POSTER SESSIONS

The LIPID MAPS meeting features two poster sessions with 56 posters. Of these, six were selected for lightning talks (see program) by the topic chairs, H. Alex Brown (NAFLD) and Joseph L. Witztum (Oxidized Lipids). 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. **L-Carnitine effects nitric oxide production in RAW 264.7 macrophage cells after LPS stimulation**
   Fugen Aktan, Asli Koc (Ankara University, Turkey).

2. **Higher-efficiency lipid profiling system using a quadrupole Orbitrap mass spectrometer and an automated search engine lipid search**
   David Peake, Takayuki Yamada, Takato Uchikata, Shigeru Sakamoto, Yasuto Yokoi, Eiichiro Fukusaki, Takeshi Bamba (Osaka University, Japan).

3. **Development of lipid metabolism assays for understanding the importance of SREBPs in cancer**
   Joseph P. Argus, Kevin J. Williams, Moses Q.Wilks, Beth N. Marbois, Yue Zhu, Dominique N. Lisiero, Horacio Sato, Robert M. Prins, Steven J. Bensinger (University of California Los Angeles, CA).

4. **Epoxyisoprostane-containing lipids regulate the inflammatory pathway in endothelial cells ***
   James R. Springstead, Wei Zhong, Sangderk Lee, Benjamin Emert, Ramei Al-Mubarak, Michael Jung, Judith A. Berliner (University of California Los Angeles, CA).

5. **Effect of fluoride chronically administered in liver and plasma of rats treated with hypercaloric diet**
   Fabrício Soares Pereira, Aline Dionízio, Heloisa Aparecida Barbosa da Silva Pereira, Cesar Augusto de Souza Valle, Flavia Godoy Iano, Marília Afonso Rabelo Buzalaf (University of São Paulo, Brazil).

6. **Proteomic analysis of liver in rats chronically exposed to fluoride and AIN-93M - hypercaloric diet**
   Heloisa Aparecida Barbosa da Silva Pereira, Aline de Lima Leite, Aline Dionízio, Janete Gualiume Vaz Madureira Lobo, Camila Perez Buzalaf, Marília Afonso Rabelo Buzalaf (University of São Paulo, Brazil).

7. **Establishment of an in vitro hepatic steatosis model using mouse hepatocyte cell line FL83B**
   Mu-En Wang, Chih-Hsien Chiu (National Taiwan University, Taiwan).

8. **Inclusion of small amounts of isotope-reinforced polyunsaturated fatty acids suppress lipid autoxidation ***
   Hui S. Tsui, Connor R. Lamberson, Libin Xu, Shauna Hill, Vadim V. Shmanai, Andrei V. Bekish, Agape M. Awad, Charles R. Cantor, Ned A. Porter, Mikhail S. Shchepinov, Catherine F. Clarke (University of California Los Angeles, CA).

9. **Genetic analysis of lipidomic profiles in Mexican American families**
   Claire Bellis, Peter J. Meikle, Jacki M. Weir, Jeremy B. Jowett, Satish Kumar, Marcio Almeida, Juan M. Peralta, Ellen E. Quillen, Michael C. Mahaney, Thomas D. Dyer, Laura Almasy, John Blangero, Joanne E. Curran (Texas Biomedical Research Institute, TX).

10. **Mechanistic insights of lipoxin formation in TLR4 primed, purinergic receptor stimulated macrophages**
    Paul C. Norris, Edward A. Dennis (University of California San Diego, CA).

11. **Phospholipase A2 superfamily of interfacial enzymes: Insight into substrate and inhibitor binding**
    Varnavas D. Mouchlis, Denis Bucher, J. Andrew McCammon, Edward A. Dennis (University of California San Diego, CA).

12. **Lipid peroxidation patterns in Alzheimer's disease and mild cognitive impairment**
    Claudio De Felice, Silvia Leoncini, Cinzia Signorini, Alessandra Pecorelli, Jean-Marie Galano, Camille Oger, Valérie Bultel-Poncé, Alexandre Guy, Stefania Boschi, Lucia Ciccoli, Giuseppe Valacchi, Thierry Durand, Joussef Hayek (AOUS of Siena, Italy).

13. **DHA dose-dependently reduces atherosclerosis: a putative role for its peroxidation metabolites, F4-neuroprostanes ***
    Cécile Gladine, John W Newman, Thierry Durand, Theresa L Pedersen, Jean-Marie Galano, Céline Demougeot, Olivier Berdeaux, Estelle Pujos-Guillot, Andrzej Mazur, Blandine Comte (Universites de Montpellier, France & INRA, France).

14. **Reducing macrophage proteoglycan sulfation increases atherosclerosis via type-I interferon signaling**
Philip L.S.M. Gordts, Erin Foley, Roger Lawrence, Risha Sinha, Chris K. Glass, Aldons J. Lusis, Joseph Witztum, Jeffrey D. Esko (University of California San Diego, CA).

15. **Studying the influence of heme oxygenase-1 on lipid metabolism in hepatocytes using an LC-ESI- and MALDI-QIT-TOF-MS/MS approach**
Gerald Stübiger, Elisa Einwallner, Omar Belgacem, Harald Esterbauer (Medical University of Vienna, Austria).

16. **Mannose-fed mice are resistant to weight gain on high fat diet**
Vandana Sharma, Jonamani Nayak, Jamie Smolin, Emily M. King, Rochelle M. Holt, Julio E. Ayala, Hudson H. Freeze (Sanford-Burnham Medical Research Institute, CA).

17. **The broad substrate specificity of the *Arabidopsis thaliana* lyso glycerophospholipid acyltransferase At1g76890 leads to alterations of the lipid composition when the enzyme is overexpressed in *Escherichia coli*.**
Teresa A. Garrett, Reuben Moncada (Vassar College, NY).

18. **Detection and structural elucidation of esterified oxy-lipids in human synovial fluid by FTICRMS and LC-IT-MS^3: discovery of esterified hydroxylated docosapentaenoic acid containing phospholipids**
Hulda S Jónasdóttira, Simone Nicolardia, Rico Derksa, Magnus Palmblada, Andreea Ioan-Facsinayb, René Toesb, Yuri EM van der Burgta, André M Deeldera, Oleg A Mayborodaa, Martin Giera (Leiden University Medical Center, The Netherlands).

19. **Desmosterol accumulation in macrophage foam cells coordinately regulates lipid metabolic and inflammatory responses**
Nathanael J. Spann [#], Lana Garmire[#], Jeffrey G. McDonald, David S. Myers, Stephen B. Milne, Norihito Shibata, Donna Reichart, Jesse N. Fox, Iftach Shaked, Daniel Heudobler, Christian R. H. Raetz, Elaine W. Wang, Samuel L. Kelly, M. Cameron Sullards, Robert C. Murphy, Alfred H. Merrill, Jr., H. Alex Brown, Edward A. Dennis, Andrew C. Li, Klaus Ley, Sotirios Tsimikas, Eoin Fahy, Shankar Subramaniam, Oswald Quehenberger, David W. Russell, Christopher K. Glass (University of California San Diego, CA).

20. **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, United Kingdom).

21. **Lipidomic profiling of rat adipose tissue after treatment with PPAR-pan agonist using sub-2µm particle CO_2 based supercritical chromatography mass spectrometry**
Giorgis Isaac, Michael D. Jones, James Langridge, John P. Shockcor, Julian L. Griffin (University of Cambridge, United Kingdom).

22. **Selective presentation of lipid ligands by CD1c**
Matthew Skaley, Daryl Cox, Wilfried Bardet, Curtis McMurtrey, Ken Jackson, Steven Cate, Jane Yaciuk, Danijela Mojsilovic, Rico Buchli, Jenny Gumperz, William Hildebrand (University of Oklahoma, OK).

23. **New approaches for nontargeted lipidomic quantitation using LC-MS and response factors for lipid classes**
Michal Holčapek, Eva Cífková, Blanka Červená, Miroslav Lísa (University of Pardubice, Czech Republic).

24. **High throughput data independent approach for qualitative and quantitative lipidomic analysis**
Xu Wang, Michael Kiebish, Paul Baker, Brigitte Simons, Christie L. Hunter (AB SCIEX, CA).

25. **Lipid class iosolation by differential mobility separation (DMS) mass spectrometry (MS)**
Paul R.S. Baker, Paul C. Norris, Aaron Armando, larry Campbell, Eva Duchoslav, Edward A. Dennis, Christie Hunter (AB SCIEX, MA).

26. **Inflammasome-mediated secretion of IL-1β in human monocytes through Toll-like receptor 2 activation: modulation by dietary fatty acids.**
Ryan G. Snodgrass, Shurong Huang, John C. Rutledge, Daniel Hwang (University of California Davis, CA).

27. **Insulin increases proliferation of fibroblast-like synoviocytes, cell model for synovitis**
Berit Johansen, Anide Johansen, Astrid J. Feuerherm (Norwegian University of Science & Technology, Norway).

28. **Dual actions of a novel bifunctional compound to lower glucose in mice with diet-induced insulin resistance**
Jane J. Kim, Michael Zimmer, William S. Lagakos, Dayoung Oh, Sarah T. Kavaler, Alice Jih, Jean E. Bemis, Jill C. Milne, Michael R. Jirousek (University of California, San Diego and Catabasis Pharmaceuticals, MA).

29. **Involvement of sphingosine 1-phosphate on palmitate-induced insulin resistance of hepatocytes via the $S1P_2$ receptor subtype**
Susann Fayyaz, Lukasz Japtok, Burkhard Kleuser (University of Potsdam, Germany).

30. **Sleeve gastrectomy in obese mice results in elevated serum bile acids and reduced hepatic steatosis**
Andriy Myronovych, Wujuan Zhang, Kenneth DR Setchell, Pinky Jha, Karen K Ryan, Michelle Kirby, Randy J Seeley, Rohit Kohli (Cincinnati Children's Hospital Medical Center, OH).

31. **The signature biomarker lipids of NASH and identification of a novel pathway for hepatic triglyceride synthesis ***
Cristina Alonso, Patricia Aspichueta, M. Luz Martínez-Chantar, José M. Mato (CIC bioGUNE, Spain).

32. **Discovery of potent multiheterocycle H-PGDS inhibitors**
Kirk M. Maxey, Fred L. Ciske, Kirk L. Olson, James B. Kramer, Adam J.Stein, Pil H. Lee, Levi L. Blazer, Daniel A. Bochar, Karie L. McGowan, Laura E. Kostrzewa, Nisha T. Palackal, Jeff K. Johnson, Gregory W. Endres, Stephen D. Barrett (Cayman Chemical Company, MI).

33. **Sputtered silver nanoparticle-assisted LDI-IM-MS for the analysis of cholesterol and 7-dehydrocholesterol in fibroblast cells from patients with Smith-Lemli-Opitz syndrome**
Michal Kliman[#], Libin Xu[#], Jay Forsythe, Zeljka Korade, Ned A. Porter, John A. McLean (Vanderbilt University, TN).

34. **Associations between plasma lipids and prediabetes and type 2 diabetes, independent of traditional risk factors**
Christopher K Barlow, Gerard Wong, Jacquelyn M Weir, Melissa A. Greeve, Gemma L MacIntosh, Laura Almasy, Anthony G Comuzzie, Michael C Mahaney, Adam Kowalczyk, Izhak Haviv, Narelle Grantham, Dianna J Magliano, Jeremy B M Jowett, Paul Zimmet, Joanne E Curran, John Blangero, Jonathan Shaw, Peter J Meikle (Baker IDI Heart and Diabetes Institute, Australia).

35. **MD-2 binding of oxidized cholesterol esters activates TLR4 signaling**
Soo-Ho Choi, Aaron Armando, Irina Kufareva, Darren Dumlao, Jungsu Kim, Felicidad Almazan, Suganya Viriyakosol, Ruben Abagyan, Edward A. Dennis, Joseph L. Witztum, Yury I. Miller (University of California San Diego, CA).

36. **Validation of an enzymatic assay for the measurement of lysophospholipid acyltransferase levels in cells**
Sarah A. Martin, Miguel A. Gijón, Robert C. Murphy (University of Colorado Denver).

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

38. **LC/MS analysis to detect different prostaglandins in SARS infected mouse lung**
Rahul Vijay, Andrew Spracklen, Stanley Perlman (University of Iowa, IA).

39. **The effect of 7-dehydrocholesterol-derived oxysterols on cholesterol and lipid biosynthesis in cells**
Libin Xu, Zeljka Korade, Karoly Mirnics, Ned A. Porter (Vanderbilt University, TN).

40. **Genetic deletion of prostacyclin IP receptor exacerbates cognitive impairment and neuronal cell death in mouse global cerebral ischemia**
Sofiyan Saleem (Sanford Burnham Medical Research Institute, CA).

41. **Intermediates of PUFA biosynthetic pathway are physiological inhibitors of the cholesterol biosynthesis**
Santhosh Karanth, Vy My Tran, Balagurunathan Kuberan, Amnon Schlegel (University of Utah, UT).

42. **Reduced dietary omega-6:omega-3 ratio and 12/15 lipoxygenase deficiency protect from high fat diet-induced steatohepatitis**
Milos Lazic, Eugenia Inzaugarat, David Povero, Alejandra Cherñavsky, Iris Chen, Mark Chen, Madlena Nalbandyan, Yury I. Miller, Ariel Feldstein, Dorothy D. Sears (University of California San Diego, CA).

43. **Molecular characterization of oxysterol binding to the EBI2 receptor**
Andreas W. Sailer, Tau Benned-Jensen, Inga Preuss, Christoffer Norn, Stephane Laurent, Christian M. Madsen, Kristine N. Arfelt, Romain M. Wolf, Thomas M. Frimurer, Francois Gessier, Mette M. Rosenkilde, Klaus Seuwen (Novartis Institutes for BioMedical Research, Switzerland).

44. **Bile acids induce diacylglycerol kinase theta-dependent phosphatidic acid production**
Kai Cai, Marion B. Sewer (University of California San Diego, CA).

45. **PMA stimulates PKCalpha-dependent phosphorylation of ASAH1 and increases enzyme activity in breast cancer cell lines**
Tania C. Escobar, Marion B. Sewer (University of California San Diego, CA).

46. **A systems biology approach to metabolic antagonism between omega-3 and omega-6 fatty acids during macrophage inflammatory response**
Shakti Gupta[#], Yasuyuki Kihara[#], Mano R. Maurya, Paul C. Norris, Edward A. Dennis, Shankar Subramaniam (University of California San Diego, CA).

47. **An omics study of oxidized phospholipid activated RAW 264.7 cells**
Mano Ram Maurya[#], Ashok Reddy Dinasarapu[#], Shakti Gupta[#], Eoin Fahy, Manish Sud, Shankar Subramaniam *(University of California San Diego, CA).*

48. **NASH associates with a Phospholipid pattern in morbidly obese female ***
Kavya Anjani, Marie Lhomme, Isabelle Dugail, Nicolas Veyrie, Philippe Lesnik, Pierre Bedossa, Anatol Kontush, Karine Clement, Joan Tordjman (INSERM, France).

49. **Quantitation of absolute rates of cholesterol synthesis and tissue cholesterol content in the liver and other major organs of mice with lysosomal acid lipase deficiency: potential applications of the model**
Jen-Chieh Chuang, Adam M. Lopez, Amal Aqul, Benny Liu, Charina Ramirez, Stephen D. Turley (University of Texas Southwestern Medical Center, TX).

50. **Dietary extra virgin olive oil down regulates oxidized lipid mediators in CCl4 induced liver injury**
Yiu Yiu Lee, Hualin Wang, Eric K.Y. Lee, Jennifer M.F. Wan, Chung-Yung Jetty Lee (University of Hong Kong, Hong Kong).

51. **Differential effects of extra virgin olive oil and corn oil in CCl_4 induced liver injury: a proteomic study**
Hualin Wang, Pinging Jiang, Wai-Hung Sit, Jennifer Man-Fan Wan (University of Hong Kong, Hong Kong).

52. **The flagellar membrane of Chlamydomonas is a specialized, highly ordered lipid domain of the plasma membrane enriched in raft lipids**
Antonio Castillo-Flores, James Evans, Scott Shaffer, Beth McCormick , George Witman (University of Massachusetts Medical School, MA).

53. **A synthetic POVPC-peptide is a model oxidized phospholipid that induces expression of inflammatory genes in macrophages and endothelial cells**
Philipp Wiesner, Erica N Montano, Ishita Shah, Oswald Quehenberger, Edward A Dennis, Sangderk Lee, Casey E Romanoski, Aldons J Lusis, Judith A Berliner, William W Turner, Michael S Vannieuwenhze, Christopher K Glass, Joseph L Witztum (University of California San Diego, CA).

54. **Phospholipids in myelin from a canine model of mucopolysaccharidosis I (MPS I): a lipidomics study**
Jennifer K. Yee, Shih-hsin Kan, Steven Q. Le, David Elashoff, Lewei Duan, N. Matthew Ellinwood, Patricia I. Dickson (Los Angeles Biomedical Research Institute at Harbor-UCLA, CA).

55. **Fatty acid metabolites as biomarkers for non-alcoholic fatty liver disease (NAFLD): A lipidomic and metabolomic approach**
Sarina Hou, Zi Li, Yunhua Zhou, Tao Meng, Huiyong Yin (Chinese Academy of Sciences, China).

56. **Lipid profiling of plasma samples from patients with chronic hepatitis C virus infection using HPLC coupled to triple quadruploe MS or LTQ-FT MS**
Feng Qu, Su-Jun Zheng, Cai-Sheng Wu, Zhi-Xin Jia, Zhong-Ping Duan, Jin-Lan Zhang (Chinese Academy of Medical Sciences & Peking Union Medical College, China).

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

1. L-Carnitine effects nitric oxide production in RAW 264.7 macrophage cells after LPS stimulation

Fügen Aktan, Asli Koc

Faculty of Pharmacy, Department of Biochemistry, Ankara University, Anakara, Turkey.

L-carnitine is an important molecule in lipid metabolism. It takes part in the transport of long–chain fatty acids to the mitochondria. It is an antioxidant molecule that suppress oxygen radical formation. Nitric oxide is a mediator of inflammatory responses. LPS stimulation of RAW 264.7 macrophage cells causes excess nitric oxide production that triggers many diseases such as atherosclerosis, diabetes, hypertension, malignancy and septic shock.In this study, we investigated whether L-carnitine inhibits nitric oxide synthesis from LPS stimulated RAW 264.7 macrophage cells. For this purpose, firstly cells were stimulated with LPS for 20h and excess nitric oxide synthesis was occured then L-carnitine was added to cells and incubated for 1 and 20h. L-carnitine inhibited NO production for both 1 and 20h incubation periods. At the end of the incubation of cells with L-carnitine for 20h after LPS, 13.09 mM and higher L-carnitine concentrations inhibited NO production. However after 20 h LPS stimulation when cells incubated with L-carnitine for 1 h, it was seen that 9.84 and higher L-carnitine concentrations exhibited inhibitor effects.As a conclusion, when L-carnitine was added to culture medium after LPS stimulation, it was seen that incubation of cells for 1h with L-carnitine has a greater inhibitor effect than 20h incubation.

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2. Higher-efficiency lipid profiling system using a quadrupole Orbitrap mass spectrometer and an automated search engine lipid search

David Peake[2], Takayuki Yamada[1], Takato Uchikata[1], Shigeru Sakamoto[2], Yasuto Yokoi[3], Eiichiro Fukusaki[1], Takeshi Bamba[1]

[1]Department of Biotechnology, Graduate School of Engineering, Osaka University, Suita, Japan [2]Thermo Fisher Scientific, San Jose, California [3]Mitsui Knowledge Industry, Tokyo, Japan.

Lipidomics studies of human disease states require accurate and quantitative lipid profiling which is challenging due to the lack of comprehensive workflows and databases for automated identification. In the present study, we developed a practical workflow for high-throughput and exhaustive lipid profiling by combining reverse-phase liquid chromatography coupled to a quadrupole Orbitrap Fourier transform mass spectrometer with automated lipid identification software. This validated method enables highly sensitive and simultaneous analysis of lipids with varying polarities such as Glycerophospholipids and Sphingolipids, by rapidly alternating the acquisition polarity of the mass spectrometer during the LC-MS analysis. In addition, data-dependent MS/MS analysis is facilitated by use of an inclusion list of targeted molecular species which improves detection limits. The m/z values of the targeted lipid molecular species, stored in the database of Lipid Search software, are added to an inclusion list in the LC-MS method. Optimization of the LC-MS/MS system using an inclusion list enables high-throughput and accurate identification of lipid molecular species existing in biological samples. In addition HPLC separation of isomeric species is essential for accurate identification of lipid molecular species that possess particular fatty acid chains. This method has high reproducibility and can be used for structural analysis of even low-abundance lipids. Using this method, over 400 lipid species were detected and identified from a sample of mouse plasma. These results indicate that the LC-MS/MS method developed in the present study enables efficient lipid profiling.

3. Development of lipid metabolism assays for understanding the importance of SREBPs in cancer

Joseph P. Argus[1,2], Kevin J. Williams[2,3], Moses Q.Wilks[4], Beth N. Marbois, Yue Zhu[1,2], Dominique N. Lisiero[1], Horacio Sato[5], Robert M. Prins[5], Steven J. Bensinger[1,2,3]

[1]Department of Molecular and Medical Pharmacology, [2]Institute for Molecular Medicine, [3]Department of Pathology and Laboratory Medicine, [4]Department of Biomathematics, and [5]Department of Neurosurgery, David Geffen School of Medicine, University of California Los Angeles, CA.

Objective: Altered lipid metabolism is characteristic of many cancers. Sterol Response Element Binding Proteins (SREBPs) are master transcriptional regulators of lipid metabolism. We have shown that inhibition of SREBPs decreases proliferation of glioma cells in vitro and in vivo. To better elucidate the mechanism of this change, we are developing assays that allow us measure lipid metabolism directly. **Methods:** We are using ^{13}C labeling, GCMS, and probabilistic modeling to determine the cellular amount of different lipid species, the ratios of several key fatty acids, the percentage of lipid that cells scavenge versus de novo synthesize, and the percentage of de novo synthesized lipid carbon that comes from glucose. Results: We have demonstrated that SREBP inhibition causes a decrease in de novo synthesis of both fatty acids and cholesterol in two glioma lines. Surprisingly, total cholesterol per cell was largely unchanged, while saturated fatty acids (SFAs) accumulated and monounsaturated fatty acids (MUFAs) decreased or were unchanged. In addition, the de novo synthesis of MUFAs was more profoundly decreased than SFAs. **Conclusion:** Our newly developed lipid metabolism assays indicate that SREBP inhibition causes a dramatic increase in the SFA to MUFA ratio. An increase in this ratio is known to cause cellular stress and apoptosis via “lipotoxicity”; this provides a mechanism for original observation that inhibition of SREBPs decreases proliferation in glioma cells.

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4. Epoxyisoprostane-containing lipids regulate the inflammatory pathway in endothelial cells *

James R. Springstead[1], Wei Zhong[2], Sangderk Lee[3], Benjamin Emert[1], Ramei Al-Mubarak[4], Michael Jung[2], Judith A. Berliner[1, 3]

[1]Department of Medicine, Cardiology, [2]Department of Chemistry and Biochemistry, and [3]Department of Pathology, University of California, Los Angeles, CA [4]Department of Chemical and Paper Engineering, Western Michigan University, Kalamazoo, MI.

Oxidation products of 1-palmitoyl-2-arachidonoyl-*sn*-glycerol-2-phosphatidylcholine (PAPC), referred to as OxPAPC, and specifically an active component, 1-palmitoyl-2-(5,6-epoxyisoprostanoyl E_2)-*sn*-glycero-3-phosphatidylcholine (PEIPC), accumulate in atherosclerotic lesions and other sites of chronic inflammation and regulate over 1000 genes in human aortic endothelial cells (HAEC). The goal of these studies was to improve the synthesis technique of PEIPC to use in future studies and to determine which isomer(s) of PEIPC were the most active in regulating inflammatory pathways in HAECs. In these studies we synthesized several optical isomers of EI, the oxidized fatty acid in the *sn-2* position of the oxidized phospholipid, PEIPC. We treated HAECs with the different isomers of EI and determined the most active optical isomer of EI, with respect to the upregulation of IL-8 and HO-1. We then synthesized the PEIPC analog of this EI isomer and, using microarray and PCR analyses, we compared gene regulation by the two molecules in HAECs. We demonstrated several similarities in gene regulation, including the upregulation of IL-8, HO-1, and ATF-3 by the two molecules. However, we also demonstrated several differences in gene regulation by the two molecules, most notably in the monocyte rectruitment pathway. PEIPC upregulates this pathway, where EI downregulates several of these genes, suggesting that it is an anti-inflammatory molecule. Preliminary MS data also suggests that EI is present in ECs under oxidative stress, suggesting that EI may be a novel endogenous regulator of inflammation in the endothelium. Overall, we have compared the gene regulation of an oxidized phospholipid PEIPC and its corresponding oxidized fatty acid analog EI and demonstrated important differences in regulation by the two molecules. These studies will further our understanding of oxidized phospholipid and fatty acid gene regulation in HAECs and provide insight that will be useful in drug discovery to treat atherosclerosis.

5. Effect of fluoride chronically administered in liver and plasma of rats treated with hypercaloric diet

Fabrício Soares Pereira, Aline Dionízio; Heloisa Aparecida Barbosa da Silva Pereira, Cesar Augusto de Souza Valle, Flavia Godoy Iano, Marília Afonso Rabelo Buzalaf

Department of Biological Sciences, Bauru Dental School, University of São Paulo, São Paulo, Brazil.

Previous studies have shown that fluoride (F) can cause alterations in the expression of hepatic proteins correlated with alterations in lipid droplets in liver. In addition, it has been reported that these alterations are dose- and time-dependent. The objective of this study was to analyze plasma and liver of rats submitted to chronic intoxication with fluoride for different treatment times and doses when a hypercaloric diet was administered. 36 21-day-old male Wistar rats were divided into 2 groups (n=18 animals/group) according with the duration of the treatment (20 days, group A, or 60 days, group B) and received the AIN-93M hypercaloric diet. Each group was divided into 3 subgroups (n=6 animals/subgroup), according with the dose of fluoride administrated through drinking water, as follows: 0 mg/L (control- subgroups A1, and B1), 15 mg/L (subgroups A2 and B2) or 50 mg/L (subgroups A3 and B3). After the experimental period, the blood and liver were collected. Fluoride analysis in plasma and liver was done. Plasma was analyzed for HDL, cholesterol and triglycerides. Histological analysis was performed in the liver. A dose-response was observed for plasma F. Liver F levels were significantly increased in high F level. Lipid droplets were present in all groups. In group A, the medium *scores* (ranging from 1-5; 1 indicates minor presence of lipids droplets) were 3.72, 3.11 and 1.28 and for group B they were 3.17, 3.50 and 2.22, for control, 15 and 50 mg/L F respectively. The cholesterol analysis did not show alterations. The HDL presented a slight increase, triglycerides and LDL a reduction by groups B2 and B3, but not significant. This can contribute to understanding the mechanisms underlying hepatoxicity induced by F and its relation to fat accumulation in liver.

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6. Proteomic analysis of liver in rats chronically exposed to fluoride and AIN-93M – hypercaloric diet

Heloisa Aparecida Barbosa da Silva Pereira, Aline de Lima Leite, Aline Dionízio, Janete Gualiume Vaz Madureira Lobo, Camila Perez Buzalaf, Marília Afonso Rabelo Buzalaf

Department of Biological Sciences, Bauru Dental School, University of São Paulo, São Paulo, Brazil.

Nutritional status is an important factor in susceptibility to toxicity. Fluoride (F) can cause systemic toxicity when ingested in excessive dose. The feed of rats with a hypercaloric diet is shown to promote accumulation of fat in liver. The present study determined the impact of F in the induction of hepatoxicity in liver of rats fed a hypercaloric diet. Weanling male *Wistar* rats (3-week years old) receiving a hypercaloric diet (AIN-93M) were divided into 3 groups and treated with drinking water containing 0, 5 or 50 mg/L F for 60 days (n=6/group). The serum and liver were collected for F analysis. Liver was also submitted to histological and proteomic analysis. Western blotting was done to confirm the proteomic data. A dose-response was observed in serum F levels. In the liver, F levels were significantly increased in animals the received the higher F level. Liver morphometric analysis did not reveal alterations in the cellular structures. Lipid droplets were present in all groups. However, animals treated with 50 mg/L F presented a slight reduction in lipid droplets. Proteomic quantitative intensity analysis detected 33, 44 and 29 spots differentially expressed in the comparisons between control *vs* 5 mg/L F, control *vs* 50 mg/L F, and 5 mg/L *vs* 50 mg/L F, respectively. In addition, 18, 1 and 5 exclusive protein spots were found in control, 5 mg/L and 50 mg/L F, respectively. Most of proteins were related to metabolic processes. In F-treated rats, changes in the apolipoprotein E (ApoE) and GRP78 expression may account for the F-induced hepatotoxicity, and also present relation to fat metabolism. This can contribute to understanding the molecular mechanisms underlying hepatoxicity induced by F and its molecular relation to fat accumulation in this tissue.

7. Establishment of an in vitro hepatic steatosis model using mouse hepatocyte cell line FL83B

Mu-En Wang, Chih-Hsien Chiu

Department of Animal Science and Technology, National Taiwan University, Taiwan

Non-alcoholic fatty liver disease (NAFLD) refers to a type of fatty liver that is not caused by alcohol intake. Although NAFLD has been studied for many years, its molecular progression mechanisms and connections to the development of chronic liver diseases still remain unclear. Up to now, there were several cell models for NAFLD research described. However, limitations and disadvantages such as the discrepancy of cancer cell metabolism, inconsistency of cell origin, low detecting sensitivity/efficiency, and high cost still exist. Due to these reasons, we established an alternative *in vitro* model based on mouse non-cancer hepatocyte FL83B, which is morphologically and physiologically similar to normal hepatocytes. Using RT-PCR, we found FL83B expresses higher level of *hmgcs2*, which is commonly expressed in normal liver tissue, than mouse hepatoma cell line Hepa1-6. Next, to induce and detect lipid accumulation in FL83B cells, we seeded cells in 96-well microplates and incubated cells with culture medium containing different concentrations (25, 50, 100, 200 and 400 □M) of BSA conjugated oleic acid (OA) or palmitic acid (PA) for 24 hours. After the incubation, cells were stained with Nile Red and Hoechst 33342, and then detected the fluorescent signals using a BioTek Synergy H1 microplate reader. By this method, we demonstrated that OA but not PA can induce a dose-dependent steatosis in FL83B cells. In addition, it is as expected that the OA induced lipid accumulation can be reduced by DGAT-1 (diglyceride acyltransferase 1) inhibitor, A922500 in a dose dependent manner. Furthermore, we found the mRNA levels of *dgat-2*, *adrp, fasn, hmgcs-1 and hmgcs-2* are elevated under OA treatment, just like what occur in NAFLD patients. According to our results, we provide an applicable *in vitro* system for NAFLD studies. We expect this model can be helpful in development of novel medicine and therapeutic strategies against NAFLD.

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8. Inclusion of small amounts of isotope-reinforced polyunsaturated fatty acids suppress lipid autoxidation *

Hui S. Tsui[1], Connor R. Lamberson[2], Libin Xu[2], Shauna Hill[1], Vadim V. Shmanai[3], Andrei V. Bekish[4], Agape M. Awad[1], Charles R. Cantor[5,6], Ned A. Porter[2], Mikhail S. Shchepinov[6], Catherine F. Clarke[1]

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Autoxidation of polyunsaturated fatty acids (PUFAs), or lipid peroxidation, is a consequence of life in an oxygen-enriched atmosphere. This process is detrimental to the cell and has been linked to age-related neurodegenerative diseases and atherosclerosis. However, vulnerable PUFAs such as linoleic acid (Lin; 18:2, n−6) and α-linolenic acid (Lnn; 18:3, n−3) are essential nutrients for humans and many animals, and are important constituents of membranes. Autoxidation of PUFAs is initiated by the facile abstraction of bis-allylic hydrogen atoms. Although yeast do not sythesize PUFAs, they avidly take up exogenously added PUFAs and incorporate them into cellular lipids with no detrimental effects. The reduced form of Q (QH_2, the hydroquinone) functions as both a primary chain terminating antioxidant and as a co-antioxidant regenerating vitamin E. We have shown that the Q-less yeast (*coq*) mutants are exquisitely sensitive to treatment with PUFAs because they lack Q/ QH_2. Yeast with low Q_6 content are much more vulnerable to PUFA stress than wild-type yeast. Replacement of the bis-allylic hydrogen atoms with deuterium atoms (termed site-specific isotope reinforcement) dramatically protects PUFAs from autoxidation. Treatment of Q-less yeast with a mixture of isotope reinforced polyunsaturated fatty acids (approximately 20%:80% isotope-reinforced D-PUFA: natural H-PUFA) rescues the hypersensitivity. Partially reinforced D-PUFAs also protect Q-less yeast mutants from lipid autoxidation mediated cell killing. The findings reported here show that either partial reinforcement or the inclusion of only a small fraction of PUFAs deuterated at the bis-allylic sites is sufficient to profoundly inhibit the chain reaction of non-deuterated PUFAs in yeast.

9. Genetic analysis of lipidomic profiles in Mexican American families

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The lipidome encompasses all fundamental lipid species. It represents a potential gold mine of clinically relevant phenotypes that may be better predictors of disease risk than those that are commonly studied. Additionally, the biologically simpler nature of such lipid species presents the hypothesis that determinants may reside closer to the causal action of genes than more complex integrated lipid measures, such as total cholesterol (TSC), triglyceride (TG), high- and low-density lipoprotein (HDL-C and LDL-C) levels, making them valuable phenotypes for finding genes involved in lipid metabolism. Implementation of a targeted capture method involving liquid chromatography electrospray ionization-tandem mass spectrometry provided precise identification and quantification of 319 lipid measures in 1202 Mexican American individuals from large pedigrees in the San Antonio Family Heart Study (SAFHS). Quantitative genetic analysis and genome-wide association analyses using over one million SNPs were performed to examine potential genetic factors involved in lipidomic profile variation. Analyses have revealed statistically significant heritabilities (when controlling for age and sex) for all species we measured within the lipidomic spectrum (n=319). Maximal heritability h^2=0.61 (p<1×10^{-15}) was calculated for the 24:1 configuration of dihexosylceramide (DHC), while the average calculated heritability (h^2=0.346) in our assayed lipids illustrates a strong genetic component influencing circulating lipid levels in Mexican Americans. Investigating genetic correlations between classical lipid measures and linoleic acid revealed a strong signal with TG (ρ_g= 0.9447) and TSC (ρ_g = 0.5553), while HDL-C was negatively correlated (ρ_g = -0.3783). Our results suggest that these canonical lipidomic profiles may represent phenotypes closer to gene action than those of classical lipid markers.

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10. Mechanistic insights of lipoxin formation in TLR4 primed, purinergic receptor stimulated macrophages

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Macrophages are critical immune cells for initiating the innate immune response, partly through generation of pro-inflammatory lipid mediators including eicosanoids. Using LC-MS lipidomics we have previously characterized the effects of fish oil omega-3 fatty acid supplementation on TLR4 and purinergic eicosanoid signaling in RAW264.7 and resident macrophages[1]. Supplementation of EPA or DHA, leads to inhibition of COX-1 and COX-2 arachidonic acid derived pro-inflammatory prostaglandins, but not inhibition of 5-LOX in these cells. Consequently, fish oil supplementation can increase production of 5-LOX AA, EPA and DHA-derived mono-hydroxylated metabolites after purinergic stimulation. More recently, we have observed endogenous formation of the pro-resolution mediators, 15R- and 15S-lipoxin A_4, which was dependent on long-term TLR4 priming followed by $P2X_7$ stimulation with mM ATP. While mostly trans-cellular mechanisms have been proposed to explain the switch to pro-resolution eicosanoid signaling, our finding suggests macrophages can do this through a novel, independent route that requires temporal and combinatorial receptor-mediated control. We have also confirmed that fish oil omega-3 fatty acids can inhibit lipoxin formation by inhibition of COX-2 derived 15-HETE; serving as an example of “pro-inflammatory” action by these molecules. [Support by NIH: U54 GM069336, R01 GM64611 & UCSD Graduate training Program in Cellular and Molecular Pharmacology National Institutes of Health Grant T32 GM007752]

1. Norris, P.C., Dennis, E. A. PNAS. 2012, 109, 8517-8522

11. Phospholipase A2 superfamily of interfacial enzymes: Insight into substrate and inhibitor binding

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Phospholipase A_2 (PLA_2) constitutes a superfamily of enzymes catalyzing the hydrolysis of the ester bond at the *sn*-2 position of phospholipids (*Chem. Rev.* **2011,** *111*, 6130-6185). The products of PLA_2 activity include free fatty acids, predominantly arachidonic acid (AA), and lysophospholipids. The AA is further metabolized by downstream enzymes (COX-1, COX-2 and 5-LO) to form a variety of pro-inflammatory lipid mediators including prostaglandins, leukotrienes and thromboxanes. Among the members of the PLA_2 superfamily, the calcium-independent group VIA-2 (GVIA-2 $iPLA_2$) and the cytosolic group IVA (GIVA $cPLA_2$) have similar sizes (~ 85 kDa) and they function through a Ser/Asp catalytic dyad located in a patatin-like *α/β*-hydrolase catalytic domain. The crystal structure of GIVA $cPLA_2$ (PDB ID: 1CJY) is available but there is none available for GVIA-2 $iPLA_2$, so a homology model was created since the enzyme shows 40 % homology with patatin (PDB ID: 1OXW) and we modeled inhibitor binding (*J. Am. Chem. Soc.* **2009,** *131*, 8083-8091 and *J. Am. Chem. Soc.* **2013,** *135*, 1330-1337). Covalent and Induced Fit docking methods (*J. Med. Chem.* **2006,** *49*, 534-53) have now been employed to predict the binding mode of the substrate and inhibitors. The substrate-enzyme and the ligand-enzyme complexes revealed key residues that play important roles in binding. Further studies of the resulting complexes with molecular dynamics simulations using NAMD have been employed to interpret experimental results from Deuterium/Hydrogen Exchange Mass Spectrometry (DXMS) (*J. Biol. Chem.* **2013,** *288*, 1806-1813). This information will be useful in developing new inhibitors with improved properties (NIH grant GM 20,501).

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12. Lipid peroxidation patterns in Alzheimer's disease and mild cognitive impairment

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Introduction: Lipid peroxidation is implicated in the pathogenesis of Alzheimer Disease (AD) and mild cognitive impairment (MCI). However, it is unclear whether it is a primary or a secondary contributor to the MCI/AD phenotype, and whether oxidative stress markers in peripheral samples could represent reliable indicators for the presence of neurodegenerative disease. **Methods:** Patients with AD and MCI were included, as well as healthy controls comparable for age and gender. Plasma was used for determination of free F2-Isoprostanes (F2-IsoPs), F2-dihomo-isoprostanes (F2-dihomo-IsoPs), F3 Isoprostanes (F3-IsoPs), F4-neuroprostanes (F4-NeuroPs) and non-protein-bound iron (p-NPBI) and erythrocytes were used for determination of intraerythrocyte NPBI (IE-NPBI). F2-IsoPs, F2-dihomo-IsoPs, and F4-NeuroPs were measured by gas chromatography/negative ion chemical ionization tandem mass spectrometry method (GC/NICI-MS/MS)**.** IE-NPBI and p-NPBI were assessed by HPLC. **Results:** Levels of IE-NPBI and p-NPBI in MCI and AD patients were significantly higher as compared to controls ($p=0.001$ and <0.001, respectively) and p-NPBI was higher in MCI than in AD. Plasma F2-IsoPs and plasma F2-dihomo-IsoPs concentrations were significantly higher in MCI and AD as compared to controls ($p<0.001$), with F2-IsoPs concentration being higher in AD as compared to MCI patients. F3-IsoPs were significantly reduced in both MCI and AD patients. No significant differences were observed for F4- NeuroPs ($p=0.216$). **Conclusions:** Increased lipid peroxidation end-products from arachidonic and adrenic acids are detectable in the peripheral blood from MCI and AD patients with distinct patterns, while lower levels of eicosapentaenoic acid oxidation end-products are observed. The data indicate that an early screening for AD is feasible and suggest a primary contributing role of lipid peroxidation in the pathogenesis of this neurodegenerative disease.

13. DHA dose-dependently reduces atherosclerosis: a putative role for its peroxidation metabolites, F4-neuroprostanes *

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Objective: Consumption of long chain n-3 PUFA is associated with reduced risks of cardiovascular disease but the role of their oxygenated metabolites is still unclear. We hypothesized that metabolites issued from the non-enzymatic oxidation of docosahexaenoïc acid (DHA, C22:6 n-3) could play a role in the prevention of atherosclerosis. **Methods and Results:** LDLR-/- mice (n=30/group) received for 20 weeks an atherogenic diet (10% lard and 0.045% cholesterol) together with daily oral gavages with a mixture of sunflower and tuna oils provided 0%, 0.1%, 1% and 2% of energy as DHA (Control, DHA1, DHA2 and DHA3 groups respectively). Supplementation with DHA dose-dependently reduced atherosclerotic plaque size (R2=0.97) as well as most cardiovascular risk factors such as plasma triglycerides and cholesterol (R2=0.97 and 0.96 respectively). Targeted lipidomic analyses were used to determine plasma and liver profiles of PUFA and their oxygenated metabolites. As expected, DHA supplementation induced dose-dependent increase of long chain n-3 PUFA (R2=0.95 and 0.99 in plasma and liver respectively) but was also associated with an increased production of n-3 PUFA's oxylipins and F4-Neuroprostanes, major peroxidized metabolites of DHA. Finally, correlation, hierarchical cluster and partial least square analysis of the overall dataset revealed that the liver content of F4-Neuroprostanes was both the variable the most negatively correlated with plaque progression and one of the two major predictive variables of plaque regression. **Conclusion:** This study shows the antiatherogenic effect of DHA could in part be achieved by one of its major peroxidation metabolites, the F4-Neuroprostanes.

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14. Reducing macrophage proteoglycan sulfation increases atherosclerosis via type-I interferon signaling

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Atherogenesis initiates by retention of atherogenic lipoproteins by proteoglycans within the vessel wall. Macrophage uptake of these atherogenic lipoproteins triggers formation of foam cells and plaque deposition. In order to examine the role of macrophage heparan sulfate proteoglycans (HSPGs) in atherogenesis, we inactivated the biosynthetic gene GlcNAc N-deacetylase/N-sulfotransferase 1 (Ndst1) selectively in macrophages by crossing *Ndst1*$^{f/f}$ mice with LysMCre+ mice. When bred onto an *Ldlr*$^{-/-}$ background and placed on an atherogenic diet, *Ndst1*$^{f/f}$*LysMCre*$^{+}$*Ldlr*$^{-/-}$ mice demonstrated increased atherosclerosis compared to Ldlr$^{-/-}$ mice. Plaque analysis also revealed significantly increased macrophage content in lesions from *Ndst1*$^{f/f}$*LysMCre*$^{+}$*Ldlr*$^{-/-}$ mice. Diminished HSPG sulfation in macrophages from *Ndst1*$^{f/f}$*LysMCre*$^{+}$ mice resulted in significantly increased expression of inflammatory genes such as CCL5, CCL7, CCL8 and ACAT2. Increased ACAT2 expression correlated with more ACAT enzyme activity and increased foam cell formation compared to wild-type macrophages. Motif analysis of promoters of up-regulated genes revealed increased Type-I interferon signaling in macrophages with reduced HSPG sulfation. Also IFN-α and IFN-β induced STAT1 phosphorylation was elevated in *Ndst1*$^{f/f}$*LysMCre*$^{+}$ macrophages. In conclusion, our data suggest that macrophage HSPGs are atheroprotective and act by maintaining Type-I interferon reception in a quiescent state either through sequestration of Type-I interferons or by forming complexes with Type-I interferon receptors.

15. Studying the influence of heme oxygenase-1 on lipid metabolism in hepatocytes using an LC-ESI- and MALDI-QIT-TOF-MS/MS approach

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Heme oxygenase-1 (HO-1) represents a key enzyme in the catabolism of heme released due to oxidative stress from hemoproteins and the respiratory chain of mitochondria (e.g. hemoglobin, cytochrom c). In this context, it is speculated that HO-1 exerts cytoprotective, anti-oxidative and anti-inflammatory properties. Nevertheless, the functions of HO-1 in pathological processes like metabolic syndrome (i.e. obesity and diabetes) and chronic liver disease (e.g. NAFLD) remains still unknown. The aim of our study is to investigate the role of HO-1 in lipid metabolism of hepatocytes using HO-1 knockout mice (LHOKO). Liver tissue and isolated hepatocytes of LHOKO and control mice (n = 5-8) were investigated. Free fatty acids (FFA), neutral- and phospholipid classes including oxidized phospholipids (OxPLs) were analyzed using LC-ESI- and MALDI-QIT-TOF-MS/MS (Shimadzu, Manchester, UK). Metabolic parameters were measured *in vivo* and mitochondrial function was determined *in vitro* using Seahorse XF Flux-Analyzer (Seahorse Bioscience, MA, USA). First results showed a significant decrease of 16:0, 18:1, and 20:4 FFA ($p<0.05$ to 0.01) and 16:0, 18:1, 18:2 and 22:6 containing TAG ($p<0.05$) in LHOKO vs. control mice indicating decreased *de novo* lipogenesis or increased catabolism. These finding correlate well with lower glucose levels, higher insulin sensitivity and lower body mass gain of LHOKO mice. No changes of PCs, LPCs, and SM, whereas a significant decrease of LPEs ($p<0.01$ to 0.001) but increase of PEs containing 18:2, 20:4 and 22:6 ($p<0.05$ to 0.01) were observed. In contrast, the levels of PS, PI and CL were found unchanged. Since PEs represent important components of liver mitochondria our findings suggest an increased mitochondrial activity. Indeed we observed a significantly increased oxygen consumption rate (OCR) and reactive oxygen species (ROS) in LHOKO vs. control mice. In summary, our preliminary data indicate important functions of HO-1 related to lipid biosynthesis and mitochondrial function in hepatocytes.

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16. Mannose-fed mice are resistant to weight gain on high fat diet

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High fat diet (HFD) causes metabolic syndrome. We studied the effects of mannose supplementation on normal diet (ND) or HFD-fed C57/BL6 mice. Mice were weaned on ND or HFD and drinking water containing either 2% mannose (HFD+M) or no mannose (HFD). At 16 weeks of age, mice on ND weighed 29.9$\pm$2.66g whereas HFD mice were heavier (40.0$\pm$3.4g). Surprisingly, HFD+M mice weighed less (29.4$\pm$3.05g) than HFD mice. 2% galactose did not affect weight gain indicating a mannose-specific effect. HFD+M mice showed decreased fat mass (22.6$\pm$4.5%) than HFD mice (31.2$\pm$3.5%). This effect of mannose on weight and fat reduction was seen even in ND fed mice. In a treadmill exercise, the speed and time at exhaustion increased significantly ($p<0.05$) for HFD+M mice (s=25$\pm$0.8 m/min, t=13.0$\pm$0.3 m) relative to HFD mice (s=22.8$\pm$0.8 m/min, t=10.8$\pm$0.6 m) showing increased fitness with mannose. Histology showed less fat in the livers from mannose-fed mice. There was no difference in water intake, energy expenditure or activity. Calorie intake was actually 20% higher for HFD+M than HFD group suggesting that the differences is neither due to increased activity and energy expenditure nor reduced food consumption. Bomb calorimeter analysis showed significantly ($p < 0.0001$) more energy content in feces from HFD+M (17.65 + 0.0995 KJ/g) mice as compared to HFD mice (16.08 $\pm$ 0.2590 KJ/g) suggesting decreased nutrient absorption. HFD+M mice were slightly glucose intolerant yet slightly insulin sensitive. Weight reduction was observed only when mice received mannose at weaning but not when provided 6 or 13 weeks post weaning, indicating that an early event might be involved. Microarray data shows that up-regulated pro-inflammatory genes in HFD consumption are lowered by mannose supplementation suggesting an anti-inflammatory effect. Our data show that providing 2% mannose to weaned mice reverses some of the adverse effects of HFD [Supported by The Rocket Fund and RO1-DK55615].

17. The broad substrate specificity of the *Arabidopsis thaliana* lyso glycerol-phospholipid acyltransferase At1g76890 leads to alterations of the lipid composition when the enzyme is overexpressed in *Escherichia coli*.

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Escherichia coli possess low levels of head-group acylated glycerophospholipids (GPLs) acyl-phosphatidylglycerol (acyl-PG) and *N*-acyl phosphatidylethanolamine that play potentially important roles in membrane function. Previously, we showed that when *Arabidopsis thaliana* At1g78690 is over-expressed in *E. coli* acyl-PG accumulates despite that *in vitro* it catalyzes the transfer of an acyl chain from acyl-CoA to the *sn*-2 position of 1-acyl GPLs to form the *sn*-1, *sn*-2, diacyl GPLs; it does not acylate PG to form acyl-PG. In addition, we recently observed that over-expression At1g78690 in *E. coli* leads to the accumulation of CL. This is intriguing because At1g78690 shares significant homology with tafazzin, a cardiolipin (CL) transacylase defective in patients with Barth syndrome. To understand the interplay among, lyso-GPL, cardiolipin, and head-group acylated GPL metabolism we have begun to characterize the *in vitro* activity of At1g78690. We assessed the ability of At1g78690 to catalyze the transfer of eicosapentaenoate from eicosapentaenoyl coenzyme A to a variety of GPL acyl acceptors including monolysocardiolipin (MLCL), dilysocardiolipin (DLCL) and three stereoisoforms of *bis*-monoacylglycerophosphate (BMP). Low levels of the predicted products were formed using MLCL and DLCL as the lipid acceptors when solubilized membranes containing At1g78690 were used as the enzyme source. In contrast, At1g78690 robustly acylates two BMP isoforms with *sn*-2 and/or *sn*-2' hydroxyls in the *R*-stereoconfiguration, as compared to the BMP isoform with the *sn*-2 and *sn*-2' hydroxyls in the *S*-stereoconfiguration, suggesting that At1g78690 is stereoselective for hydroxyls with *R*-stereochemistry. The acylation of BMPs, MLCLs, and DLCLs by At1g78690 may explain the accumulation of acyl-PG and CL when At1g78690 is overexpressed in cells. No significant tafazzin-like transacylation of cardiolipin was detectable with At1g78690. Current work is focusing on isolating At1g78690 in order to use pure protein to completely elucidate the mechanism by which At1g78690 so strikingly alters the lipid composition of *E. coli*.

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18. Detection and structural elucidation of esterified oxy-lipids in human synovial fluid by FTICRMS and LC-IT-MS^3: discovery of esterified hydroxylated docosapentaenoic acid containing phospholipids

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Here we present the application of a cross-platform approach combining rapid direct infusion high-resolution/accurate mass FTICRMS with in-depth data-dependent LC-MS^2 and LC-MS^3 analysis for lipid profiling. Using this approach we profiled human synovial fluid samples from osteo- and rheumatoid arthritis patients. Multivariate statistical analysis revealed esterified oxylipids to be present in a subset of the patient samples. Employing LC-MS^2 and LC-MS^3 analysis of these species we were able to clarify hypothesized lipid structures initially based on the accurate mass measurements performed on the FTICRMS platform. LC-MS^3 analysis of intact esterified oxy-lipids and LC-MS^2 analysis of the hydrolysis products allowed for the detection of positional isomers. Using the described approach we were able to elucidate the structure of hydroxylated docosapentaenoic acid-containing diacyl-phosphatidylcholine type phospholipids.

19. Desmosterol accumulation in macrophage foam cells coordinately regulates lipid metabolic and inflammatory responses

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Inflammation and macrophage foam cells are characteristic features of atherosclerotic lesions, but the mechanisms linking cholesterol accumulation to inflammation and LXR-dependent response pathways are poorly understood. To investigate this relationship, we utilized lipidomic and transcriptomic methods to evaluate the effect of diet and LDL receptor genotype on macrophage foam cell formation within the peritoneal cavities of mice. Foam cell formation was associated with significant changes in hundreds of lipid species and unexpected suppression, rather than activation, of inflammatory gene expression. We provide evidence that regulated accumulation of desmosterol underlies many of the homeostatic responses observed in macrophage foam cells, including activation of LXR target genes, inhibition of SREBP target genes, selective reprogramming of fatty acid metabolism and suppression of inflammatory response genes. These observations suggest that macrophage activation in atherosclerotic lesions results from extrinsic, pro-inflammatory signals generated within the artery wall that suppress homeostatic and anti-inflammatory functions of desmosterol.

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20. 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.

21. Lipidomic profiling of rat adipose tissue after treatment with PPAR-pan agonist using sub-2µm particle CO_2 based supercritical chromatography mass spectrometry

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The typical chromatographic methods for analyzing fatty acids and neutral lipids are gas chromatography after derivitization and liquid chromatography-tandem mass spectrometry (LC/MS/MS). However, there are shortcomings associated with each of these methods. For example, GC methods require derivatization of the fatty acids to methyl esters (FAME), which is burdensome, time consuming, and there is a risk of re-arrangement of the fatty acids during derivitization which leaves doubt as to whether the esters formed are from free fatty acids or intact complex lipids. In LC/MS/MS methods, the runs typically involve labor intensive and time consuming sample preparation, and utilize toxic organic solvents, which are expensive to purchase and dispose, and may contain contaminants even for the analytical grade solvents. We have developed rapid, high throughput and efficient method for the separation and analysis of free fatty acids and neutral lipids using sub-2µm particle CO_2 based supercritical chromatography. The organic extract from the tissue containing lipids is directly injected onto the system showing a significant saving in solvent, cost and sample preparation time. The analysis of adipose tissue extract using the CO_2 based supercritical chromatography mass spectrometry produced a very good separation of the free fatty acids and neutral lipids in less than 10 minutes. The separation mechanism is mainly based on the number of carbon chains and the number of double bonds on the acyl chain. The datasets were processed using TransOmics Informatics for Metabolomics and Lipidomics a new software tool that provides automatic peak detection followed by principal component analysis (PCA). Preliminary results showed there is a group separation between the control and PPAR-pan agonist treated samples. Some potential biomarkers that contribute to the group difference have been identified.

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22. Selective presentation of lipid ligands by CD1c

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Introduction: CD1c molecules sample lipids within the cell and present these lipid ligands at the cell surface. Once at the cell surface, CD1c restricted T cells review the CD1c-lipid complex in order to discriminate healthy from diseased cells. It has been previously shown that the CD1c of cancerous thymocytes present lipids for recognition by cytotoxic T cells. Unfortunately, little is known pertaining to the lipid ligands that are bound and presented by the CD1c of a T cell lymphoma or leukemia. Methods: In order to understand the nature and breadth of intracellular lipid ligands presented by CD1c, a recombinant soluble CD1c was constructed, stably expressed in the SUP-T1 T lymphoma cell line, and the stable transfectants were cultured in a hollow-fiber bioreactor for sCD1c protein production. Soluble CD1c was affinity purified, lipid ligands were extracted from the purified CD1c protein, and the CD1c ligands were analyzed by mass spectroscopy. Results: More than117 lipid species were molecularly identified as ligands for CD1c. From this expansive group of ligands it can be seen that the CD1c of a T cell lymphoma presents lipids with nine predominant head group moieties. In regards to lipid moiety diversity, an array of radyl group linkages and carbon chain lengths ranging from 14-22 carbons were observed, as were various degrees of unsaturation. Conclusions: These data establish a lipid profile for the CD1c of a T cell lymphoma. Such data can be transitioned to support immune assays that elucidate reactive CD1c cancer ligands, comparisons with ligands from other structurally distinct CD1 molecules, and comparisons to CD1c ligands from alternative cell lineages. Realizing the nature of biologically relevant CD1c ligands contribute to understanding the role of CD1 ligands in T cell initiated immunity.

23. New approaches for nontargeted lipidomic quantitation using LC-MS and response factors for lipid classes

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Targeted lipidomic quantitation methods are mostly based on SRM transitions measured on triple quadrupoles with internal standards for each lipid class. The goal of our work is the development of universal nontargeted LC-MS method applicable for multiple lipid classes. Hydrophilic interaction liquid chromatography (HILIC) allows the separation of polar lipid classes (phospholipids and sphingolipids), while nonpolar lipid classes (triacyglycerols, diacylglycerols and cholesterol esters) are separated in the normal-phase system. The quantitation of lipid classes in both systems is performed by the same approach described further. Calibration dependencies are measured for representative standards of individual classes containing C18:1 fatty acid. The ratio of calibration slope of class representative (e.g., triolein as the representative triacylglycerol) to the calibration slope of internal standard (dioleoylglycol for nonpolar lipids and sphingosyl PE-d17:1/12:0 for polar lipids) determines the response factor of particular lipid class. The concentration of lipid class in real samples is calculated by the lipid class peak area multiplied by response factor and related to the internal standard. Our approach is based on the fact that ratios of lipid class extraction recoveries are comparable within the range of studied samples and the simplification that differences among individual lipid species inside the class can be neglected. The data on the NIST plasma and other biological samples shows a good correlation among our nontargeted approach, targeted SRM method on triple quadrupole and ^{31}P NMR. The quantitation of lipid species inside classes can be achieved by two-dimensional LC-MS, MS/MS or negative-ion ESI. The potential of our method for detailed lipidomic characterization will be illustrated on clinical samples of dissected cancer tumor tissues after the surgical intervention and patients suffering from cardiovascular diseases. This work was supported by the project No. 203/11/0022 sponsored by the Czech Science Foundation.

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24. High throughput data independent approach for qualitative and quantitative lipidomic analysis

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Numerous studies have demonstrated the biological importance of lipid metabolism in energy storage, cell membrane structure and signaling. Changes in the abundances of lipids can closely correlate to progression of numerous conditions such as cancer, neurodegeneration, and metabolic diseases. The Infusion MS/MS^{ALL} Workflow, a novel data-independent acquisition (DIA) strategy for lipid molecular characterization, enables in-depth analysis by direct infusion lipid extracts in high throughput qualitative and quantitative screening mode. A fully automatic workflow has been developed using the Infusion MS/MS^{ALL} workflow on a QqTOF system with a flow injection sample introduction strategy. The overall workflow provided a streamlined strategy for automation and throughput generating extensive quantification and lipid identification data of diverse sample types. The high density information content of this DIA strategy enables comprehensive profiling of multiple lipid extracts at a rate of 7.5 minutes per sample. The preliminary results show the identification of 1563, 402, 1759, 1638, and 1601 lipid species in plasma, meibum, brain, heart, and lymphoblast respectively, covering diverse lipids classes, which include glycerophospholipid, diphosphatidylglycerol lipid, glycerolipid, sphingolipid and steroid lipids. The identified lipid peak intensities, across all samples, were then normalized to the spiked internal standard from same lipid class, and exported for comparative profiling. After principle component analysis (PCA) and t-test analysis, numerous statistically significant changes ($p < 0.01$) were observed. Additionally, cardiolipins, ether linked phosphocholines and phosphoserines were identified with relative higher abundance in brain extract comparing with other samples. Many lyso-phosphocholines were found to be higher in plasma than other tissues. The majority of monoalkyl diacylglycerols were seen in plasma and heart.

25. Lipid class isolation by differential mobility separation (DMS) mass spectrometry (MS)

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Lipids mediate diverse physiological processes such as metabolic homoestasis and inflammation and are implicated in pathology of inflammatory-related diseases. Lipidomics is an emerging field aimed at identifying global lipid profile changes in biological samples. The human lipidome contains >100,000 different molecular species found within a small mass range; consequently, isobaric overlap makes unambiguous identification and quantitation of lipid species difficult. Methods that utilize DMS coupled to a triple quadrupole linear ion trap mass spectrometer to isolate lipid classes for MS analysis have been investigated. Preliminary infusion experiments indicated DMS, using a mixture of purified standards, isolated all 6 phospholipid sub-classes. Translation of the method to a complex mixture generated clean spectra for each phospholipid sub-class with minimal contamination. Using heart tissue extract, an experiment designed to compare the spectra from DMS isolated PC and a precursor ion scan specific to PC (i.e., precursor ion of +184) generated nearly identical MS results, confirming the power of DMS to resolve components of a complex mixture without chromatography. Product ion analysis with and without DMS generated very different fragmentation patterns. For MS/MS spectra acquired with no DMS, there were multiple unexplained fragments due to the presence of multiple isobaric lipids species. In contrast, using DMS and MS/MS resulted in simpler product ion spectra for easier identification and reduced isobaric contribution to product ion formation for increased quantitative rigor. In addition to resolving phospholipid sub-classes, major lipid classes were also separated. Triglycerides were easily separated from polar lipids, and strikingly, the sphingomyelins (SM) were resolved from PC molecular species. The latter observation is significant considering these two classes cannot be resolved using triple quadrupole strategies alone, and their masses overlap significantly. These data indicate DMS positioned at the entrance to the mass spectrometer significantly simplifies and improves lipidomic analysis by mass spectrometry.

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26. Inflammasome-mediated secretion of IL-1β in human monocytes through Toll-like receptor 2 activation: modulation by dietary fatty acids.

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Many studies have shown that TLR4 and TLR2 deficient mice are protected from high fat diet-induced inflammation and insulin resistance, suggesting that saturated fatty acids derived from the high fat diet activate TLR-mediated proinflammatory signaling pathways and induce insulin resistance. However, evidence that palmitic acid, the major dietary saturated fatty acid, can directly activate TLR has not been demonstrated. Here we present multiple lines of evidence showing that palmitic acid directly activates TLR2, a major TLR expressed on human monocytes, by inducing heterodimerization with TLR1 in a NADPH oxidase-dependent manner. Dimerization of TLR2 with TLR1 was inhibited by the n-3 fatty acid docosahexaenoic acid. Activation of TLR2 by palmitic acid leads to expression of pro-IL-1β that is cleaved by caspase-1, which is constitutively present in monocytes, to release mature IL-1β. Our results reveal mechanistic insight about how palmitic acid activates TLR2 and induces inflammasome-mediated-IL-1β production in human blood monocytes which can trigger enhanced inflammation in peripheral tissues, and suggest that these processes are dynamically modulated by the types of dietary fat we consume.

27. Insulin increases proliferation of fibroblast-like synoviocytes, cell model for synovitis

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Rheumatoid Arthritis is an autoimmune disease that causes inflammation of the joints and surrounding tissue. The presence of pro-inflammatory cytokines leads to hyper proliferation of synoviocytes and the synovial production of hormones which promotes angiogenesis and joint destruction. Metabolic inflammation is a silent inflammation proven to be caused by overeating. Recent science also claims that the macronutrient composition of the diet plays a role in metabolic inflammation. A typical western diet, where carbohydrates are the main source of energy has proven to activate the genes for transcription factors like NF-kB, STAT3/5 and CEBPA. Insulin is a growth hormone and also a strong candidate for linking the high carbohydrate diet to metabolic inflammation. This suggested link inspired us to test the involvement of insulin in the hyper proliferation of synoviocytes. Through the use of a fibroblast like synoviocyte cell line, as a cell model for synovitis, we tested the pro-inflammatory properties of insulin in RA.

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28. Dual actions of a novel bifunctional compound to lower glucose in mice with diet-induced insulin resistance

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Docosahexaenoic acid (DHA 22:6n-3) and salicylate (salicylic acid) are both known to exert anti-inflammatory effects. In this study, we investigated the effects of a novel bifunctional compound, CAT-1004, a chemical conjugate of DHA and salicylate which is hydrolyzed intracellularly in target tissues to release the individual components, DHA and salicylate. We demonstrate that CAT-1004 acts synergistically compared to DHA and salicylate to reduce inflammation mediated along the NFκB pathway in cultured macrophages. Notably, we find oral administration of the bifunctional compound mitigates hyperglycemia in high-fat diet (HFD)-induced insulin resistance by two different mechanisms. In mice with diet-induced obesity, the compound lowers blood glucose by reducing hepatic insulin resistance. It also has an immediate glucose-lowering effect that is attributed to enhanced glucagon-like peptide-1 (GLP-1) secretion; this effects is abrogated by the administration of Exendin(9-39), a GLP-1 receptor antagonist. Administration of this compound also significantly delays the onset of diabetes in rodents by preserving pancreatic beta cell function and increasing insulin secretion. These results suggest that CAT-1004 may effectively treat individuals with Type 2 diabetes and insulin resistance. This strategy could also be employed in other disease conditions characterized by chronic inflammation.

29. Involvement of sphingosine 1-phosphate on palmitate-induced insulin resistance of hepatocytes via the $S1P_2$ receptor subtype

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Hepatic insulin resistance is a major cause in the pathogenesis of type 2 diabetes which is characterized by the classical triad of hyperinsulinemia, hyperglycemia and hypertriglyceridemia. Data indicate that enhanced serum fatty acids availability induces lipid accumulation in liver followed by the development of hepatic insulin resistance. It has been suggested that bioactive lipid intermediates are formed in response to saturated but not unsaturated fatty acids, which contributes to an impaired insulin signalling. Indeed, ceramides, which can be formed by a *de novo* pathway from the fatty acid precursor palmitate, have been indicated to interrupt several putative targets of insulin signalling. Once generated, ceramides can be further metabolized to a extensive array of bioactive sphingolipid-metabolites involved in the modulation of further cellular functions. In this way, ceramides can metabolized to the bioactive molecule sphingosine 1-phosphate (S1P). The complexity of S1P-mediated actions can be explained by the fact that it functions not only inside the cell but also acts as a ligand of five G protein-coupled receptors, namely $S1P_{1-5}$, when it is secreted into the extracellular environment. To examine whether S1P-signaling is also relevant in hepatocytes, primary rat and human cells were used. Measurement of palmitate metabolism indicated that an increase of S1P occurred in response to the fatty acid. To examine the role of S1P on insulin signaling, not only glucokinase expression but also PI3K/Akt phosphorylation was studied. Both, insulin-mediated glucokinase expression and PI3K/Akt phosphorylation, was drastically reduced in the presence of S1P. Real-time PCR revealed that all five S1P receptors are present in rat as well as human hepatocytes. The S1P2 was identified as the crucial receptor subtype to inhibit insulin-mediated pathways. Thus, PI3K/Akt phosphorylation in response to insulin was not influenced by S1P, when the S1P2 antagonist JTE013 was present.

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30. Sleeve gastrectomy in obese mice results in elevated serum bile acids and reduced hepatic steatosis

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Bariatric surgery patients such as Roux-en-Y-Gastric Bypass and vertical sleeve gastrectomy (VSG) have increased serum bile acid levels and reduced hepatic steatosis. This improvement in hepatic steatosis is recognized to be beyond what can be explained by weight loss alone. Our aim was to investigate the role of bile acids by performing VSG in a diet induced obese mouse model.Our results indicated that VSG mice lost the most weight compared to all other groups, viz.; Sham surgery (S), Sham surgery with pair feeding (S-PF), and a surgery naïve control group (NC) in the first week after surgery and at sacrifice NC and S group mice gained significantly more body weight compared to S-PF and VSG mice. Fasting plasma bile acid levels were significantly higher in VSG mice at 2 and 4 weeks after surgery. A strong correlation was observed in VSG mice between plasma total bile acid levels and body weight change at 2 weeks after surgery. VSG resulted in a change in serum bile acid composition with the increase of cholic and tauroursodeoxycholic acids. Hepatic triglycerides were significantly lower in VSG group compared to NC, S-PF and S groups. Further lipogenic and bile acid synthesis genes were down regulated in VSG mice. In conclusion, mice having VSG surgery lost more body weight and had reduced triglyceride accumulation in the liver in addition to increased serum bile acid levels. These results were independent of caloric intake and body weight as the Sham-PF mice had no such changes. Our novel data suggest a potential role for bile acids in the signaling of genes responsible for reduction of hepatic steatosis observed after VSG in mice.

31. The signature biomarker lipids of NASH and identification of a novel pathway for hepatic triglyceride synthesis *

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We have developed an LC/MS-based platform that allows the semi-quantitative determination of around 850 lipids (fatty acids, glycerophospholipids, glycerolipids, sphingolipids and sterol lipids), 123 polar metabolites including central carbon metabolism, and 41 amino acids and small peptides[1,2]. We have used this platform to determine the sera metabolite profile of 467 biopsied individuals with normal liver histology (n=90) or diagnosed with non-alcoholic fatty liver disease (NAFLD, n=377). Among the NAFLD patients, a histopathological diagnosis of steatosis was established in 246 patients and of non-alcoholic steatohepatitis (NASH) in 131. This has allowed us to define a robust BMI-dependent lipidomic signature that reliably/accurately differentiates liver steatosis from NASH. This constitutes the first NAFLD-associated serum lipidomic signature in humans. We used also this platform to analyze the liver lipid profile in wild-type mice and in glycine *N*-methyltransferase (GNMT)-knockout mice. In the case of GNMT-deficient mice, which have high hepatic S-adenosylmethionine (SAMe) and spontaneously develop NAFLD[3], their lipidomic signature agrees with an increased flux from phosphatidylethanolamine (PE) to phosphatidylcholine (PC), via the PE *N*-methyltransferase (PEMT) pathway, followed by its hydrolysis to diglycerides and accumulation of TG. Importantly, feeding a methionine-deficient diet to reduce the flux from PE to PC reverted the lipid profile to that of wild-type mice and prevented NAFLD. This demonstrates that PC synthesized via PEMT is a novel source of hepatic TG.

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32. Discovery of potent multiheterocycle H-PGDS inhibitors

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Hematopoietic-type Prostaglandin D Synthase (H-PGDS) is responsible for the enzymatic production of the pro-inflammatory mediator Prostaglandin D_2 (PGD_2). H-PGDS is expressed in muscle fibers from patients with Duchenne Muscular Dystrophy (DMD) and polymyositis, and has been reported to play a role in the etiology of the muscle necrosis associated with these conditions. The drug targeting of H-PGDS with potent enzyme inhibitors is therefore a reasonable approach to the potential discovery and development of therapies for treatment of DMD and other inflammation-related disorders. Herein we describe the discovery of potent H-PGDS inhibitors through rational and structure-based drug design, which were identified using Cayman's new H-PGDS Fluorescence Polarization Binding Assay (FPBA) and functional assays.

33. Sputtered silver nanoparticle-assisted LDI-IM-MS for the analysis of cholesterol and 7-dehydrocholesterol in fibroblast cells from patients with Smith-Lemli-Opitz syndrome

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Cholesterol (Chol) is a major lipid membrane component that plays important structural and functional roles in biological processes. In human Smith-Lemli-Opitz Syndrome (SLOS), mutations to the gene encoding 3β-hydroxysterol-Δ^7-reductase (DHCR7), which catalyzes the conversion of 7-dehydrocholesterol (7-DHC) to cholesterol, lead to elevated levels of 7-DHC and reduced levels of cholesterol in tissues. Hydrophobic olefins, including cholesterol and related sterols, steroids, etc., are difficult to ionize in mass spectrometry (MS) analysis and Lewis acid-complexation has been employed here to facilitate the ionization process (Rentel et al., 1998; Bayer et al., 1999). Several animal SLOS models exist, but the ideal approach for high-throughput small molecule intervention studies are cell lines that posses the SLOS genetic makeup. Here we introduce a strategy for the detection and relative quantitation of cholesterol and 7-DHC directly from plated and trypsinized cells using sputtered silver nanoparticle (NP)-assisted LDI and ion mobility – mass spectrometry (IM-MS). Ion mobility structure-based separation allows the removal of isobaric chemical noise that prevents reproducible detection and quantitation of cholesterol and 7-DHC in traditional LDI-MS platforms. We have determined the optimal silver sputter coating thicknesses and detection limits for standards and trypsinized cells, and have used this approach to obtain images of plated cells that were grown directly on ITO-coated glass plates. The sputtered Ag was found to perform better in LDI-IM-MS than using $AgNO_3$ and colloidal Ag nanoparticles. Sputtered silver NP-assisted LDI-IM-MS was found to provide a rapid and reproducible way to profile and image intact cells by IM-MS.

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34. Associations between plasma lipids and prediabetes and type 2 diabetes, independent of traditional risk factors

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The relationship between the lipid metabolism and Pre- and Type 2 Diabetes is poorly defined. We performed plasma lipid profiling on 117 newly diagnosed diabetics, with 234 non-diabetic, controls (age and sex matched), 64 of whom were categorized as being prediabetic and 170 of whom were classified as normal glucose tolerant drawn from the Australian Diabetes, Obesity and Lifestyle (AusDiab) Study. Lipidomic analysis involved measuring 259 lipids including sphingolipids, phospholipids, glycerolipids and cholesterol esters by LC-MS. Analysis of replicate quality control plasma samples across the cohort had a median CV of 10.3% with 90% of lipids below 17.0%. Logistic regression analysis demonstrated associations with T2D (135 lipids) and prediabetes (134 lipids) against NGT, after adjusting for multiple covariates, known to be risk factors for type 2 diabetes. Lipidomic analysis of an independent cohort of 1076 samples taken from the San Antonio Familial Heart Study validated the majority (90%) of the significant associations observed in the AusDiab cohort. In addition to the expected associations with diacylglycerols, triacylglycerols and cholesterol esters, there were positive associations with ceramide, dihydroceramide, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol. Additionally there were significant negative associations with the ether-linked phospholipids; alkyl- and aklenyl-phosphatidylcholine. The lipid profiles associated with prediabetes and T2D were closely aligned strongly suggesting the aberrant lipid profile associated with T2D largely established in the prediabetes group. We subsequently performed a longitudinal study drawing on 604 samples from the AusDiab cohort. Logistic regression showed that 90 lipids were associated with incident diabetes (progression from NGT or prediabetes to diabetes) further supporting the proposition that substantial lipidomic changes precede the development of T2D.

35. MD-2 binding of oxidized cholesterol esters activates TLR4 signaling

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Oxidation of low-density lipoprotein (LDL) is one of the major causative mechanisms in the development of atherosclerosis. In a previous study, we found that oxidized cholesterol esters (OxCE) are biologically active components of minimally oxidized LDL (mmLDL) that induce TLR4/MD-2-dependent signaling and proinflammatory responses in macrophages [JBC 2008, 283:10241]. In addition, mmLDL and OxCE induce recruitment of spleen tyrosine kinase (Syk) to TLR4/MD2, macropinocytosis and intracellular lipid accumulation. In the current study, we found that MD-2 binds free cholesterol (FC) and CE and that FC/MD-2 complexes can be found *in vivo*. In addition, we identified a type IV bicyclic endoperoxide/hydroperoxide of cholesteryl arachidonate (BEP-CE) as a specific OxCE, which activates macrophages in a TLR4/MD-2-dependent manner. Using a computational modeling approach, we identified K58, R90, and C133 as amino acid residues in MD-2, involved in BEP-CE binding. Using individual K58A, R90A and C133A mutants of MD-2, we confirmed the involvement of these amino acids in BEP-CE binding. BEP-CE binding to MD-2 induced TLR4 dimerization, activation of Syk, ERK1/2, JNK and c-Jun, and uptake of dextran and native LDL by macrophages. The latter resulted in intracellular lipid accumulation and macrophage foam cell formation. In summary, our results suggest that BEP-CE is an endogenous ligand that binds MD-2 and activates TLR4. Because TLR4/MD-2 expression in circulating monocytes is elevated in CVD patients and BEP-CE is found in human plasma and atherosclerotic lesions, BEP-CE-induced and TLR4/MD-2-mediated monocyte/macrophage activation may contribute to chronic inflammation and lesion progression in human atherosclerosis.

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36. Validation of an enzymatic assay for the measurement of lysophospholipid acyltransferase levels in cells

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Lysophospholipid acyltransferases (LPATs) are enzymes involved in recycling free fatty acids back into phospholipids via a coenzyme A-dependent mechanism. Previous work shows that blocking LPCAT3 (MBOAT5) or LPIAT1 (MBOAT7) with thimerosal leads to greatly enhanced eicosanoid production in stimulated human neutrophils by inhibiting the reacylation of arachidonic acid (AA) into phospholipids and substantially increasing its availability. The development of a facile assay to determine LPAT activity could be a useful tool to investigate the regulation of AA metabolism. Using microsomes from murine RAW264.7 cells, we have compared traditional enzymatic activity measurements of LPAT activity by radiochemical methods, using single lysophospholipid species and arachidonoyl-coenzyme A, to a multiple substrate choice assay where one lysophospholipid from each of six major classes (PA, PC, PE, PG, PI and PS) and eight fatty acyl coenzyme A esters (14:0, 16:0, 18:0, 18:1, 18:2, 20:4, 20:5 and 22:6) are commixed with the microsomes containing various LPATs. The complex mixture of products of this assay is analyzed using liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS). The results show that the multiple substrate choice assay provides similar results to the well-established radiochemical assay regarding kinetics and microsome dosage for AA acylation into phospholipids, without the need to use expensive and potentially hazardous radioactive compounds. In addition, this approach provides a single assay to monitor AA incorporation into six different phospholipid classes, offering an overview of the various AA acyltransferase activities that may be present in any complex biological sample. Finally, this assay offers insight into many LPAT activities involving not only AA, but other acyl chains as well, presenting a much broader picture of phospholipid remodeling activities in cells. In summary, this assay will aid in our understanding of the role that LPATs have in phospholipid remodeling and in regulating levels of free fatty acids.

37. Investigating the 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%, 2.5 mol%, 5 mol%, and 10 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.

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38. LC/MS analysis to detect different prostaglandins in SARS infected mouse lung

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Eicosanoids are arachidonic acid metabolites that play a variety of roles in regulating immune responses, especially in lungs. Our lab recently reported an age related defect in the migration of respiratory dendritic cells (DCs) to the draining mediastinal lymph nodes, which consequently resulted in reduced recruitment of virus-specific CD8 T cells to the lung following SARS-CoV (Severe Acute Respiratory Syndrome) infection. Aged mice had higher levels of PGD2 in the broncho alveolar lavage fluid (BALF) as detected by ELISA. Since precise quantification of the low levels of prostaglandins present in the BALF and lungs require a high-resolution method of detection, we developed an UPLC-MRM/MS method for simultaneous quantification of different eicosanoids, such as PGD2, PGE2, PGF2a and 5(S) HETE from the lungs and BALF of mice without derivatization. A relatively simple solid phase extraction (SPE) technique was used to extract prostaglandins from the mouse lung homogenate. We used a short UPLC reversed-phase column (1.7um particles) for separating the analytes, which allowed for shorter run times compared to the HPLC columns. This method was employed to quantify and compare the level of prostaglandins in the lungs of SARS infected aged and young mice and showed repeatability and tight retention times. We found that higher levels of pulmonary prostaglandins, such as PGD2, PGE2 and PGF2a were present in the lungs and BALF of uninfected, aged mice relative to young mice but that levels did not change substantially during SARS-CoV infection. In summary, this UPLC based MRM/MS method, which allows robust and sensitive profiling and quantification of pulmonary prostaglandins, will be useful for various immunological and pharmacological studies.

39. The effect of 7-dehydrocholesterol-derived oxysterols on cholesterol and lipid biosynthesis in cells

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7-Dehydrocholesterol (7-DHC) is accumulated in individuals affected with Smith-Lemli-Opitz syndrome (SLOS), a cholesterol biosynthesis disorder that is caused by mutations in the gene encoding 3β-hydroxysterol-Δ^7-reductase (DHCR7). SLOS is characterized by multiple congenital malformation and developmental disabilities (including autism). We reported that 7-DHC is the most oxidizable lipid molecule known to date, leading to over a dozen oxidation products (i.e., oxysterols). A number of novel 7-DHC-derived oxysterols have recently been identified in SLOS cell and rodent models, but the biological actions of these oxysterols have not been elucidated. Here we report that selected 7-DHC-derived oxysterols act as direct inhibitors of cholesterol biosynthesis at different enzymatic stages. First, Neuro2a cells were treated with individual oxysterols at biologically relevant concentrations (1 – 5 μM) in cholesterol-free medium, and the levels of cholesterol precursors were quantified by GC and HPLC-MS. The sterol profiles suggest that the activities of 3β-hydroxysterol-Δ^8,Δ^7-isomerase (Ebp) and/or Dhcr7 in cholesterol biosynthesis were inhibited. Second, in gene expression studies by qPCR, we found that individual oxysterols upregulate the expression of cholesterol biosynthesis-related genes in Neuro2a cells, such as *Srebp2*, *Hmgcr*, *Ebp*, and *Dhcr7*. Other lipid biosynthesis-related genes, such as *Fasn* and *Scd*, are also mildly upregulated with the treatment of selected oxysterols. This feedback response suggests that these 7-DHC-derive oxysterols act as direct inhibitors of the biosynthesis, which may contribute to the aggravation of the biochemical defect in SLOS. On the other hand, when the cells were treated with oxysterols in cholesterol-rich medium, the gene expression changes were attenuated or reversed. We propose that the observed expression changes in genes related to cholesterol and lipid biosynthesis may be a result of oxysterol-mediated interplay between SREBP-2 and SREBP-1 pathways.

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40. Genetic deletion of prostacyclin IP receptor exacerbates cognitive impairment and neuronal cell death in mouse global cerebral ischemia

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Transient global cerebral ischemia causes delayed neuronal death in the hippocampal CA1 region. It also induces an up regulation of cyclooxygenase 2 (COX-2), which generates several metabolites of arachidonic acid, known as prostanoids, including Prostaglandin I_2 (PGI_2). The present study investigated whether PGI2 IP receptor plays a role in brain injury after global cerebral ischemia. Adult male wild-type (WT) or IP knockout (IP KO) mice underwent a 12-minute bilateral common carotid artery occlusion (BCCAo) or a sham operation. Behavior tests (neurologic deficit and T-maze) were performed 3 and 7 days after BCCAo. Sham and ischemic mice were euthanized after 7 days of reperfusion to evaluate the morphological reactions of neurons, astrocytes and microglia through immunohistochemistry. In, sham WT and IP KO mice, no significant differences in behavior or neuronal cell death were observed. Interestingly, in IP KO ischemic mice, neurologic and cognitive deficits significantly increased ($p<0.05$) and death of hippocampal CA1 pyramidal neurons was delayed ($p<0.01$) as compared to WT mice. In addition, Microglia were activated at 7 days after 12 min ischemia in IP KO mice ($p<0.05$) as compared to WT mice in the CA1 hippocampal region. These data suggest that genetic deletion of the PGI2 IP receptor exacerbates ischemic brain injury, so blocking PGI2 signaling by treatment with IP receptor agonists could be a useful tool to prevent delayed pyramidal neuronal cell death.

41. Intermediates of the PUFA biosynthetic pathway are physiological inhibitors of the cholesterol biosynthesis

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Consumption of a diet very high in polyunsaturated fatty acids (PUFA) is associated with low levels of serum cholesterol and triacylglycerol. Similarly, in mice a diet rich in docosahexaenoic acid (DHA), a major, biologically active PUFA that mammals are incapable of synthesizing, lowers serum cholesterol, hepatic cholesterol and activity of the rate-limiting enzyme of cholesterol biosynthesis 3-hydroxy-3-methylglutaryl Coenzyme A reductace (Hmgcr). Despite these observations, conventional pharmaceutical PUFA doses lower serum triacylgycerol, but not serum cholesterol. Here we show that the intermediates (Coenzyme A thioesters) of PUFA biosynthesis are competitive inhibitors of Hmgcr. Since zebrafish are capable of synthesizing n:3 and n:6 PUFAs from plant-derived dietary fatty acids, this model organism provides a unique opportunity to investigate the effect of PUFAs on cholesterol synthesis. We fed *slc16a6a* mutants (which are incapable of secreting ketone bodies during fasting because they lack the hepatocyte b-hydroxybutyrate transporter protein Slc16a6a) a normal fat, high protein ketogenic diet to trigger massive triacylglycerol-only hepatic steatosis marked by lower cholesterol accumulation and decreased Hmgcr activity. Reminiscent of treatment with statin drugs, Hmgcr protein abundance, maturation of Sterol regulatory element binding factors 1 and 2, and relative incorporation of mevalonate into cholesterol were increased in *slc16a6a* mutants. Stearidonyl-CoA, eicosatetraenoyl acid-CoA, eicosapentaenoic acid, eisosapentaneyl-CoA docosahexaenoic acid and docosahexaenyl-CoA were increased in mutant livers. Finally, human HMGCR was inhibited in vitro by CoAs of PUFAs with kinetic parameters suggesting physiological plausibility, with the K_i of each putative inhibitor being lower than the K_M the substrate HMG-CoA. These results elucidate a mechanism for PUFA-mediated cholesterol lowering. Since one fifth of patients receiving statins can not tolerate these drugs, our results provide a rational basis for lowering cholesterol with an alternative HMGCR inhibitor.

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42. Reduced dietary omega-6:omega-3 ratio and 12/15 lipoxygenase deficiency protect from high fat diet-induced steatohepatitis

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Obesity is associated with metabolic perturbations including liver and adipose tissue inflammation leading to insulin resistance and type 2 diabetes. Omega-6 fatty acids (ω-6) can promote inflammation and omega-3 fatty acids (ω-3) can reduce inflammation as they can be metabolized, respectively, to pro- and anti-inflammatory eicosanoids. 12/15-lipoxygenase (12/15-LO) produces some of these metabolites and is induced by high fat diet (HFD). 12/15-LO knockout (KO) mice are protected from insulin resistance, adipose tissue inflammation, and fatty liver induced by an ω-6-enriched HFD. We investigated the effects of altering dietary ω-6:ω-3 ratio on HFD-induced tissue inflammation in wild-type (WT) and 12/15-LO KO mice. WT and 12/15-LO KO groups were each fed one of two iso-caloric HFDs with 45% kcal from fat for 15 weeks: soybean oil-rich HFD as model of high dietary ω-6:ω-3 ratio (11:1, HFHI) or HFD wherein soybean oil was replaced with fish oil to decrease the ω-6:ω-3 ratio to 2.7:1 (HFLO). We assessed insulin action, adipose and liver pathophysiology and inflammatory gene expression in the mice. WT mice fed HFLO exhibited reduced liver but not adipose tissue inflammation compared to WT mice fed HFHI, as evidenced by decreased hepatic expression of IFN-γ ($P< 0.01$), TNF-α ($p< 0.05$), IL-12p40 and -18 ($p< 0.05$), and CCR7 ($p< 0.05$). Fatty liver, adipose tissue inflammation, and adiposity were not different between HFLO- and HFHI-fed WT groups. KO mice were protected from fatty liver and liver inflammation (decreased IFN-γ, $p< 0.05$) and lymphocyte homing (decreased CCL19, $p< 0.05$) induced by HFHI. Only KO mice were protected from HFHI-induced insulin resistance. High dietary ω-6:ω-3 ratio, common in Western diet, increases HFD-induced nonalcoholic steatohepatitis and may exacerbate this pro-inflammatory microenvironment. Lowering dietary ω-6:ω-3 ratio significantly reduced HFD-induced hepatic inflammation. In addition, we conclude that inhibiting 12/15-LO activity may reduce diet-induced steatohepatitis and enhance insulin action.

43. Molecular characterization of oxysterol binding to the EBI2 receptor

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Oxysterols are oxygenated metabolites of cholesterol that are emerging as a physiologically important group of molecules. Although they regulate a range of cellular processes, only few oxysterol-binding effector proteins have been identified and the knowledge of their binding mode is limited. We and others have recently identified 7α, 25-dihydroxycholesterol (7α, 25-OHC) as a potent and selective agonist of EBI2[1,2] (Epstein-Barr virus induced gene 2; aka GPR183), a G protein-coupled receptor that is required for humoral immune responses diseases. In order to elucidate the molecular structure of the oxysterol binding site in the EBI2 receptor, we have used receptor modeling followed by site-directed mutagenesis to identify residues critical for ligand interaction. We find that 7α, 25-OHC binds to EBI2 via hydrogen bonding between its three OH-groups and the receptor residues R87 in TM-II (position II:20/2.60), Y116 in TM-III (III:13/3.37) and Y260 in TM-VI (VI:16/6.51). Mutating these residues to Ala and/or Phe, results in a severe decrease in agonist binding and receptor activation. In addition, Y112 (position III:09/3.33) is also involved in 7α,25-OHC binding but via hydrophobic interactions. EBI2 homology modeling suggests that R87 interacts with the 25-OH group, Y116 with the 3-OH and Y260 with the 7α-OH. In addition to the mutagenesis study, we have isolated tool compounds which can be used to address the physiological role of the oxysterol/EBI2 pathway in health and disease.

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

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44. Bile acids induce diacylglycerol kinase theta-dependent phosphatidic acid production

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Diacylglycerol kinases (DGKs) are intracellular lipid kinases that catalyze the conversion of diacylglycerol into phosphatidic acid (PA), which positively modulates mammalian target of rapamycin (mTOR). We have found that chenodeoxycholic acid and the synthetic farnesoid X receptor (FXR) ligand GW4064 induce DGKθ mRNA and protein expression in the HepG2 cell line and in primary human hepatocytes. Reporter gene studies using 1.5 kB of the DGKθ promoter fused to the luciferase gene revealed that bile acids increase DGKθ transcriptional activity and mutation of putative a FXR response element attenuated the ability of GW4046 and FXR to increase DGKθ reporter gene activity. Consistent with this finding, chromatin immunoprecipitation (ChIP) assays demonstrated that bile acid signaling increased the recruitment of FXR to the DGKθ promoter, concomitant with increased acetylation of histone H3. Further, GW4064 increased the cellular concentration of PA in a time-dependent manner. We also found that GW4064 and PA promote the phosphorylation of mTOR, Akt, and FoxO1, and that silencing DGKθ expression significantly abrogated ability of GW4046 to promote the phosphorylation of these target genes. Moreover, GW4064-mediated suppression of gluconeogenic gene expression and glucose production was dependent on DGKθ expression. Taken together, our results establish DGKθ as a pivotal mediator of bile acid-stimulated modulation of the glucose production.

45. PMA stimulates PKCalpha-dependent phosphorylation of ASAH1 and increases enzyme activity in breast cancer cell lines

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Ceramide and sphingosine-1-phosphate (S1P) are part of a large family of sphingolipids which have been extensively studied for their opposing roles in the regulation of various aspects of tumorigenesis. Ceramide inhibits cell growth and promotes apoptosis, while S1P induces cell proliferation and migration. Thus, the relative concentration of these two molecules plays a pivotal role in determining cell fate. Acid ceramidase (ASAH1) is a lipid hydrolase that directly regulates ceramide metabolism by catalyzing its degradation to sphingosine (SPH), which is then phosphorylated to form the potent mitogen S1P. ASAH1 plays a key role in cellular homeostasis by controlling the balance between ceramide and S1P. Interestingly, aberrant expression of ASAH1 in various human cancers, including breast cancer, prompted the emergence of ASAH1 as a potential chemotherapeutic target. Notably, inhibitors of ASAH1 such as B13 and LCL464 were shown to cause ceramide accumulation and prevent tumor growth. Given the emergence of targeting ASAH1 in cancer therapeutics, we sought to define the factors that regulate the expression and function of ASAH1 in breast cancer. We show that phorbol 12-myristate 13-acetate (PMA) stimulates the phosphorylation of ASAH1 at threonine-287 (T287) in multiple breast cancer cell lines, including MCF-7, MDA-MB-468, MDA-MB-231, and BT-20. Mutation of T287 reduced the stability of the protein, and *in vitro* ceramidase activity assay showed an increase in enzymatic activity in PMA treated MDA-MB-231 cells. PMA-stimulated phosphorylation of ASAH1 was dependent on protein kinase C-alpha. Interestingly, PKC-alpha expression levels, which has been reported as a marker for breast cancer aggressiveness, was 6-fold greater when compared to benign MCF10A mammary epithelial cells. Significantly, PMA was unable to induce the phosphorylation of ASAH1 in MCF10A cells, or increase enzymatic activity suggesting that phosphorylation of ASAH1 may contribute to increased ceramide turnover, S1P production, increased cell proliferation and the development of a cancerous phenotype.

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46. A systems biology approach to metabolic antagonism between omega-3 and omega-6 fatty acids during macrophage inflammatory response

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Eicosanoids, such as prostaglandins and leukotrienes are one of the major classes of lipids which are derived from arachidonic acid (AA), a 20-carbon polyunsaturated fatty acid (ω6 PUFA). Eicosanoids play important roles in pain, fever, inflammation and related disorders including cardiovascular diseases. The ω3 PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are thought to be potentially effective in preventing and treating cardiovascular diseases through competitive metabolism with AA. A systems biology approach, based on integrating existing mechanistic knowledge and novel high-throughput data, provides a powerful strategy for quantitative understanding of the molecular basis of lipid metabolic networks in mammalian cells. Previously, we have developed mathematical models of AA metabolic network in RAW264.7 cell and mouse bone-marrow derived macrophage. Here, we have developed a quantitative computational model of the competitive metabolism of AA and EPA via COX pathway through a two-step matrix-based approach to estimate the rate constants. The model was developed by using lipidomics datasets that were experimentally obtained from PUFA-supplemented ATP-stimulated RAW264.7 macrophages. The resulting model fits the experimental data well for all species and demonstrates that the integrated metabolic and signaling network and the experimental data are consistent with each other. The robustness of the model is validated through parametric sensitivity and uncertainty analysis. We further validated the model by predicting accurately the results from another independent experiment, using the parameters estimated previously. Work supported by the NIH grant GM U54 069338 to the LIPID MAPS Consortium.

47. An omics study of oxidized phospholipid activated RAW 264.7 cells

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Oxidized low density lipoproteins (OxLDLs) that accumulate during oxidative stress are recognized and cleared by macrophages using cell surface scavenger receptors, including CD36. However, the unregulated uptake of OxLDL by macrophages within the arterial wall leads to atherosclerosis. POVPC (1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphocholine), a major oxidized phospholipid, generated in highest concentration from LDL, has been used to study the role of macrophages in atherosclerosis. In this study we examined how metabolism of POVPC by macrophages affects its pro-inflammatory response. For this, LIPID MAPS consortium has generated high-throughput transcriptomic and lipidomic data from RAW 264.7 cells treated with POVPC-peptide (a synthetic hexapeptide/phosphatidylcholine construct) and/or ATP over 0-24 hour duration. The analysis of variance (ANOVA) identified the effects of ATP and POVPC on lipid levels. Enrichment analysis of the regulated genes revealed that several processes such as inflammation, immunity and cell survival, ER stress (up-regulated) and sterol biosynthesis (down-regulated) are affected in a time-dependent manner. Additionally, protein misfolding and endoplasmic reticulum stress results in substantial up-regulation of Trib3, Atf3/4 and Chac1 genes. Transcription factor - target gene mapping also reveals that inflammation related genes and their targets such as NF-kB1, c-Jun, c-Fos, IL-6/10 and Stat1 are up-regulated temporally. The temporal regulation is observed for c-Jun and c-Fos to Ptgs2 early on and Cebpδ to Ptgs2 at later time points. Toll-like receptor signaling and cytokine-cytokine receptor signaling are overall up-regulated. The targets of sterol regulatory element binding proteins (SREBP1/2) are up-regulated early on but down-regulated during late phase. Liver X receptor (LXR) targets are down-regulated (especially, Abca1 and Abcg1) contributing to some intracellular accumulation of cholesterol. Further, for the eicosanoids metabolism, joint interpretation of lipid and transcriptomic data suggests that despite 8-fold increase in cyclooxygenase-2 (COX-2, Ptgs2) mRNA levels, levels of prostaglandins do not change substantially because much of the flux from arachidonic acid is still through COX-1. Work supported by the NIH grant GM U54 069338 to the LIPID MAPS Consortium.

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48. NASH associates with a phospholipid pattern in morbidly obese female *

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Non-alcoholic fatty liver disease (NAFLD) is the accumulation of liver fat which associates with inflammation and hepatocyte ballooning in severe forms (i.e. nonalcoholic steatohepatitis, NASH). This obesity-related liver condition can progress to cirrhosis, hepatocellular carcinoma and liver failure. We previously showed that the accumulation of inflammatory cells in visceral adipose tissue associates with NASH in morbid obesity. This suggests that there are putative molecules linking visceral fat to liver injuries via the portal venous system that need to be discovered. We examined in this context the lipidomic profile in systemic and portal blood, collected from obese women undergoing bariatric surgery. Subjects were divided into NASH or no-NASH based on NASH activity score ≥ 5 of liver biopsies. In NASH patients, there was an increased circulating concentrations of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylglycerol (PG), ceramides (Cer) suggesting alterations in phospholipid (PL) metabolism. Elevated long chain PGs with reduced unsaturation and elevated medium chain Cer species characterized phospholipid profile in systemic blood from NASH patients. Interestingly in these subjects, PG and PE were also elevated in portal blood suggesting contribution of visceral adipose tissue or gut to the circulatory PL pool. Lipid species significantly elevated in NASH in systemic and portal blood were also significantly associated with bioclinical variables such as percent fat mass and serum levels of liver enzymes - ASAT, ALAT, GGT and ApoB. Our work suggests relationships between PL pattern and liver injury in morbid obesity.

49. Quantitation of absolute rates of cholesterol synthesis and tissue cholesterol content in the liver and other major organs of mice with lysosomal acid lipase deficiency: potential applications of the model

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Background: Lysosomal acid lipase (LAL) hydrolyzes cholesteryl esters (CE) and triacylglycerols (TAGS) in lipoproteins that are internalized via receptor-mediated endocytosis. Mutations in the LAL gene result in cholesteryl ester storage disease (CESD), or Wolman's disease (WD). Aim: (1) To quantitate in vivo the absolute rates of cholesterol synthesis and tissue cholesterol contents in the major organs of young adult *lal*$^{-/-}$ and matching *lal*$^{+/+}$ mice and (2) to measure the lifespan of these mice. **Methods:** Litters from *lal*$^{+/-}$ parents (FVB strain) (from Dr. G. Grabowski) were weaned at 21 days on to a low cholesterol, low fat rodent chow diet and maintained on this until studied (in fed state) at 7 wks of age. Results: At 7 wks, relative liver weight in the *lal*$^{-/-}$ mice was double that in *lal*$^{+/+}$ controls. The whole body (WB) cholesterol content (mg/kg bw) in the *lal*$^{-/-}$ mice (9526±385) was 4.2-fold greater than in their *lal*$^{+/+}$ controls (2270±31) and the proportion (%) of WB cholesterol contained in the liver of the *lal*$^{-/-}$ mice (63±1.4) was 10-fold more than in the controls (6.3±0.2). In the *lal*$^{-/-}$ mice the proportion (%) of liver cholesterol present as CE was 91.4±0.4 vs 18.9±1.8 in controls. Hepatic TAG (mg/liver) was elevated 4.1-fold in the mutants (72.6±8.3 vs 17.7±3.9). WB cholesterol synthesis (CS) was elevated 3.5-fold in the mutants. In the *lal*$^{-/-}$ mice, the liver contributed 68.2±0.5% to WB cholesterol synthesis vs 21.7±2.3% in the controls. The median age of survival of the *lal*$^{-/-}$ mice was 313 days. **Conclusions:** These data represent the first complete whole body measurements of cholesterol production and content in an animal model for CESD. The *lal*$^{-/-}$ mouse is currently being used to evaluate the impact of chronic treatment with ezetimibe, a potent intestinal cholesterol absorption inhibitor, on tissue cholesterol sequestration in CESD.

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50. Dietary extra virgin olive oil down regulates oxidized lipid mediators CCl_4 induced liver injury

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Dietary oils rich in unsaturated fatty acids demonstrate various effects in chronic liver injury. Some reports indicated that extra virgin olive oil has better benefits against carbon tetrachloride (CCl_4) induced liver injury, but the details are still unclear. It is well established part of liver injury induced by CCl_4 is mediated by lipid peroxidation. In this study we investigated the effect of different dietary fat in rats with and without CCl_4. Oxidized lipid mediators of arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid and cholesterol liberated through lipoxygenase and cytochrome P450 enzymatic and non-enzymatic pathways were determined. Fischer 344 male rats were fed normal laboratory chow plus 20% (w/w) corn oil (CO) or extra virgin olive oil (EVOO) or lard (LD). The animals in each dietary group were hypodermically given CCl_4 (1:1 with soybean oil, 0.1 ml/100g bodyweight) twice a week for 4 weeks and the control with soybean oil only. Oxidized lipid mediators (OLM) namely 5(S)-, 8(S)-, 11(S)-, 12(S)-, 15(S) and 20-HETEs, F_2-isoprostanes, F_3-isoprostanes, resolvin D1, 7β-hydroxycholesterol, 7-ketocholesterol, 27-hydroxycholesterol and the precursor levels were measured by liquid chromatography tandem mass spectrometry. Liver of rats fed with EVOO had significantly reduced ($p<0.01$) HETE products, F_2-isoprostanes, 7 β - hydroxycholesterol and 7-ketocholesterol levels compared to control, CO and LD. Treatment of CCl_4 alone substantially elevated ($p<0.01$) liver OLM in particular 5(S)- and 9(S)-HETEs, F_2-isoprostanes, 7 β - hydroxycholesterol and 7-ketocholesterol compared to the control. However, some of these OLM in rat livers fed with EVOO significantly ($p<0.01$) reduced (HETE products, F_2-isoprostanes, F_3-isoprostanes, 7□-hydroxycholesterol) below the control levels. The levels remained low even when adjusted by the precursor concentration. This effect was not seen in rat livers fed with CO or LD where OLM levels remained high. This study showed dietary EVOO is capable of reducing oxidized lipid mediators in rat liver even when injured by CCl_4.

51. Differential effects of extra virgin olive oil and corn oil in CCl_4 induced liver injury: a proteomic study

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Introduction: Dietary oils rich in unsaturated fatty acids present various effects in chronic liver injury. In order to explore the molecular mechanisms of health benefit of extra virgin olive oil (EVOO) against carbon tetrachloride (CCl_4) induced liver injury, we constructed a 2-dimensional gel (2DG) based proteomics approach in the present study. **Methods:** Fischer 344 male rats were divided into four groups: corn oil normal group (CO-), olive oil normal group (OO-), corn oil CCl4 (CO+) group and olive oil CCl4 (OO+) group. Rats were fed normal laboratory chow plus 20% (w/w) of corn oil or EVOO for the entire experimental period. Rats in CCl4 treatment groups were given subcutaneous injection of CCl4 (1:1 with soybean oil, 0.1 ml/100g body weight) twice a week for four weeks, rats in normal groups were given soybean oil only. MDA index and α-SMA, TGF-β gene expression level in liver were measured and hepatic protein proteomes were compared between two dietary oil groups with CCl_4 treatment. **Results:** The CCl_4 administrated animals fed EVOO showed weaker fibrogenesis activity and lower lipid peroxidation than corn oil feeding group. The identified differential expressed proteins including thioredoxin domain-containing protein 12, peroxiredoxin-1, thiosulfate sulfurtransferase, Calcium-binding protein 1, phosphohistidine phosphatase 1, homogentisate 1,2-dioxygenase, heterogeneous nuclear ribonucleoprotein F, seryl-tRNA synthetase, Annexin A2 and heat shock cognate 71 kDa protein which has a higher expression level in OO+ group while the expression of COQ9, cAMP-dependent protein kinase type I-alpha regulatory subunit, phenylalanine hydroxylase and glycerate kinase are lower in OO+ group compared with CO+ group. **Conclusions:** The histological and biochemical results proved the benefits of EVOO, and proteomic results indicate the different effects of extra virgin olive oil and corn oil in CCl_4 induced liver injury due to the difference of antioxidant effects, hepatocellular function regulation and general protein expression modification.

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52. The flagellar membrane of Chlamydomonas is a specialized, highly ordered lipid domain of the plasma membrane enriched in raft lipids

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Cilia are important for human health. Although numerous studies have demonstrated that ciliary and flagellar membranes contain unique proteins and thus represent unique domains of the plasma membrane, no studies exist comparing the lipids of ciliary *vs*. plasma membranes. Because specific lipids may be important in sorting and targeting ciliary proteins at the trans-Golgi network, in intraflagellar transport, and in the functioning of membrane proteins, we are studying the lipidome of the *Chlamydomonas* flagellum. Wild-type and cell-wall mutant cells were grown under controlled conditions and deflagellated by treatment with dibucaine. The lipid composition of whole cells, cell bodies, flagella, and cell body plasma membrane (PM) were isolated by two-phase partitioning and compared by mass spectrometry. The analyses showed that all compartments have distinct lipid profiles. The most abundant fatty acids (FAs) in flagella, representing 95% of the total, were the short-chain saturated palmitic (37%) and stearic (22%) acids, and the unsaturated oleic (15%) and γ-linolenic (21%) acids. γ-linolenic acid is highly enriched in flagella *vs*. to the PM (2%) and to cell bodies (5%). The ratio of saturated to unsaturated FAs in flagella was higher (1.5) than in cell bodies (0.4), but lower than in PM (7.4). The raft lipids phosphatidylethanolamine, ergosterol, stigmasterol, and β-sitosterol, and five unidentified lipids are enriched in flagella ~25-35x *vs*. to the PM, suggesting that the flagellar membrane is highly ordered, a prediction confirmed by *in vivo* two-photon microscopy using the membrane polarity sensitive probe C-Laurdan. These results show for the first time that the flagellar membrane differs from the plasma membrane in lipid composition. The lipid raft-like composition of the flagellar membrane may have an essential role in the assembly and function of flagella.

53. A synthetic POVPC-peptide is a model oxidized phospholipid that induces expression of inflammatory genes in macrophages and endothelial cells

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POVPC (1-Palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphocholine) is an oxidized phospholipid (OxPL) found in OxLDL and other inflammatory settings. It has pro-inflammatory effects on macrophages (MAC) and endothelial cells (EC), promoting inflammation and atherosclerosis. POVPC possesses a reactive aldehyde in the *sn-2* position, which renders it highly reactive and prone to structural changes and is therefore difficult to study. We developed a synthetic POVPC-peptide adduct at the *sn-2* side chain, which is soluble, stable and not prone to further oxidation, to study inflammatory effects on MAC and EC. The POVPC-peptide induced substantial expression of the pro-inflammatory cytokines Ccl2, Ccl3, Ccl4, Cxl2 and Tnf-alpha in murine bone marrow derived macrophages and RAW 264.7 cells. Control peptide alone was inactive. Moreover, we stimulated human monocyte derived MAC and EC with either POVPC-peptide or oxidized PAPC (OxPAPC), a mixture of OxPLs, and analyzed gene expression with microarray analysis. Both were more bioactive in EC vs. MAC, with POVPC-peptide > OxPAPC. In contrast, OxPAPC was more active in MACs. The gene expression patterns in human and murine macrophages were similar, whereas expression patterns between human MAC and EC were very different. Overall, POVPC-peptide stimulated ~ 50% of highly regulated genes stimulated by OxPAPC. The POVPC-peptide prominently increased the MAC and EC expression of Cox-2, an enzyme known to play a central role in eicosanoid production. Thus, we studied the effect of POVPC-peptide on eicosanoid production in RAW macrophages. While POVPC-peptide alone modestly induced eicosanoid secretion, co-stimulation along with ATP, which raises intracellular arachidonic acid levels and provides substrates for Cox-2, robustly stimulated eicosanoid production. This work shows that the POVPC-peptide adduct is a stable model of an OxPL that is highly bioactive. It induced pro-inflammatory responses in MAC and EC similar to OxPAPC and should be valuable in further study of the role of OxPL in inflammation and atherosclerosis.

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54. Phospholipids in myelin from a canine model of mucopolysaccharidosis I (MPS I): a lipidomics study

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Background: Mucopolysaccharidosis (MPS) I is a lysosomal storage disease of α-L-iduronidase deficiency. Clinical manifestations include visceral organomegaly, skeletal and joint abnormalities, and mental retardation. Our data on the corpus callosum of MPS I canines indicates myelin deficiency. Neurologic symptoms in the MPS I canines are improved by intrathecal-enzyme replacement therapy (IRT). We hypothesized that the myelin of MPS I canines would demonstrate an abnormal phospholipid profile, which would be normalized by IRT. The objective of this study was to characterize the polar lipid profile of MPS I myelin with and without IRT using a lipidomic approach. **Methods:** Myelin was isolated from the corpus callosum of adult 1) MPS I canines, 2) MPS I canines after intrathecal recombinant human alpha-l-iduronidase treatment (0.05mg/kg every 3 months from age 4-21 months) and 3) heterozygous clinically-normal controls (n=4 each group). Total lipids were extracted for liquid chromatography/tandem mass spectrometry analysis of polar lipids. Quantities of polar lipid species were compared among the three groups. Principal components analysis (PCA) was also applied to the data set. **Results:** 260 polar lipid species were detected in at least half of the samples. Polar lipid species in MPS I were low in many classes, including phosphatidylcholine (PC), sphingomyelin, ether-linked PC (ePC), phosphatidylethanolamine (PE), lysoPE, ePE, phosphatidylinositol, phosphatidylserine (PS), ePS, and phosphatidic acid. IRT normalized some species, but did not substantially improve those in the ePC and PS classes. PCA analysis separated the lipidomics profiles into the three experimental groups. **Conclusions:** Myelin of the corpus callosum in MPS I canines is characterized by low quantities of polar lipid species, distributed across most of the phospholipid classes. IRT does not completely restore the lipid profile. Further studies are needed to demonstrate whether the hypomyelination of canine corpus callosum in MPS I is associated with decreased phospholipid production versus increased turnover.

55. Fatty acid metabolites as biomarkers for non-alcoholic fatty liver disease (NAFLD): a lipidomic and metabolomic approach

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With an increasing incidence of NAFLD worldwide, intensive research has focused on the molecular mechanisms that underlie the pathogenesis of this disease and biomarkers that can predict the onset and progression of NAFLD. Emerging evidence suggests that oxidative stress and chronic inflammation contribute to the development of NAFLD. Omics techniques including lipidomics and metabolomics have primarily focused on lipid/fatty acid signatures as potential biomarkers in plasma lipidome that may reflect the development of NAFLD. We hypothesize that the downstream metabolites of fatty acids may play equally or even more important role in mediating the signaling pathways that lead to NAFLD. In this presentation, we summarize our efforts to develop and validate a lipidomic and metabolomic approach to systematically analyze metabolites of fatty acids in cellular models as well as in plasma, liver tissue, and urine of a rodent model of NAFLD. The identified biomarkers from these studies are also cross-validated in patients with different stages of NAFLD. Our results show that metabolites derived from enzymatic oxidation and lipid peroxidation of arachidonic acids and linoleic acid are elevated significantly in cellular and animal model. Our data demonstrate that metabolites derived from fatty acids may be potential biomarker for the pathogenesis of NAFLD. This work is supported by grants from the National Basic Research Program of China (2012CB5249) and National Natural Science Foundation of China (31170809).

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56. Lipid profiling of plasma samples from patients with chronic hepatitis C virus infection using HPLC coupled to triple quadrupole MS or LTQ-FT MS

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Although many studies demonstrated that lipid metabolism, especially sphingolipids, participate in several aspects of the hepatitis C virus (HCV) life cycle, but how the lipid profile changes during development of the disease are currently unknown in human. Meanwhile noninvasive biomarkers for liver inflammation are needed urgently. Here we reported a reliable and efficient platform consisted of HPLC-QQQ and HPLC-LTQ/FT to simultaneously and quantitatively profile sphingolipids (SPs), diacylglycerols (DGs), triacylglycerols (TGs), glycerophosphocholines (PCs), and glycerophosphoethanolamines (PEs) in human plasma samples (HCV Patient group: n=120, containing five groups with 0~4 biopsy confirmed inflammation grade; control group: n=11). LTQ/FT was used for screening lipid composition based on retention time, mass resolution of 200,000, mass accuracy below 2 ppm and isotope distribution by lipid data analyzer. Each lipid category contained at least one internal standard and one standard curve. Owing to low sensitivity, LTQ/FT can not detect SPs because of very low abundance. To complement this, SPs were detected and quantified by HPLC-QQQ based on retention time and fragmentation pattern of corresponding synthetic standards from standard curve and quality control samples. Statistical analysis consisted of Mann-Whitney U test, correlation analysis and receiver operating characteristic (ROC) curve analysis. Orthogonal partial least squares discriminant analysis (OPLS-DA) was applied for potential biomarkers determination. As a result, 44 SPs, 1 DG, 43 TGs, 24 PCs and 5 PEs were successfully identified and quantified. Fifteen of them correlated with biopsy determined inflammation grade (p<0.05). By OPLS-DA, Cer(d18:1/24:0), Cer(d18:1/18:1)-1-P, PE(38:4), and PC(38:4) were selected as potential biomarkers with area under the ROC curves greater than 0.95. This study demonstrated that combination of two mass spectrometers with complementary characteristics could successfully quantify 117 lipids in human plasma sample. Four lipid potential biomarkers could be further used for the evaluation of inflammation grade of chronic HCV infection noninvasively.