KO zebrafish can be a useful animal model to study mechanisms involved in the development of atherosclerosis, acute pancreatitis and, possibly, defects of hematopoiesis.

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## 40. AIBP inhibits inflammation and reduces foam cell formation

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ApoA-I binding protein (AIBP) is a conserved, ubiquitously expressed and secreted protein, with largely unknown functions. In a recent study, we have demonstrated that AIBP accelerates cholesterol efflux from endothelial cells (EC), decreases lipid rafts and inhibits VEGFR2 signaling, which in turn impairs angiogenesis in vitro and in zebrafish. The role of AIBP in atherosclerosis is unknown. Here we show that AIBP promoted cholesterol efflux from macrophages. This result led us to hypothesize that AIBP has an atheroprotective function. We generated a transgenic zebrafish with conditional expression of Aibp and fed them a high cholesterol diet. In the hypercholesterolemic transgenic zebrafish, induced expression of Aibp diminished vascular lipid accumulation. We also created an AIBP knockout mouse. Naïve AIBP-/- mice manifested increased expression of proinflammatory cytokines in the peritoneum compared to control mice. Peritoneal macrophages isolated from wild type and AIBP-/- mice were treated with mmLDL or OxLDL, and increased accumulation of free cholesterol and cholesterol esters was found in AIBP-/- macrophages. We further documented that AIBP was expressed in murine atherosclerotic lesions, likely in macrophages and vascular smooth muscle cells but not in EC. We performed a bone marrow transplantation experiment, transferring wild type or AIBP-/- bone marrow (BM) into irradiated LDLR-/- mice, and feeding recipient mice a high-fat diet. Mice that received AIBP-/- BM had increased plasma IL6 protein and peritoneal MCP-1 mRNA levels compared to control recipients. Furthermore, IgM Ab titers to MDA-LDL were higher, and IgM Ab titers to Cu-OxLDL and mmLDL trended higher in mice that received AIBP-/- BM. Collectively, the data demonstrate that AIBP regulates macrophage cholesterol efflux, vascular lipid accumulation, inflammatory and immune responses. Further studies in systemic AIBP null mice on an atheroprone background are warranted to determine the role of AIBP in atherosclerosis. Our studies of the roles of AIBP in atherosclerosis may lead to development of therapeutic approaches for prevention and treatment of atherosclerotic cardiovascular diseases.

## 41. Apolipoprotein A-I Binding Protein deficiency promotes development of high-fat diet-induced metabolic syndrome

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Apolipoprotein A-I Binding Protein (AIBP) is a conserved protein that is found in the mitochondria, cytoplasm, and nucleus, in addition to being secreted. Our laboratory recently demonstrated that extracellular AIBP-mediated cholesterol efflux controls angiogenesis in zebrafish. While secreted AIBP has been shown to regulate cholesterol efflux, little is known about its intracellular functions. To investigate these, our laboratory has generated *Aibp*$^{-/-}$ mice, which are viable and fertile. A recent paper suggests that AIBP is an NAD(P)H-hydrate epimerase, giving it a presumptive role in mammalian NAD+ homeostasis. We hypothesized that by maintaining an appropriate cellular NAD+ level, AIBP indirectly regulates SIRT1, an NAD+-dependent histone deacetylase with targets including inflammatory and metabolic regulators such as NF-κB, AP-1, and Akt. SIRT1 expression and function were decreased in *Aibp*$^{-/-}$ mice, as determined by *Sirt1* mRNA transcript levels and acetylation status of SIRT1 targets. The presumptive role of AIBP in NAD+ homeostasis indicates a link between energetic status and AIBP activity, suggesting that metabolic disturbances should exacerbate the effects of *Aibp* knockout. Indeed, *Aibp*$^{-/-}$ mice fed a 45% high-fat diet (HFD) gained significantly more weight than wild type mice, despite consuming a similar quantity of food. In contrast, chow-fed knockout mice did not weigh more than their wild type counterparts. On the HFD, knockout mice also had decreased VO2 consumption and VCO2 production, but did not exhibit differences in heat production or respiratory exchange ratio. HFD-fed *Aibp*$^{-/-}$ mice were also glucose intolerant and had increased M1 pro-inflammatory macrophage infiltration in their white adipose tissue, indicating the development of metabolic syndrome. A better understanding of AIBP's regulation of inflammation and metabolism will provide new mechanistic insights and therapeutic targets for metabolic disorders.

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## 42. Alterations of lipid metabolism in preeclampsia: lipid characterization in the maternal circulation and placenta

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Preeclampsia (PET) is linked to obesity and increased adipocyte lipolysis, and is a leading cause of pregnancy-related morbidity. Women with PET have reduced high-density lipoprotein (HDL) levels and increased plasma triacylglycerol (TG), low-density lipoprotein (LDL) and non-esterified fatty acid levels compared to healthy pregnancy. In women with PET their maternal erythrocytes and fetal cord blood have lower polyunsaturated fatty acid (PUFA) levels compared to healthy controls, suggesting a reduced supply of PUFA in the fetal circulation. To investigate changes in lipid metabolism in PET, we quantified apolipoprotein B (apoB), TG and total cholesterol (TC) in maternal very low-density lipoprotein (VLDL) isolated from plasma of PET and control subjects. Fatty acids (FA) extracted from VLDL particles were quantified by gas chromatography (GC). Quantitative lipidomics was performed on placental samples. Lipids were extracted from placental tissue and analyzed using an AB Sciex QTRAP 5500 with an Advion Nanomate nano-electrospray source. Targeted precursor-ion and neutral-loss scans were used to identify and quantify approximately 120 lipid species. Maternal VLDL apoB and therefore VLDL particle number was doubled in PET compared to controls. TG and TC levels were also substantially higher in PET patients. However, TG and TC levels per VLDL particle were unchanged. This suggests the maternal system increases VLDL synthesis in PET, but the composition is unchanged. This was supported by VLDL FA quantification, which showed unchanged FA levels per particle. In placental tissue, quantification of seven lipid classes, including cholesteryl ester, free cholesterol, TG, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin was performed. Preliminary analysis indicates higher total lipid levels in PET placenta compared to controls. This included a build-up of storage lipids, i.e. TG and cholesteryl ester, as well as increased phosphatidylcholine. Further data will be presented using principal component analysis to elucidate lipid species that contribute to variations between PET and controls.

## 43. Protection of mitochondrial function in mammalian cells by deuterated polyunsaturated fatty acids

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Oxidative stress is thought to contribute to etiology of a broad range of diseases (e.g., cardiovascular, neurodegenerative, immune, etc.). A critical step in its development is lipid peroxidation, initiated by hydrogen abstraction at bis-allylic sites of polyunsaturated fatty acyls (PUFA); this sets in motion a chain reaction that generates multiple toxic products. Synthetic PUFAs with hydrogens at the bis-allylic carbons replaced with deuterium (d-PUFAs) inhibit the chain process. Kinetic isotopic effect that slows down the abstraction step is assumed to be primarily responsible for this inhibition.

These mechanisms are of particular relevance to mitochondria, which are both a major source and one of the primary targets of oxidative stress. Therefore we tested protection of bioenergetic mitochondrial function by d-PUFAs. Bioenergetic status of intact cells was assayed using high content, high throughput, non-invasive extracellular flux analysis technique (Seahorse Biosciences). Triggers of oxidative stress with various modes of action (i.e., tert-butylhydroperoxide, ethacrynic acid and iron (II)) damage both mitochondrial membrane integrity and respiratory capacity. This damage can be almost completely prevented by incorporation into the cells of d-PUFAs with completely deuterated bis-allylic positions; deuteration of non-bis-allylic positions is ineffective. Paradoxally, partially deuterated linoleic acid that still carries one hydrogen atom at the bis-allylic position is also protective. Similarly, incorporation of PUFA mixtures in which just 20-50% are deuterated (fully or partially) are protective. The extent of this protection by partial bis-allylic deuteration is much greater that could be explained by “dilution” effect. These results suggest an existence of a secondary protective mechanism(s) additional to inhibition of abstraction off the bis-allylic sites.

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## 44. Identification of oxidized phospholipids in bronchoalveolar lavage fluid exposed to ozone

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Ozone is a powerful oxidant and a common pollutant known to cause adverse respiratory effects. Chemical reactions with unsaturated phospholipids in the respiratory tract lining fluid have been identified as one of the first important steps in the mechanisms mediating ozone toxicity. Thus, an essential part in understanding ozone toxicity is the identification of lipid ozonation products that derive from reactions of ozone with the phospholipid pool. In this study, we exposed human bronchoalveolar lavage (BAL) to low levels of ozone (60, 150 and 300 ppb) for 60 minutes. Ozonized BAL samples with and without derivatization were analyzed by liquid chromatography electrospray ionization tandem mass spectrometry in the full scan (positive and negative mode), precursor ion scan (*m/z* 184) and neutral loss mode (*m/z* 172). Data processing, including the selection of variables (peak labels containing *m/z* and retention time) unique for ozonized and derivatized samples, respectively, was accomplished using principal component analysis (PCA). Resulting PCA score plots showed a linear dose-dependent increase, with apparent changes in BAL samples exposed to as low as 60 ppb ozone compared to non-exposed BAL samples, and a clear separation between ozonized samples before and after derivatization. Corresponding loadings plots showed that more than 30 glycerophosphocholine species decreased due to ozonation and a total of 16 glycerophosphocholine and 5 glycerophosphoglycerol oxidation products were formed with the major part being identified as chain-shortened aldehyde products. The most abundant oxidation products included 1-palmitoyl-2-(9’-oxo-nonaoyl)-glycerophosphocholine and 1-palmitoyl-2-(9’-oxo-nonaoyl)-glycerophosphoglycerol.

## 45. Characterization and quantification of hopanoids in *Burkholderia multivorans* and *Rhodopseudomonas palustris* TIE-1 *

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Hopanoids are lipid molecules of the isoprenoid family found in some bacteria. They play a role similar to cholesterols from eukaryotes in helping cells to deal with environmental stresses such as high temperature, pH variations, and the presence of detergents or antibiotics. Furthermore, potential applications for hopanoids as biomarkers have been proposed because their carbon skeletons are well preserved in fossils dated back to at least 1.7 billion years ago. To better understand the biological roles of hopanoids and better interpret microbial footprints from ancient fossil records, a robust method to quantify hopanoids and identify their localization in bacterial membranes is needed. Here we present the application of LC-MS/MS and GC-MS techniques for the identification and quantification of lipids in cell membranes from two organisms, *Burkholderia multivorans* and *Rhodopseudomonas palustris* TIE-1. Clinical isolates of *B. multivorans* from cystic fibrosis (CF) patients were treated with 256 μg/mL fosmidomycin, an antibiotic that targets the non-mevalonate pathway of isoprenoid synthesis, which is required for hopanoid biosynthesis. The treated cells showed up to 49% hopanoid decrease and a 64-fold decrease of the minimum inhibitory concentration of a membrane-disrupting antimicrobial agent, colistin, to as low as 8 μg/mL. This suggests blocking hopanoid biosynthesis could make colistin treatment of CF lung infections more effective. In addition, we isolated membranes from a model organism for hopanoid studies, *R. palustris* TIE-1. Extended ($C_{35}$) hopanoids such as bacteriohopanetetrol have different distributions between inner and outer membranes compared to short hopanoids ($C_{30}$) such as diplopterol. The application of robust lipidomic analyses to the study of hopanoids in diverse bacteria will improve our understanding of these molecules in both clinical and environmental contexts.

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## 46. Membrane homeostasis of *R. palustris* in the absence of hopanoids

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The characteristic carbon rings of hopanoid lipids are preserved over geological times and can be detected as molecular fossils in sedimentary rocks. Bacterial hopanoids are ancestral forms of cyclic triterpenoids and *in vitro* behave similar to sterols, yet their physiological functions remain poorly understood. Here we report the lipid composition of the anoxygenic phototroph *Rhodopseudomonas palustris* TIE-1, a model bacterium for studying hopanoid function. Liquid chromatographytandem mass spectrometry (LC-MS/MS) enabled the simultaneous detection of native hopanoids and other polar lipids, providing insights into the chemical milieu in which hopanoids occur. The deletion of genes for the biosynthesis of hopanoids caused distinct changes in the lipid composition, which were quantified for chemoheterotrophic and photoautotrophic growth conditions. These adaptations rationalize how the bacterium can grow in the absence of hopanoids and more broadly might also shed light on membrane homeostasis in bacterial species that do not utilize hopanoids. Based on this work the potential of lipidomics for the study of microbial communities in environmental samples is discussed.

## 47. Tight control of inwardly rectifying potassium channel activity through the balance between PI(4,5)$P_2$ and other anionic phospholipids in the membranes

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Inwardly rectifying potassium (Kir) channels regulate cell excitability and potassium homeostasis in multiple tissues. Crystal structures and our recent functional analyses together show that Kir2.1 channels have two distinct anionic lipid binding sites: the crystallographic PIP2 binding site at the C linker ('Primary) and a non-specific site at the N-terminal end of slide helix ('Secondary') where anionic lipid (PL(-)) binding is required for high $PIP_2$ sensitivity. Our previous docking results (D'Avanzo et al., 2013, Lee et al., 2013) suggested that, besides this synergistic mechanism, any PL(-)s could potentially play inhibitory roles by competiting with PIP2 at the 'Primary' site. To test this prediction we performed the following assays with purified human Kir2.1 channels reconstituted in synthetic liposomes. First, Kir2.1 channel activity was measured with a fixed $PIP_2$ level and increasing amounts of various PL(-)s. The level of inhibition correlated well with the binding affinity of these lipids at the 'Primary' site. The 'Secondary' PL(-) activatory site is primarily generated by residue K64. K64C mutant channels are insensitive to PL(-) and only weakly PIP2-actived, but high PIP2 sensitivity is regenerated by tethering of this residue to the membrane by decyl-MTS modification (Lee et al., 2013). The level of inhibition by PL(-) was therefore also compared between Kir2.1 Control and 'Secondary' site mutant (K64C, tethered to the membrane by decyl-MTS modification) channels. Inhibition by PL(-)s was now enhanced. This is consistent with the likelihood that PL(-) species are actually at limiting concentrations, such that PL(-) binding to the Primary site is augmented in these mutant channels as a consequence of the loss of the separate 'Secondary' PL(-) binding site. Such interplay between $PIP_2$ and other PL(-)s on Kir2.1 channel gating can be predicted by a mechanistic two-site binding model.

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## 48. Total lipid extraction made easy – the new BUME methods for rapid automated chloroform-free lipid extraction of biofluids and tissue samples

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**AIM:** The aim of this work was to develop new rapid and automated chloroform-free methods for total lipid extraction from biological samples and to replace the need for the laborious gold-standard chloroform-based method [1]. **METHODS:** Instead of building on current chloroform-based methods and trying to substitute chloroform we based the development work on the capabilities of a standard 96-well robot. For biofluids we defined the ideal automated protocol based on repeated solvent extraction steps with small volumes of solvents aiming at 100% automation. Non-chloroform solvents and solvent mixtures were then screened for high lipid recoveries, spontaneous and clear phase separation and full compatibility with automation. For tissue samples, our rapid semi-automated all-in-one-tube protocol for tissue collection, homogenization and extraction [2] was than evaluated for lipid extraction utilizing the developed lipid extraction protocol for bio-fluids [3]. Lipids investigated included cholesterol, cholesterol ester, triglyceride, diglyceride, phospholipids (PC, PE, PS, PA, PG, LPC), sphingomyelin, ceramide, dihydroceramides, and glucosylceramides. **RESULTS & DISCUSSION**: A fully automated protocol for biofluids – the BUME method - was developed delivering lipid recoveries identical to the Folch method for the investigated lipids for 10-100 µl biofluids in the 96-well format [3].The lipid extraction protocol was successfully applied to our generic protocol for tissue samples and a semi-automated all-in-one-tube process defined and validated [4]. The BUME method for tissue delivered identical lipid results for all investigated lipids except for PS, PA and PG where the BUME method delivered significantly higher recoveries than the Folch method. The developed BUME lipid extraction protocols now allow high-quality high-through-put sample preparation matching new fast lipidomics methods.

[1]Folch, J. et al (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biological Chemistry* ***226*** *(1):594-610*, [2]Bengtsson, C. et al (2011). Design of small molecule inhibitors of acetyl-CoA carboxylase 1 and 2 showing reduction of hepatic malonyl-CoA levels in vivo in obese Zucker rats. *Bioorg Med Chem.* ***19****(10):3039-53,* [3]Löfgren, L. et al (2012). The BUME method: a novel automated chloroform-free 96-well total lipid extraction method for blood plasma. *J. Lipid Research* ***53****(8):1690-700.*, [4]Löfgren, L. et al (2014). The BUME method – a new rapid and simple chloroform-free method for the extraction of total lipids from animal tissue. *Manuscript in preparation.*

## 49. Analysis of sterols and sterol derivatives by SFC-APPI-MS/MS

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**AIM:** High levels of plasma cholesterol are a major target for pharmacological research and drug development. Plasma cholesterol levels are related to intestinal absorption of dietary cholesterol, synthesis of new cholesterol, and elimination through bile acid synthesis and excretion. Cholesterol homeostasis is tightly linked to sterols and sterol derivatives both as receptor ligands and as biomarkers. The aim of this study was to show whether supercritical fluid chromatography coupled with atmospheric photoionisation tandem mass spectrometry would be a suitable complement to methods based on reversed phase chromatography and to explore its compatibility with atmospheric photo ionization mass spectrometry. **METHODS:** Plasma samples were analysed by liquid/liquid extraction (1) followed by supercritical fluid chromatography and tandem mass spectrometric detection, using atmospheric pressure photo ionization with methanol as moile phase B and make-up solvent. Xylene 2% was used as dopant. **RESULTS & DISCUSSION**: A new analytical methodology was successfully developed for the separation and detection of biomarkers of cholesterol homeostasis, using 25 µl of plasma sample. SFC-APPI-MS/MS showed baseline separation, low detection limits and reversed elution order compared with reversed phase UPLC-MS/MS. SFC-APPI-MS/MS was shown to be a suitable complimentary technique to UPLC-MS/MS due to its complete orthogonality vs the latter technique.

[1]Löfgren, L., M. et al. (2012) The BUME method: a novel automated chloroform-free 96-well total lipid extraction method for blood plasma. *J. Lipid Res*. **53**: 1690–1700.

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## 50. The dual effects of vitamin E on the degree of lipid peroxidation in the membrane system

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Membranes are vital part of all forms of life. Lipid peroxidation in membranes is of great importance because it modifies the structural and dynamic properties of the membranes, which in turn, influences the membranes' function. Lipid peroxidation in membranes plays a central role in many pathologic processes, including cancer, Alzheimer's disease, atherosclerosis, and type II diabetes. Vitamin E is an important lipid-soluble vitamin that acts as antioxidant and protects against lipid peroxidation in membranes. In this study, different amounts of vitamin E (0 mol%, 1 mol%, 2 mol%, 5 mol%, 10 mol%, and 20 mol%) are incorporated into the model membrane system to examine how vitamin E affects the degree of lipid peroxidation in the membrane using UV-VIS Spectroscopic technique. The data shows that as the amount of vitamin E increases in the model membrane system, the absorbance decreases. Henceforth, the degree of lipid peroxidation decreases. One remarkable feature of all biological membranes is their dynamic properties or fluidity. Therefore, this work is also focused on studying how the incorporation of different amounts of vitamin E into the membrane system affects the fluidity of the membrane utilizing Fluorescence Polarization (FP) Spectroscopic technique. The results indicate that as the amount of vitamin E increases in the membrane, the FP value increases. An increase in the FP value implies a decrease in the fluidity of the membrane. The attained results indicate that vitamin E serves as structural antioxidant. Vitamin E is highly effective at preventing lipid peroxidation because its structure decreases the availability of oxygen, as well as, creates a steric hindrance to the radical chain reactions. This study shows that the 1-2 mol% of vitamin E in the membrane is optimum to convey its dual function.

## 51. Aged related increase in secretory phospholipase A2 group II D is associated with an anti-inflammatory environment in the lungs

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Age-dependent defects in the immune system have been well documented and shown to correlate with worse outcomes after infections. In the 2002-2003 epidemic caused by the Severe Acute Respiratory Syndrome-coronavirus (SARS-CoV), more than half of aged patients (> 65 years) died, while no mortality was observed in patients under 24 years of age. We recently showed that higher levels of PGD2 were present in the lungs of aged mice compared to young mice, which resulted in a poor anti-virus T cell response and increased mortality. To study the factors associated with the increase in levels of PGD2 in aged mice, we used qRT-PCR to examine the mRNA levels of different phospholipases, COX-1, COX2 and PGD2 synthase and mass spectrometry to determine the levels of different eicosanoids in the lungs, before and after infection with SARS-CoV.
We found increased expression of the secretory phospholipase A2-IID (PLA2G2D) in the lungs of both naïve and infected aged compared to young mice and showed that these differences were primarily localized to cells expressing CD11c (alveolar macrophages and dendritic cells). Microarray analysis showed upregulation of many anti-inflammatory genes in the CD11c+ cells from the lungs of aged mice. Mass spectrometric analysis showed higher levels of different lipid mediators such as PGE2, PGF2a, 6 ketoPGF1a, AA, EPA and DHA in addition to PGD2 in the lungs of uninfected aged compared to young mice. Lipid profiling of SARS-CoV infected lungs revealed an increase in levels of various pro inflammatory lipid mediators in the lungs of young mice but not in aged mice. Also infected aged compared to young animals showed a higher ratio of anti-inflammatory to pro inflammatory lipid mediators in the lungs. Collectively, these results suggest that aged mice harbor an anti-inflammatory environment in the lungs compared to young mice and that this contributes to a non-protective immune response after SARS-CoV infection.

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## 52. Sphingosine kinase 1 regulates adipose proinflammatory responses and insulin resistance

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Adipose dysfunction resulting from chronic inflammation and impaired adipogenesis has increasingly been recognized as a major contributor to obesity-mediated insulin resistance, but the molecular mechanisms that maintain healthy adipocytes and limit adipose inflammation remain unclear. Here, we used genetic and pharmacological approaches to delineate a novel role for sphingosine kinase 1 (SK1) in metabolic disorders associated with obesity. SK1 phosphorylates sphingosine to form sphingosine 1 phosphate (S1P), a bioactive sphingolipid with numerous roles in inflammation. SK1 mRNA expression was increased in adipose tissue of diet-induced obese (DIO) mice and obese type 2 diabetic humans. In DIO mice, SK1 deficiency increased markers of adipogenesis and adipose gene expression of the anti-inflammatory molecules IL-10 and adiponectin, and reduced adipose tissue macrophage (ATM) recruitment and proinflammatory molecules TNF-α and IL-6. These changes were associated with enhanced insulin signaling in adipose and muscle and improved systemic insulin sensitivity and glucose tolerance in SK1-/- mice. Specific pharmacological inhibition of SK1 in WT DIO mice also reduced adipocyte and ATM inflammation and improved overall glucose homeostasis. These data demonstrate the importance of the SK1/S1P axis in adipose inflammatory responses and suggest the pathway could be an attractive target for the development of treatments for obesity-related metabolic disorders.

## 53. Characterization of the lipoxygenase-allene oxide synthase pathway in the stress responses of coral

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Natural and human-induced stress factors influence coral ecosystems worldwide. Representing an early evolutionary arm of *Animalia*, corals contain polyunsaturated fatty acids and the potential to express a repertoire of lipid signaling genes in response to stress. Using the easily cultured soft coral *Capnella imbricata* as the stress-response model, we have characterized genes of arachidonic acid metabolism and examined how their activity and expression are influenced by acute incision wounding and by thermal stress. By homology-based PCR, we cloned two cDNAs from *Capnella* encoding allene oxide-lipoxygenase (AOS-LOX) fusion proteins. The two isoforms were heterologously expressed in *E. coli* and their catalytic activities characterized by HPLC-UV, LC-MS, and NMR. Both isoforms initially convert arachidonic acid to 8*R*-hydroperoxy-eicosatetraenoic acid (8*R*-HPETE). AOS-LOX-a then forms the unstable 8,9-epoxy allene oxide, detected as its a-ketol hydrolysis product, whereas AOS-LOX-b exhibits a novel hydroperoxide lyase activity with formation of the cleavage product 8-oxo-(6*E*)-octenoic acid. Wounding of cultured *Capnella* results in elevated $\alpha$-ketol concentrations and decreased hydroperoxide lyase products. Thermal stress, the normal 23°C increased to 28°C (modest stress) or 31°C (severe), shows upregulation of *AOS-LOX-a* and the heat shock proteins *Hsp70* and *Grp78* as determined by qRT-PCR, in association with elevated $\alpha$-ketol concentrations. Moreover, transcriptome studies on reef-building corals report up-regulation of AOS-LOX mRNA in response to white band disease, elevated UV radiation and temperature. Taking into account all the data available, we suggest that the AOS-LOX pathway might be involved in general stress and survival response throughout *Cnidaria*.

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## 54. Lipidomic profiles of human adipose tissue associated with insulin resistance

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Insulin resistance is the pathological state where insulin action is impaired in target tissues such as muscle, liver, and adipose tissue. Obesity-induced insulin resistance is associated with adipose tissue inflammation and is a primary defect leading to type 2 diabetes. Insulin sensitivity can be improved with thiazolidinedione (TZD) administration, but the mechanism of how TZDs work in the body is not fully understood. Seventy-two human subjects participated in a three month TZD treatment study. We generated lipidomic profiles, rates of glucose disposal (Rd), and gene expression profiles on all subjects immediately before and after this time period. We developed a classifier model using elastic net regularization that classified subjects as insulin-resistant or insulin-sensitive, given their adipose tissue lipidomic profile (AUC = 0.935 pre-treatment, 0.850 post-treatment). The fatty acids that robustly contribute to the model are docosahexanoic acid (DHA, 22:6n3), myristic acid (14:0), arachidic acid (20:0), behenic acid (22:0), nervonic acid (24:1n9), and lignoceric acid (24:0), with DHA being the most heavily weighted component. These model components suggest that the presence of very long chain saturated fatty acids and the omega-3 polyunsaturated fatty acid DHA are positively associated with insulin sensitivity. With respect to polyunsaturated fatty acids, we observed acylated form-specific distributions, correlations with Rd, and sex-specific levels. On-going work includes ontology and pathway analysis on expression of genes correlated with individual lipid concentrations (Spearman coefficient >|0.5|), with the goal of relating baseline lipidomic profiles to insulin sensitivity after TZD intervention. We have initially found that adipose tissue inflammation is differentially associated with fatty acid subtypes, specifically, positively associated with very long chain omega-6 fatty acids and negatively associated with very long chain saturated fatty acids. We ultimately aim to drive towards a broader relationship between the lipidomic profile, gene expression, TZD effectiveness, and insulin resistance.

## 55. Effects of diet intervention on plasma eicosanoid profiles in obese human subjects

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Eicosanoids, the metabolic products of dietary polyunsaturated fatty acids (PUFAs), play a key role in modulating inflammation-related diseases. Previous studies show that specific dietary components can modify conditions such as insulin resistance, type 2 diabetes and cardiovascular disease. We conducted lipidomic analyses of plasma free eicosanoids and their parent omega-6 (n-6) and omega-3 (n-3) PUFAs in samples from a recent pilot randomized, controlled diet study. Thirty-nine obese subjects were randomized to one of two 12-week, 1500 kcal/day diet groups. The active diet included reduced glycemic index bread products, fish oil capsules (2.4g EPA+DHA/day), and delphinidin polyphenol capsules (300mg/day). Both diet groups lost fat mass but only the active diet group exhibited significant reductions in insulin resistance (HOMA-IR, $p<0.05$). Plasma samples from study weeks 0, 6, and 12 were analyzed via mass spectrometry to measure concentration of 41 lipid molecules. Statistical analysis of these lipids allows us to draw inferences about diet effects on n-6 to n-3 balance. We hypothesized that subjects consuming the active diet would express more anti-inflammatory eicosanoids derived from the n-3 PUFA supplements. We assessed intervention-induced change in arachidonic acid-to-EPA ratio and overall ratio of n-6 to n-3 molecules in subjects from each diet group. Bootstrap-based t-tests confirmed that both ratios fell significantly faster in the active vs. the placebo diet group ($p < 0.02$). The main drivers of this effect were that, relative to the placebo diet group, the active diet group exhibited a steady decrease in adrenic acid and an increase in EPA and its products 9- and 12-HEPE during the 12 weeks. This result demonstrates that an n-3 enriched diet induces measurable changes in plasma samples. On-going studies are targeted to assessing possible relationships between the lipids and other subject parameters (e.g., sex, ethnicity, fasting insulin) to identify molecules of interest for future study.

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## 56. LipoxinA4 and Benzo-LipoxinA4 attenuate adipose inflammation and rescue obesity-induced kidney disease *

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**Background**: Visceral obesity and adipose inflammation is considered a driving force of systemic disease, *e.g.* liver cirrhosis and chronic kidney disease (CKD). Inflammatory resolution is actively regulated by specialized pro-resolving mediators (SPMs), including the lipid $LXA_4$. Impairment of SPMs may underlie development of obesity-related pathology (Neuhofer,Diabetes,2013). Here we explored the therapeutic potential of $LXA_4$ in experimental obesity-induced systemic disease. **Method:** Male C57BL/6 or AdiponectinKO mice were fed a standard (10% fat) or HFD (60% fat) diet for 12 wks. HFD induced obesity and CKD, as evident by increased albuminuria. $LXA_4$ (5ng/g), benzo-$LXA_4$ analogue (1.7 ng/g) or the positive control AICAR (500 mg/g) were given intraperitoneal as interventional therapeutics 3 times weekly, between wk 5-12. **Results:** LXs mediated systemic protection against obesity-induced disease. Specifically, LXs attenuated CKD, as evident by a reduction of glomerular expansion, mesangial matrix and urine $H_2O_2$($p<0.05$), although renal MΦ infiltration remained unaffected. Furthermore, the interventional treatment attenuated liver hypertrophy and triglyceride accumulation. Unlike the positive control, LXs did not rescue obesity-induced impairment of glucose-tolerance, suggesting that protection occurred independent of rescued insulin-sensitivity. The adiponectin/AMPK pathway has been linked to obesity-induced disease, but the KO-study suggests that LX-mediated protection was not adiponectin dependent. Interestingly, LXs modified visceral adipose fibrosis, which may limit adipose hypertrophy and disease (Divoux,Diabetes,2010). A shift of MΦ phenotype has been suggested to be a key link between adipose inflammation and disease. LXs did not alter total number of adipose MΦ, but acted in a pro-resolving manner by increasing M2 MΦ (SFD 58%; HFD 28%; $LXA_4$ 43%, Benzo$LXA_4$ 40%). **Conclusion:** Collectively these data suggests that LXs, through reduction of inflammation and modulation of MΦ phenotype, may have therapeutic potential in obesity-induced pathologies, such as liver cirrhosis and CKD. ***Acknowledgment:*** *Financial support was obtained from Marie Curie IOF, National Institutes of Health and Science Foundation Ireland.*

## 57. Application of sequencing, fatty acid profiling, and metabolomics investigation to explore pathogenesis and treatment strategy for anorexia nervosa *

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Individuals with anorexia nervosa (AN) restrict eating and become emaciated. AN tend to have an aversion to foods rich in fat. Epoxide Hydrolase 2 (*EPHX2*) was uncovered as a novel AN susceptibility gene, through a series of complementary genetic study designs (GWAS, exon-based sequencing, single-locus association and replication studies) in 1205 AN and 1948 controls. We assessed lipidomics and metabolomics targets of *EPHX2* to evaluate the biological functions of *EPHX2* and their influence on AN risk. *EPHX2* codes for soluble epoxide hydrolase (sEH) which converts epoxides to the corresponding diols. We measured both the polyunsaturated fatty acids (PUFAs) substrates of sEH as well as associated eicosanoids in AN and gender- and race-matched controls using the GC/MS and LC/MS/MS systems. PUFA and eicosanoid markers were tested as potential biomarkers for AN, whereas eicosanoid ratios (e.g. DiHETrE-to-EpETrE ratios) were calculated as proxy markers of *in vivo* sEH activity. Several free- and matrix-bound forms of PUFAs were differentially elevated in ANs compared to controls. PUFA ratios further confirmed that AN displayed elevated n-3 PUFAs and may differ from controls in PUFA elongation and desaturation processes. A number of eicosanoid markers from precursor ARA, LA, ALA, and DHA PUFAs were associated with AN risk, and the diol:epoxide ratios suggest the sEH activity is higher in AN compared to controls. We identified the presence of biomarker-to-disease interactions, which further support the potential utility of these markers as prognostic biomarkers for AN. This study shows that *EPHX2* influences AN risk through biological interaction with the PUFA pathway and both PUFAs and sEH activity contribute to the pathogenesis of AN. It demonstrates that joint investigation of genetic risk factors with their biological non-genetic partners (e.g. diet, stress) can unravel mechanistic functions and may lead to improved understanding of pathophysiology and new treatment strategies for AN.

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## 58. Monoacylglycerol lipase mediates fever via hypothalamic prostaglandin $E_2$ production

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Hypothalamic prostaglandin $E_2$ ($PGE_2$) mediates fever through EP3 prostaglandin E receptor. Metabolic pathway for $PGE_2$ production involves cyclooxygenase 2 (COX-2) and microsomal prostaglandin E synthase 1 (mPGES1), but the source of precursor arachidonic acid (AA) has not been confirmed. We performed lipopolysaccharides (LPS)-induced fever model using cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$) knockout (KO) and monoacylglycerol lipase (MGL) KO mice to determine which of the two precursors, phospholipids or 2-arachidonoylglycerol (2-AG), is critical in fever mechanism. Febrile response upon LPS administration was abolished in MGLKO mice, whereas it was normal in $cPLA_2\alpha$KO mice, suggesting MGL is critical in febrile reaction. Involvement of the cannabinoid receptor type 1 (CB1) was examined by analyzing CB1KO and CB1-MGL double KO mice. The results demonstrated that 2-AG-CB1 pathway is not involved in fever. Increase in blood cytokines (TNFα, IL1β and IL-6) were similar in MGL WT and KO mice, but hypothalamic $PGE_2$ levels were decreased in MGLKO mice. Moreover, intracerebroventricular injection of $PGE_2$ caused fever normally in MGLKO mice. Thus, our results demonstrate that MGL mediates fever via hypothalamic $PGE_2$ production.

## 59. Development of glycerophospholipid profiling methods using ternary gradient liquid chromatography/high-speed triple quadrupole mass spectrometry

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Glycerophospholipids are major components of cellular membranes and changes in profile of the glycerophospholipids may reflect various cellular functions and properties. Based on the polar headgroup at the *sn-3* position of the glycerol backbone, glycerophospholipids are divided into distinct classes and each class consists of numerous molecular species due to a variety of the hydrocarbon chains and the type of linkage to the glycerol backbone at *sn-1* and *sn-2* positions. Typically, two liquid chromatography/mass spectrometry (LC/MS) strategies have been used for global phospholipid profiling. One is head-group selective MS/MS (precursor/neutral loss) scan analysis by triple quadrupole mass spectrometers. The other is accurate-mass based analysis by time-of-flight mass spectrometers or Fourier-transform mass spectrometers. Selected-reaction monitoring (SRM) -based method by triple quadrupole mass spectrometer is preferred for sensitive and accurate analysis and has been used for targeted analysis to quantify limited number of molecules but not explored as a major strategy for global phospholipid profiling due to its difficulty in target coverage. Recent advances in high-speed triple quadrupole mass spectrometer have provided the means for rapid and sensitive analyses of cellular lipids. Here, we evaluate scan-based and SRM-based global phospholipid analyses using LC/MS methods with ternary gradient LC system and high-speed triple quadrupole mass spectrometer and discuss the potentials of these methods.

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## 60. Deficiency of monoacylglycerol lipase attenuates diet-induced obesity in an endocannabinoid system-independent manner

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Monoacylglycerol lipase (MGL) is an enzyme that hydrolyzes monoacylglycerols to produce a free fatty acid and glycerol. The enzyme is known to play a role in degrading 2-arachidonoylglycerol (2-AG), an endogenous ligand for cannabinoid type 1 receptor (CB1) in the central nervous system, and has been suggested to regulate CB1-mediated endocannabinoid functions including food intake and energy comsumption. MGL is also expressed in the peripheral tissues and may have some functions dependently or independently of CB1 signaling, but such mechanisms have not been fully demonstrated so far. Here, we examined a possible role for MGL in lipid homeostasis using knockout mice.

MGL knockout mice were resistant to high fat diet-induced obesity, glucose intolerance and fatty liver, despite normal food intake. To determine whether the phenotype depends on CB1 signaling, we prepared a double-knockout mice for CB1 and MGL. Although CB1 deficiency by itself prevented obesity, double knockout mice showed an additional effect, suggesting a CB1-independent mechanism. We examined fat absorption upon oral fat administration and found that MGL knockout mice have reduced triglyceride uptake into plasma, which was apparently CB1 independent. Thus, our results demonostrate a possible important role for MGL in lipid absorption.

## 61. Expression of diacylglycerol kinase theta during the organogenesis of mouse embryos

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Diacylglycerol kinase (DGK) is an enzyme that phosphorylates diacylglycerol (DG) to produce phosphatidic acid (PA). In other words, the enzyme can attenuate PKC activity by reducing the amount of DG and activate several enzymes including mTOR and PIP5K by producing PA. In addition, the DG/PA turnover by DGK affects contents of other many lipids including phosphatidylinositol and phosphatidylinositol phosphates. So far, ten mammalian isoforms of DGK have been cloned and divided into five groups. One of the isoforms, DGKθ has a unique domain structure and is the sole member of type V DGK. However, physiological function of DGKθ is still unknown. Therefore, we performed immunohistochemical staining on paraffin sections of mouse embryos to reveal the spatial and temporal expression of DGKθ. At an early stage of development (E10.5 and 11.5), the expression of DGKθ was prominently detected in the brain, spinal cord, dorsal root ganglion, and limb bud, and was also moderately detected in the bulbus cordis and the primordium of the liver and gut. At later stages (E12.5 and 14.5), DGKθ expression persisted or increased in the neocortex, epithalamus, hypothalamus, medulla oblongata, and pons. DGKθ was also evident in the epidermis, and nearly all epithelia of the oropharyngeal membrane, digestive tract, and bronchea. At prenatal developmental stages (E16.5 and E18.5), the expression pattern of DGKθ was maintained in the central nervous system, intestine, and kidney, but was attenuated in the differentiated epidermis.These results suggest that DGKθ may play important physiological roles not only in the brain, but also in diverse organs and tissues during the embryonic stages.

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## 62. Oxidative stress induces endothelial dysfunction: Role of sterol regulatory element binding protein 2 and microRNA-92a

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Oxidative stress, elevated in pathophysiological conditions such as hypertension, hyperlipidemia, and aging affects endothelial homeostasis by impairing endothelial nitric oxide synthase (eNOS)-derived NO bioavailability and promoting inflammatory response. We recently demonstrated that atheroprone flow activates sterol regulatory element binding protein (SREBP) 2 and induces endothelial innate immunity, evidenced by NLRP3 inflammasome activation. Transgenic mice overexpressing endothelium-specific SREBP2 (EC-SREBP2) manifest accelerated atherosclerosis synergized by hyperlipidemia. In the current study, we investigated whether oxidative stress activates SREBP2 in endothelial cells (ECs), contributing to endothelial dysfunction. Several oxidative stress-inducing stimuli, i.e. hydrogen peroxide, Angiotensin II (AngII), and oxidized-LDL, all activates SREBP2 in ECs. Furthermore, SREBP2 transactivates microRNA-92a (miR-92a), previously shown to be dysregulated in ischemia and atherosclerosis. Chromatin immunoprecipitation, together with gain- and loss-of-function assays revealed that oxidative stress-activated SREBP2 induces miR-92a, which in turn targets several key molecules including Kruppel-like factor (KLF) 2, KLF4, and Sirtuin 1. As a result, NLRP3 inflammasome is activated and eNOS inhibited. In EC-SREBP2 mice, locked nucleic acid (LNA) inhibition of miR-92a improves vasodilatory function. In an AngII-challenged model, LNA-92a ameliorates atherogenesis in EC-SREBP2 with an $ApoE^{-/-}$ background. Collectively, these findings suggest a novel link between oxidative stress and the endothelial inflammation involving SREBP2-miR-92a-inflammasome. This newly defined pathway could be a therapeutic target to intervene endothelial dysfunction during the onset of atherosclerosis.

## 63. Peroxisomal Atg37 binds Atg30 or palmitoyl-CoA to regulate phagophore formation during pexophagy

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Autophagy is a membrane trafficking pathway that sequesters proteins and organelles into autophagosomes. The selectivity of this pathway is determined by autophagy receptors, such as the *Pichia pastoris* autophagy-related protein 30 (Atg30), which controls the selective autophagy of peroxisomes (pexophagy) through the assembly of a receptor protein complex (RPC). However, how the pexophagic RPC is regulated for efficient formation of the phagophore, an isolation membrane that sequesters the peroxisome from the cytosol, is unknown. Here we describe a new, conserved, acyl-CoA binding protein, Atg37, that is an integral peroxisomal membrane protein required specifically for pexophagy at the stage of phagophore formation. Atg30 recruits Atg37 to the pexophagic RPC, where Atg37 regulates the recruitment of the scaffold protein, Atg11. Palmitoyl-CoA competes with Atg30 for Atg37 binding. The human ortholog of Atg37, acyl-CoA binding domain containing protein 5 (ACBD5), is also peroxisomal and is required specifically for pexophagy. We suggest that Atg37/ACBD5 is a new component and positive regulator of the pexophagic RPC.

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## 64. Runx1-mediated hair follicle stem cell activation and skin tumorigenesis by regulation of lipid metabolism *

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Runx1 is a conserved transcription factor important for development and cancer. Runx1 is essential for hematopoietic stem cell and hair follicle stem cell emergence during embryogenesis, and for leukemia and skin squamous cell carcinoma (SCC) growth and survival. Moreover, our lineage tracing data show that Runx1-expressing hair follicle cells act both as long-term hair follicle stem cells as well as cells of origin for skin SCC. In normal adult skin homeostasis Runx1 promotes activation of hair follicle stem cells from G0 quiescence and timely hair growth. Recently, we demonstrated that during G0 quiescence Runx1 initiates stem cell conversion to a progenitor cell fate, more prone to respond to tissue growth signals. To understand how is this accomplished we performed microarray experiments in sorted hair follicle stem cells from mice induced for one day to express elevated levels of Runx1 in the skin epithelium. Runx1 elevation in stem cells resulted in broad gene expression changes, of which ~40 genes had been previously implicated in lipid metabolism. The latter can be classified in 6 sub-categories: (1) Fatty acids; (2) Cholesterol biosynthesis; (3) Prostaglandins; (4) Phosphoinositides; (5) Phosphatidylserine; and (6) Phosphocholine. We plan to examine global changes in lipid production in freshly skin isolated hair follicle stem cells, with Runx1 elevation or deletion. In addition, we will dissect the role of our candidate genes in modulating hair follicle stem cell lipidome and examine structural versus signaling roles. Very little is known about lipid regulation of early steps in tissue stem cell activation and differentiation in general, and virtually nothing is known about lipid regulation and hair follicle stem cells. However, some of our candidate genes play documented roles in cancer. These data provides a possible connection between Runx1 roles in stem cell activation/early differentiation and cancer cell growth/survival via regulating lipid metabolism.

## 65. Gender-specific prostaglandin production

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Lipid production may be different in males and females, indicating that males and females may require different therapeutic treatments during disease. Historically, medical studies have neglected the examination of females and, for this reason, biomedical science is less relevant to females than males. A purported rationale is that mechanisms that lead to disease are thought to be different to those involved in reproductive physiology and thus, differences between males and females are unimportant. Furthermore, it is expensive to study both males and females. We have previously found that female mice produce different levels of urinary prostaglandins compared to male mice, thus indicating a sex-related difference in lipid production. In this study, we used genetically modified male and female mice (*Funk et al.,* Nature Medicine 2006) that produce a COX2 enzyme containing a tyrosine to phenylalanine mutation at Tyr385 ($COX2^{Y385F}$). This mutation renders the enzyme unable to form a key intermediate radical required for complete arachidonic acid metabolism. We collected urine and elicited peritoneal macrophages from wild-type and $COX2^{Y385F}$ mice that were stimulated with lipopolysaccharide and interferon gamma. Our results measuring potential sex-related differences in prostaglandin production will be reported.

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## 66. Molecular structures of phospholipids with very long chain fatty acids in skin fibroblasts of Zellweger Syndrome

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The ratio of C26:0/C22:0 fatty acids in patient lipids is widely accepted as a critical clinical criterion of peroxisomal diseases, such as Zellweger syndrome and X-linked adrenoleukodystrophy (X-ALD). However, phospholipid molecular species with very long chain fatty acids (VLCFA) have not been precisely characterized. In the present study, the structures of such molecules in fibroblasts of Zellweger syndrome and X-ALD were examined using LC-ESI-MS/MS analysis. In fibroblasts from Zellweger patients, a large number of VLCFA-containing molecular species were detected in several phospholipid classes as well as neutral lipids, including triacylglycerol and cholesteryl esters. Among these lipids, phosphatidylcholine showed the most diversity in the structures of VLCFA-containing molecular species. Some VLCFA possessed longer carbon chains and/or larger number of double bonds than C26:0-fatty acid (FA). Similar VLCFA were also found in other phospholipid classes, such as phosphatidylethanolamine and phosphatidylserine. In addition, VLCFA-containing phospholipid species showed some differences among fibroblasts from Zellweger patients. It appears that phospholipids with VLCFA, with or without double bonds, as well as C26:0-FA might affect cellular functions, thus leading to the pathogenesis of peroxisomal diseases, such as Zellweger syndrome and X-ALD.