POSTER SESSIONS

The LIPID MAPS meeting features two poster sessions with 63 posters. Of these, only five could be selected for short talks this year (see program) by the poster committee (Edward A. Dennis, Alfred H. Merrill, Jr., Christian R.H. Raetz and David W. Russell). We received so many high quality, relevant abstracts that making these selections was very difficult and was based in part on fitting with the program. The poster presenters are also cross referenced in the attendee list.

1. **Mechanism of extracellular production of bioactive lysophospholipids using a newly developed LC-MS/MS method**
   Asuka Inoue, Michiyo Okutani, Daisuke Saigusa, Akira Shuto, Shin-ichi Okudaira, Naohito Suzuki, Yoshihisa Tomioka, Junken Aoki (Tohoku University, Sendai City, Japan).
2. **Eosinophils contribute to the resolution of acute peritonitis by producing pro-resolving lipid mediators**
   Makoto Arita, Tomohiro Yamada, Yukako Tani, Yosuke Isobe, Hiroyuki Arai (University of Tokyo, and PRESTO JST, Japan).
3. **Mapping the HEPG2 lipidome as effected by niacin using products of all acquisition on an new generation Q Q TOF**
   Phillip Sanders, David Yurek, Ming-Shang Kuo, Lyle Burton, Brigitte Simmons, Eva Duchoslav, Ron Bonner (Eli Lilly & Co. and AB Sciex).
4. **Information independent MS/MS data collection of all precursors using time-of-flight mass spectrometry**
   Brigitte Simons, Tanya Gamble, Lyle Burton, Eva Duchoslav, Philip Sanders, Ron Bonner (AB SCIEX and Lilly Corp).
5. **Isolation and structural analysis of leukotriene A epoxides: insights into the mechanism of the lipoxygenase-catalyzed transformation**
   Jing Jin, Yuxiang Zheng, William E. Boeglin, Alan R. Brash (Vanderbilt University).
6. **The oxidation and subsequent hydrolysis of linoleoyl-ω-hydroxyceramide is fundamental in epidermal barrier formation**
   Christopher P. Thomas, Yuxiang Zheng, William E. Boeglin, Valerie B. O'Donnell, Alan R. Brash (Vanderbilt University Medical Center).
7. **Specifically oxidized ceramide esters: required intermediates in formation of the skin permeability barrier ***
   Yuxiang Zheng, Huiyong Yin, William E. Boeglin, Peter M. Elias, David R. Beier, Alan R. Brash (Vanderbilt University Medical Center).
8. **EP2 signaling does not restore resolution of experimental Lyme arthritis in cyclooxygenase-2-deficient mice**
   Anna M. Ritzman, Jennifer M. Hughes-Hanks, Charles R. Brown (University of Missouri).
9. **Effect of leukotriene-deficiency on phagocytosis in mouse leukocytes**
   Yan Zhang, Charles R. Brown (University of Missouri).
10. **Activation of lysophosphatidic acid receptors reduces the infarct brain volume of rats after ischemic stroke**
    Hay-Yan J. Wang, Ping-Ju Tsai, Hsuan-Wen Wu, Cheng Bin Liu, Li-Hwa Chen (National Sun Yat-Sen University, Taiwan).
11. **Bioinformatic and comparative determination of tumor cardiolipin remodeling mechanisms using lipidomic data**
    Lu Zhang[#], Robert J.A. Bell[#], Michael A. Kiebish, Thomas N. Seyfried, Xianlin Han, Richard W. Gross, Jeffrey H. Chuang (Boston College).

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

12. **Lysophosphatidic acid (LPA) signaling as an initiating cause of fetal hydrocephalus**
Yun C. Yung, Tetsuji Mutoh, Mu-en Lin, Kyoko Noguchi, Richard R. Rivera, Ji Woong Choi, Marcy A. Kingsbury, Jerold Chun (The Scripps Research Institute).

13. **Characterization of CC1736, a conserved START domain protein and putative coenzyme Q-binding protein**
Christopher M. Allan, Shauna Hill, Susan Morvaridi, Ryoichi Saiki, Wei-Siang Liao, Kathleen Hirano, Tadashi Kawashima, Catherine F. Clarke (University of California, Los Angeles).

14. **Stabilization of catalytic Coq polypeptide complex in *Saccharomyces cerevisiae* by over-expression of the Coq8 protein kinase**
Cuiwen He, Letian Xie, Jia Yan Chen, Catherine F. Clarke (University of California, Los Angeles).

15. **Isotope-reinforced polyunsaturated fatty acids profoundly slow lipid chain autoxidation in yeast**
Shauna Hill, Kathleen Hirano, Vadim V. Shmanai, Beth N. Marbois, Randy To, Dragoslav Vidovic, Adrei V. Bekish, Mikhail Shchepinov, Catherine F. Clarke (University of California, Los Angeles).

16. **Aromatic precursors in the *S. cerevisiae* coenzyme Q biosynthetic pathway**
Beth Marbois, Catherine F. Clarke (University of California, Los Angeles).

17. **Yeast *coq* null mutants harboring multi-copy *COQ8* accumulate novel intermediates in coenzyme Q biosynthesis**
Letian X. Xie, Jia Yan Chen, Shota Watanabe, Cuiwen He, Beth Marbois, Fabien Pierrel, Catherine F. Clarke (University of California, Los Angeles).

18. **The use of stable isotopic labeled glycerol and oleic acid to differentiate the hepatic functions of diacylglycerol acyltransferase-1 and -2 ***
Jenson Qi, Wensheng Lang, John G. Geisler, Ping Wang, Ioanna Petrounia, Charles Smith, Hossein Askari, Shariff Bayoumy, Eugene Grant, Gary W. Caldwell, Yin Liang, Michael Gaul, Margery A. Connelly (Janssen Pharmaceutical Companies of Johnson and Johnson, LLC).

19. **Identification of 1-deoxydihydroceramide biosynthesis by mammalian cells**
Hyejung Park, M. Cameron Sullards, Alfred H. Merrill, Jr., Catherine E. Costello (Boston University).

20. **A protective role for LXR in diabetic nephropathy**
Monika Patel, Xiaoxin Wang, Tao Jiang, Moshe Levi, Carolyn L Cummins (University of Toronto, Canada).

21. **Essential role of ELOVL4 in very long chain fatty acid synthesis and retinal function**
Richard Harkewicz, Hongjun Du, Zongzhong Tong, Hisham Alkuraya, Matthew Bedell, Woong Sun, Xiaolei Wang, Guy Hughes, Xinran Wei, Peter X. Shaw, Edward A. Dennis, Kang Zhang (University of California, San Diego).

22. **Specificity of eicosanoid production depends on the TLR-4 stimulated macrophage phenotype**
Paul C. Norris, Donna Reichart, Darren S. Dumlao, Christopher K. Glass, Edward A. Dennis (University of California, San Diego).

23. **Identification of oxidative modifications in phosphatidylserine polar head using lipidomic approach**
Elisabete Maciel, Raquel Silva, Cláudia Simões, Pedro Domingues, Rosário M. Domingues (University of Aveiro, Portugal).

24. **The influence of PE glycation, oxidation and glycoxidation on monocyte stimulation**
Cláudia Simões, Ana Silva, Rosário M. Domingues, Pedro Domingues, Artur Paiva (Univ. of Aveiro, Portugal).

25. **Novel serum albumin-bound lipids correlate with newly diagnosed type II diabetes**
Jared Bowden, Penggao Duan, Edward Dratz (Montana State University).

26. **Contribution of H3K4 methylation by SET-1A to interleukin-1–induced cyclooxygenase 2 and inducible nitric oxide synthase expression in human osteoarthritis chondrocytes**
Fatima Ezzahra El Mansouri, Sarah Salwa Nebbaki, Hassan Fahmi (University of Montreal, Canada).

27. **Valproic acid suppresses interleukin-1β-induced microsomal prostaglandin E2 synthase-1 expression in chondrocytes through upregulation of NAB1**
Sarah Salwa Nebbaki, Nadia Zayed, Fatima Ezzahra EL Mansouri, Nadir Chabane, Mohit Kapoor, Hassan Fahmi (University of Montreal, Canada).

28. **Detection of breast cancer-associated lipids in hair**
Dharmica A H Mistry, Peter W. French (Macquarie University and SBC Research Pty Ltd,. Australia).

29. **The putative *N*-acyl PE synthase from *Arabidopsis thaliana* is a lyso-glycerophospholipid acyltransferase ***
Teresa A. Garrett, Evgeny Bulat (Vassar College).

30. **Down-regulation of sterol biosynthesis network by interferon**
Mathieu Blanc, Kevin A Robertson, Paul Lacaze, Wayne Hsieh, Thorsten Forster, Birgit Strobl, Matthias Müller, Markus R Wenk, Peter Ghazal (University of Edinburgh, UK).

31. **New pool of brain esterified prostanoids**
Stephen Brose, Mikhail Y. Golovko (University of North Dakota).

32. **Cholestenoic acids and motor neuron development?**
William J. Griffiths, Michael Ogundare, Spyridon Theofilopoulos, Ernest Arenus, Yuqin Wang (Swansea University, United Kingdom).

33. **Oxysterols in newborn mouse brain**
Anna Meljon, Yuqin Wang, William J. Griffiths (Swansea University, UK).

34. **HSP70/70B' induced by lipoprotein immune complexes sequester lipids in the endosomal compartment: impact on oxidative stress and macrophage survival**
Mohammed Al Gadban, Kent J. Smith, Waleed O. Twal, Dezirea Jones, Gabriel Virella, Maria F. Lopes-Virella, Samar M. Hammad (Medical University of South Carolina).

35. **Oxidized low density lipoprotein immune-complexes protect monocytes from apoptosis and stimulate increased cytokine release through acid sphingomyelinase activation**
Jean-Philip Truman, Mohammed Al Gadban, Kent J. Smith, Nalini Mayroo, Gabriel Virella, Maria F. Lopes-Virella, Alicja Bielawska, Yusuf A. Hannun, Samar M. Hammad (Medical University of South Carolina).

36. **Lipid metabolome of fibroblasts with peroxisomal diseases ***
Kazuaki Yokoyama, Chiho Nishizawa, Toru Nagai, Kazutaka Ikeda, Masashi Morita, Ken Karasawa, Noriko Satoh, Ayako Harada, Nobuyuki Shimozawa, Tsuneo Imanaka, Ryo Taguchi, Keizo Inoue (Teikyo University, Japan).

37. **Eicosanoid profiling reveals shunting towards PGD_2 pathway in MPGES1 knock-out mice**
Helena Idborg, Marina Korotkova, Karina Gheorghe, Petter Olsson, Elena Ossipova, Patrick Leclerc, Per-Johan. Jakobsson (Karolinska Institutet, Sweden).

38. **The metabolic fate of fatty acids in yeast: to store or not to store, that is the question**
Oskar L. Knittelfelder, Harald F. Hofbauer, Gerald N. Rechberger and Sepp D. Kohlwein (University of Graz, Austria).

39. **Glucose induced lipid stress and inflammation, drivers of insulin resistance development**
Berit Johansen, Hans-Richard Brattbakk, Ingerid Arbo, Mette Langaas, Martin Kuiper (Norwegian University of Science and Technology, Norway).

40. **Five-lipoxygenase/leukotriene pathway inhibition by caffeic acid phenethyl ester and propolis**
Maria-Pia Rossi, Lucia A. Giliberti, Maria-Filomena Caiaffa, Luigi Macchia (University of Bari, Italy).

41. **Expression of $cPLA_2$ in macrophages is critical for lung cancer metastasis**
Mary Weiser-Evans, Jay Amin, Amber Sorenson, Miguel Gijon, Robert Murphy, and Raphael Nemenoff (University of Colorado).

42. **Steady-state and dynamic analytical approaches for identifying lipid biomarkers and understanding the pharmacology of lipid-modifying drug targets**
Thomas Roddy, Stephen Previs, David McLaren, Michael Lassman, Haihong Zhou, Douglas Johns, Jose Castro-Perez, Brian Hubbard, Andrew Nichols (Merck & Co).

43. **Sensitive detection of nonpolar lipids in human meibomian gland secretions via shotgun electrospray ionization-mass spectrometry**
Jianzhong Chen, Kari B. Green-Church, Kelly K. Nichols (Ohio State University).

44. **Investigating the effects of lipid peroxidation on the dynamic properties of model membrane system**
Stewart Gruey, Nisreen Nusair (Walsh University).

45. **Monitoring the dynamics of oxygen and free radicals within neuronal membranes by EPR techniques**
Sameh S. Ali, Brian Head, David Roth, Hemal Patel (University of California, San Diego).

46. **Discovery and biological characterization of novel non-cytidine liponucleotides**
Jeremy Henderson, Ziqiang Guan, Greg Laird, Reza Kordestani, Christian R.H. Raetz (Duke University).

47. **A pathway for lipid A biosynthesis in *Arabidopsis thaliana* resembling that of *Escherichia coli***
Chijun Li, Ziqiang Guan, Dan Liu, Christian R. H. Raetz (Duke University Medical Center).

48. **Sterol analysis in humans: the Dallas Heart Study**
Jeffrey G. McDonald, Daphne D. Head, David W. Russell, Daniel D. Smith, Ashlee R. Stiles, Bonne M. Thompson (University of Texas Southwestern Medical Center).

49. **Reversal of foam cell infiltration with non-macrophage targeted rescue of Niemann-Pick C disease**
Andrés D. Klein, Manuel E. Lopez, Matthew P. Scott (Stanford University School of Medicine).

50. **A role for DGKθ in cAMP-dependent steroidogenic gene transcription in the adrenal cortex**
Kai Cai, Marion B. Sewer (University of California, San Diego).

51. **Acid ceramidase is a novel transcriptional coregulator of steroidogenic genes in human H295R adrenocortical cells**
Natasha C. Lucki, Sibali Bandyopadhyay, Elaine Wang, Alfred H. Merrill, Donghui Li, Marion B. Sewer (Georgia Institute of Technology and University of California, San Diego).

52. **An automated workflow for rapid processing and analysis of lipidomic datasets ***
Christian Klose, Michal A. Surma, Mathias J. Gerl, Felix Meyenhofer, Julio L. Sampaio, Andrej Shevchenko, Kai Simons (Max-Planck-Institute of Molecular Cell Biology and Genetics, Germany).

53. **Development of a kinetic model for eicosanoid metabolism in bone-marrow derived macrophages**
Shakti Gupta[#], Yasuyuki Kihara[#], Mano Ram Maurya, Oswald Quehenberger, Aaron Armando, Christopher K Glass, Edward A. Dennis, Shankar Subramaniam (University of California, San Diego).

54. **Kinetic modeling of eicosanoid and sphingolipid pathways**
Shakti Gupta, Mano Ram Maurya, Alfred H Merrill, Jr, Christopher K Glass, Edward A. Dennis, Shankar Subramaniam (University of California, San Diego).

55. **Blood lymphocytes lysophosphatidylcholine homeostasis in acute lymphoblastic leukemia**
Gohar V. Hakobyan, Tamara B. Batikyan, Elizaveta S. Amirkhanyan, Yuri V. Tadevosyan ([1]Institute of Molecular Biology, NAS RA, Armenia).

56. **Changes in blood limphocyte lipids fatty acid content modification by arachidonic acid in breast cancer**
Mihran P. Lazyan, Hasmik H. Davtyan, Tamara B. Batikyan, Rafik A. Kazaryan, Knarik A. Alexanyan, Hayrapet M. Galstyan, Yuri V.Tadevosyan (Institute of Molecular Biology, NAS RA, Armenia).

57. **Lipid second messengers generation by stimulated blood lymphocytes in ovarian cancer**
Tigran R Torgomyan, Tamara B Batikyan, Rafik A. Kazaryan, Knarik A. Alexanyan, Hayrapet M. Galstyan, Yuri V. Tadevosyan (Institute of Molecular Biology, NAS RA, Armenia).

58. **A multiplexed lipid biomarker for non-invasive diagnosis of endometriosis**
Yie Hou Lee, Bo Zheng, Jagath C. Rajapakse, Seong Fei Loh, Jerry Chan, Steven R. Tannenbaum (Singapore-MIT Alliance for Research and Technology (SMART)).

59. **Characterizing the enzymatic function of Pxt, the *Drosophila* prostaglandin G/H synthase 1**
Andrew J. Spracklen, Tina L. Tootle (University of Iowa).

60. **Lipidomics in studies lifestyle associated diseases**
Leon Coulier, Kitty Verhoeckx, Michiel Balvers, Ivana Bobeldijk, Suzan Wopereis, Renger Witkamp, Ben van Ommen, Elwin Verheij (TNO and Wageningen University, The Netherlands).

61. **Sulfoglucuronosyl paragloboside expression mediates apoptosis of human cerebromicrovascular endothelial cells in inflammation**
Robert K. Yu, Somsankar Dasgupta, Guanghu Wang (Medical College of Georgia).

62. ***In situ* glycerophospholipid imaging with C_{60} SIMS**
Melissa Passarelli, Anita Durairaj, Nicholas Winograd (Penn State University).

63. **Spinal 12-lipoxygenases (12-LOX) contribute to inflammatory hyperalgesia**
Ann M. Gregus, Darren S. Dumlao, Bethany L. Fitzsimmons, Xiao-Ying Hua, Edward A. Dennis, Tony L. Yaksh (University of California, San Diego).

POSTER ABSTRACTS

1. Mechanism of extracellular production of bioactive lysophospholipids using a newly developed LC-MS/MS method

Asuka Inoue[1], Michiyo Okutani[1], Daisuke Saigusa[2], Akira Shuto[1], Shin-ichi Okudaira[1], Naohito Suzuki[2], Yoshihisa Tomioka[2], Junken Aoki[1,3]

[1]Laboratory of Molecular and Cellular Biochemistry, [2]Laboratory of Oncology, Graduate School of Pharmaceutical Sciences, [3]CMeD, Graduate School of Medicine, Tohoku University, Sendai City, Japan, 980-8578.

Lysophospholipids have been established as important lipid mediators for various physiological and pathophysiological conditions. These bioactive lysophospholipids include lysophosphatidic acid (LPA) and lysophophatidylserine (LPS). Concentrations of LPA and LPS in plasma are at levels of 10 to 100 nM, which is >1000-fold lower than their precursors, making it difficult to quantify these bioactive molecules. In addition, acyl position (at *sn*-1 or *sn*-2 position of glycerol backbone) and acy moiety (length and saturation) are known to have distinct roles in several conditions. Thus it is important to separately quantify these lysophospholipid species. In this study, we have developed a new LC-MS/MS method and analyzed LPA and LPS species in multiple tissues from enzyme-deficient mice. We found that Autotaxin (ATX) and phosphatidic acid (PA)-selective phospholipase $A_1\alpha$ (PA-PLA$_1\alpha$; also known as LIPH) were responsible for steady state LPA production in plasma and hair follicle tissues, respectively. Depletion of ATX in mouse plasma resulted in ~95% reduction of plasma LPA concentration. In control plasma, 2-acyl-LPA was ~50% whereas 2-acyl-LPC was ~20%, suggesting that ATX prefers 2-acyl-LPC for its substrate. In PA-PLA$_1\alpha^{-/-}$ hair follicle tissues, which have defects in hair formation, 2-acyl-LPA was reduced by more than 90%. We also found that mice deficient for phosphatidylserine (PS)-specific phospholipase A_1 (PS-PLA$_1$ showed reduction of LPS in serum, especially LPS with polyunsaturated acyl moieties. Furthermore, HEK293 cells expressing LPA receptor (LPA$_6$ also known as P2Y5) and LPS receptor (GPR34) were activated by treatment of recombinant PA-PLA$_1\alpha$ and PS-PLA$_1$, respectively, suggesting that PA-PLA$_1\alpha$ and PS-PLA$_1$ hydrolyze PA and PS, respectively, on the outer leaflet of plasma membrane. In summary, we found that ATX and PA-PLA$_1\alpha$ extracellularly produce LPA *in vivo* and PS-PLA$_1$ produces LPS *in vivo* and that this mechanism is capable of activating lysophospholipid receptors *in vitro*.

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2. Eosinophils contribute to the resolution of acute peritonitis by producing pro-resolving lipid mediators

Makoto Arita, Tomohiro Yamada, Yukako Tani, Yosuke Isobe, Hiroyuki Arai

Graduate School of Pharmaceutical Sciences, University of Tokyo, and PRESTO JST, Japan.

Acute inflammation in healthy individuals is self-limiting and has an active termination program. The mechanisms by which acute inflammation is resolved are of interest. In murine zymosan-induced peritonitis, we found that eosinophils are recruited to the inflamed loci during the resolution phase of acute inflammation. In vivo depletion of eosinophils caused a resolution deficit, namely impaired lymphatic drainage with reduced appearance of phagocytes carrying engulfed zymosan in the draining lymph node, and sustained numbers of polymorphonuclear leukocytes in inflamed tissues. Liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based lipidomics of the resolving exudates revealed that locally activated eosinophils in the resolution phase produced 12/15-lipoxygenase-derived mediators including protectin D1 (PD1) from docosahexaenoic acid. The resolution deficit caused by eosinophil depletion was rescued by eosinophil restoration or the administration of PD1. Eosinophils deficient in 12/15-lipoxygenase were unable to rescue the resolution phenotype. The present results indicate that mouse eosinophils and eosinophil-derived lipid mediators including PD1 have a role in promoting the resolution of acute inflammation, expanding the roles of eosinophils in host defense and resolution.

3. Mapping the HEPG2 lipidome as effected by niacin using products of all acquisition on an new generation Q Q TOF

Phillip Sanders[1], David Yurek[1], Ming-Shang Kuo[1], Lyle Burton[2], Brigitte Simmons[2], Eva Duchoslav[2], Ron Bonner[2]

[1]Eli Lilly & Company, Indianapolis, IN, USA; [2]AB Sciex, Toronto, ON, Canada.

Recent developments in instrumentation and software have made it possible to acquire an array of data that can be mined for information of interest as opposed to designing an experiment to capture it directly. Data collected in an untargeted manner was mined for both expected and unexpected changes in the lipidome caused by niacin as reflected by the incorporation of a stable isotope label. An equivalent experiment using conventional acquisition methods was not possible.

To demonstrate this new paradigm, HEPG2 cells were grown in the presence and absence of a stable labeled fatty acid and with and without the presence of niacin to observe the flux of the label into the various lipid pathways. Data were acquire on the Sciex TripleTOF 5600 using a scan function where precursor masses are iterated in 1 amu increments from m/z 200 to m/z 1000. Given the speed of the TOF it was possible to acquire ms/ms spectra for all 800 precursor ions in just under 3 minutes where each spectrum is the average of approximately 18 scans/precursor mass. All data were acquired using unit mass resolution for the precursor mass and approximately 40000 resolution of the product ion spectra, with collision energy ramping for each ms/ms event.

The presence of the stable label enhances the ability to recognize remodeling of the lipidome cause by niacin. The dataset was mined to visualize common lipid species through selected ion or neutral loss plots using native software.

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4. Information independent MS/MS data collection of all precursors using time-of-flight mass spectrometry

Brigitte Simons[1], Tanya Gamble[1], Lyle Burton[1], Eva Duchoslav[1], Philip Sanders[2], Ron Bonner[1]

[1]AB SCIEX, Concord ON CAN; [2]Lilly Corp, Indianapolis, IN USA.

A simplified technique of stepping through a set mass range fragmenting everything, without applying criteria or any prioritization, may offer advantages when profiling lipids in complex biological extracts in an untargeted fashion. In this MS/MS^{ALL} workflow, a unit-resolved window defined in Q1 steps through a given mass range, CID on, with all product ions stored enabling the recall of any precursor ion spectrum and lipid-class specific profiling post-acquisition. This technique is similar to multiple precursor ion or neutral loss scanning on a QqQ-type instrument at high speed but is carried out with high resolution and accurate MS/MS mass information. Novel data visualization tools provide the capabilities of filtering and profiling of any precursor characterized by a particular fragment or neutral loss in quantitative applications. A yeast lipid extract was subjected to this acquisition whereby a product ion spectrum for every mass between 200 and 1000 m/z following a high resolution TOF MS scan – in both polarities. Through the nanoelectrospray infusion analysis of very small lipid extract volumes, global lipid profiling experiments of yeast shows the identification of species from 6 different lipid classes and 15 sub-classes encompassing a total of 179 lipids in positive mode and 337 lipids confirmed in negative mode in a total of two 1.8 min acquisitions. Quantitatively, the lipid class internal standards show excellent response and reproducible measurements with CVs < 10%. This single acquisition approach offers reproducible quantitation when measuring the response of lipid species against their lipid class –specific internal standard through a wide dynamic range.

5. Isolation and structural analysis of leukotriene A epoxides: insights into the mechanism of the lipoxygenase-catalyzed transformation

Jing Jin, Yuxiang Zheng, William E. Boeglin, Alan R. Brash

Department of Pharmacology, Vanderbilt University, Nashville, TN 37232, USA.

A pivotal role of Leukotriene A (LTA) epoxides is established in formation of bioactive mediators, including the leukotrienes, eoxins, lipoxins, resolvins, maresins and (neuro)protectins. LTA biosynthesis is attributed to a lipoxygenase (LOX)-catalyzed reaction with fatty acid hydroperoxides (HPETEs). Due to their extreme instability, LTA-type intermediates of LOX catalysis have not been isolated and their structure has not been analyzed directly. We hypothesize that transformation of the fatty acid hydroperoxide to LTA epoxide depends on participation of the lipoxygenase non-heme iron in catalyzing both the initial hydrogen abstraction and in facilitating cleavage of the hydroperoxide moiety. This postulate implies that the hydrogen abstracted and the hydroperoxide lie in suprafacial relationship, which in turn, dictates that the *cis* or *trans* epoxide configuration of the LTA product depends on the pro-*R* or pro-*S* chirality of the H-abstraction (an inherent property of the specific lipoxygenase) and the *R* or *S* chirality of the HPETE substrate. To test this hypothesis, we have developed methods for isolation and direct structural analysis of LTA epoxides. We expressed human 15-LOX-1 in *E. coli* and purified the protein by nickel affinity chromatography by using an N-terminal His-tag. Reaction of 15*S*-HPETE with purified human 15-LOX-1 was performed in a biphasic hexane/pH 7.5 aqueous system. After 1.5 min of vortex mixing at 0 °C, UV spectroscopy of the hexane phase showed a decrease of substrate and appearance of a new chromophore with λ_{max} at 278 nm characteristic of the LTA epoxide. Rapid esterification (diazomethane) and RP-HPLC (pH 8) or SP-HPLC (with 0.5% TEA) produces 14,15-LTA_4 methyl ester in 10-20 microgram quantities, suitable for NMR analysis. The results demonstrate the feasibility of enzymatic synthesis, isolation and characterization of LTA epoxides and provide the experimental basis for analysis of LTA epoxide intermediates in formation of eoxins, lipoxins, resolvins, and other novel products.

This work was supported by NIH grant GM-15431.

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6. The oxidation and subsequent hydrolysis of linoleoyl-ω-hydroxyceramide is fundamental in epidermal barrier formation

Christopher P. Thomas[1], Yuxiang Zheng[1], William E. Boeglin[1], Valerie B. O'Donnell[2] Alan R. Brash[1]

[1]Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN, USA [2]Infection, Immunity and Biochemistry, Cardiff University, Cardiff, UK.

The outer epidermis of mammalian skin functions as a barrier vital to maintaining life on dry land. Our group has shown that linoleoyl-ω-hydroxyceramide is oxygenated by the consecutive actions of 12*R*-lipoxygenase and epidermal lipoxygenase-3 forming 9*R*-HPODE and its derivatives that occur naturally in pig and mouse epidermis. These products are absent in 12*R*-lipoxygenase null mice, which die shortly after birth from transepidermal water loss. We propose that oxygenation of linoleoyl-ω-hydroxyceramide is required to facilitate ester hydrolysis of the oxidized linoleate, allowing covalent bonding of the ceramide to protein via the free ω-hydroxyl, helping to seal the barrier. Mouse 12*R*-LOX only reacts with linoleate esters although free 9*R*-HODE is found in murine epidermis, indicating release by a specific hydrolase. One candidate is $sPLA_2$-IIF, a secretory phospholipase, in which the mouse knockout is associated with reduced free 9-HODE in the epidermis and impaired barrier function (K. Yamamoto et al, Keystone Conference Abstract 337, June 2010). We have expressed catalytically active $sPLA_2$-IIF in *E.coli* and the enzyme is now being screened for activity with a range of oxidized phospholipids and ceramides possibly involved in barrier function. Phospholipid substrates have been synthesized via several routes including auto-oxidation yielding the racemic linoleic acid phosphatidylcholine and phosphatidylethanolamine derivatives, 9 and 13-HPODE. The use of the rat liver micosomal acyl-transferase enzyme has allowed the coupling of 5*S*, 12*R,S* and 15*S* HETE acids to a lyso-PC substrate of choice and the oxidation of linoleic acid in SLPE and SLPC by linoleate 9*R*-LOX from Anabaena sp. PCC 7120, has been utilized to generate phospholipids containing 9*R*-HODE. Additionally, the analysis of epidermal lipids is being extended to human skin in which genetic studies strongly implicate the actions of 12*R*-lipoxygenase and epidermal lipoxygenase-3 in barrier function, and the structures of the oxidized ceramides and lipids in human epidermis will be reported. *Supported by a Marie Curie Fellowship (to CPT) and NIH grant AR-51963 (ARB).*

7. Specifically oxidized ceramide esters: required intermediates in formation of the skin permeability barrier *

Yuxiang Zheng[1], Huiyong Yin[1], William E. Boeglin[1], Peter M. Elias[2], David R. Beier[3], Alan R. Brash[1]

[1]Department of Pharmacology and the Vanderbilt Institute of Chemical Biology, Vanderbilt University Medical Center, Nashville, TN, USA; [2]Department of Dermatology, UCSF, San Francisco, CA, USA; [3]Division of Genetics, Harvard Medical School, Boston, MA, USA.

Basic research on the mammalian skin permeability barrier strives to define the basis of its construction, with translational benefits that include rationalizing transdermal drug delivery, cosmetic interests, and potential treatments for the ichthyoses, diseases of defects in barrier-related genes.

Barrier lipids include epidermal-specific ceramides with a C28-C36 N-acyl fatty acid that is ω-hydroxylated and esterified mainly with linoleic acid. This Cer-EOS (Esterified Omega-hydroxy-Sphingosine) undergoes hydrolysis of the linoleate moiety and covalent coupling of the free ω-hydroxyl to protein glutamates, a crucial step in sealing the water barrier. Based on the known (but not previously understood) requirement for linoleate in Cer-EOS, and barrier defects associated with lipoxygenase (LOX) deficiencies in both humans and mice, we hypothesized that LOX-catalyzed oxidation of the linoleate in EOS is required to facilitate the ester hydrolysis and allow coupling of the free ω-hydroxyceramide to protein. Supporting this idea, we have characterized specific oxygenation of EOS by the epidermal enzymes 12*R*-LOX and eLOX3 and identified the same products in epidermis. Pig epidermis contains EOS with linoleate-derived 9*R*-HODE, 9-ketone and the epoxyalcohol, 9*R*,10*R*-epoxy-13*R*-hydroxy-octadec-10*E*-enoate, and murine epidermis contains mainly EOS with the corresponding epoxyketone, 9*R*,10*R*-epoxy-13-keto-octadec-10*E*-enoate.

Using *12RLOX* null mice, which die within hours after birth due to a severe barrier abnormality, we find (i) a complete absence of the specific LOX products in the epidermis, (ii) only 1% of the wild-type level of ω-hydroxyceramides bound to protein, and (iii) an absence of the characteristic corneocyte lipid envelope (composed of ω-hydroxyceramides) on electron microscopy. We conclude that the activity of the LOX enzymes renders EOS susceptible to hydrolysis of the (oxidized) linoleate, freeing the ω-hydroxyceramide and allowing its subsequent esterification to protein, vital events in sealing the epidermal permeability barrier. Together, these studies illuminate specific oxidations required for barrier construction, and point to potential topical treatments for the ichthyoses. *Supported by NIH grant AR-51968.*

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8. EP2 signaling does not restore resolution of experimental Lyme arthritis in cyclooxygenase-2-deficient mice

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Our lab has previously shown that genetic depletion of cyclooxygenase (COX)-2 in *Borrelia burgdorferi* (*Bb*)-infected mice led to an inability to resolve joint inflammation. This defect was not due to a failure to clear the *Bb* from the joint or to produce *Bb*-specific antibodies. However, the mice produced drastically reduced levels of prostaglandins (PGs), including PGE_2. PGE_2 signals through a variety of receptor subtypes, termed EPs. EP2 has been shown to be an important receptor for PGE_2 in inflammatory diseases such as collagen-induced arthritis and is highly expressed on inflammatory cells such as macrophages. Therefore, we sought to determine whether a lack of PGE_2 signaling through EP2 led to the defective resolution of experimental Lyme arthritis observed as a result of COX-2-deficiency. We have previously used lipidomic analysis to identify two peaks of PGE_2 production during the course of Lyme arthritis in wild type mice. We treated COX-$2^{-/-}$ mice with 5μM of the EP2 agonist, Butaprost, during these time points. Our results suggest that PGE_2 signaling through EP2 is not solely responsible for the resolution of Lyme arthritis, as Butaprost treatment did not rescue a wild-type phenotype in COX-$2^{-/-}$ mice. We conclude that the defective resolution seen as a result of COX-2 inhibition is likely due to the deficit of other "pro-resolution" prostaglandins, such as PGD_2 or $15dPGJ_2$, which we will address in future studies.

9. Effect of leukotriene-deficiency on phagocytosis in mouse leukocytes

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Leukotrienes (LTs) are lipid mediators derived from arachidonic acid via the 5-lipoxygenase (5-LO) pathway that are involved in leukocyte chemotaxis, activation and phagocytic activity. Macrophage clearance of apoptotic cells (efferocytosis) is an important step in the resolution of inflammation. Recently we found that 5-LO$^{-/-}$ mice develop Lyme arthritis similar to wild-type mice, but have a failure in arthritis resolution. Thus, we investigated the effect of 5-LO-deficiency on phagocytosis of *B. burgdorferi (Bb)* and efferocytosis in the Lyme arthritis model. Macrophages from 5-LO$^{-/-}$ mice were significantly defective in the uptake and killing of *Bb* and in efferocytosis of apoptotic PMN. Direct uptake of un-opsonized *Bb* by PMN from 5-LO$^{-/-}$ mice was not affected while uptake of opsonized *Bb* was significantly impaired. In addition, macrophages from LTB_4 receptor-deficient BLT-1$^{-/-}$ mice were also unable to efficiently take up *Bb*, but their efferocytosis of apoptotic PMN was unaffected. Addition of exogenous LTB_4 augmented phagocytic abilities in both of the macrophages from both 5LO$^{-/-}$ and BLT-1$^{-/-}$ mice. This result suggests that LTB_4 plays an important role in macrophage phagocytosis of *Bb* and other receptors may be involved in this process. Moreover, macrophages from TLR-2$^{-/-}$ or COX-2$^{-/-}$ mice demonstrate a defective phagocytic phenotype similar to 5-LO$^{-/-}$ cells, implying a possible linkage between 5-LO, TLR-2 and COX-2 pathway. These studies demonstrate that the contribution of leukotrienes to the regulation of inflammation is complex and suggests that augmented clearance of apoptotic cells may important to boost therapeutic effectiveness.

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10. Activation of lysophosphatidic acid receptors reduces the infarct brain volume of rats after ischemic stroke

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Lysophosphatidic acids (LPAs) is a constituent of lysophospholipids (LPLs), a class of lipid that mediates a wide variety of biological functions. Activation of LPAs' G-protein coupled receptors (GPCRs) triggers diverse intracellular signaling pathways that in turn evoke a wide variety of cellular responses. Nevertheless, to date, the LPA receptor (LPAR)-mediated effects to the adult central nervous system (CNS) remains inconclusive. In this study, we investigated the effects of VPC 31143(R) (VPC), a LPAR 1/3 agonist, to the CNS of rats after ischemic stroke. Intraperitoneal injection of VPC (0.8 mg/kg) 30 minutes after permanently occluding the right middle cerebral artery of the rats significantly reduced the mortality rate of the animals 24 hours after stroke. Such intervention also significantly reduced the cerebral infarct volume by 27%, and improved the overall neurological performance of the rats at the same time point after infarction. Further studies revealed that low dose VPC (0.25 mg/kg) was already protective against the ischemic insults to the brain. Preliminary Western blot analysis showed the upregulation of phospho-Akt (p-Akt) protein in the ischemic rat brain treated with low dose VPC. Such upregulation could be one of the underlying mechanisms contributed to the protective effects of VPC against ischemic stroke. Our current results suggest that activation of LPARs may serve as a new means of intervention to attenuate the deleterious impacts of ischemia, and possibly other types of traumatic insults, to the CNS.

11. Bioinformatic and comparative determination of tumor cardiolipin remodeling mechanisms using lipidomic data

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Recent technological advances in high-throughput lipidomics have resulted in the accrual of large amounts of data facilitating the systematic determination of the mechanisms regulating lipid metabolic flux. We developed a computational approach to infer lipid synthesis and remodeling behavior based on data sets from multidimensional mass spectrometry based shotgun lipidomics (MDMS-SL). We focused on the mitochondrial-specific lipid cardiolipin (CL), an important mitochondrial phospholipid containing four acyl chains. Detailed analysis of lipidomic data demonstrate that the four acyl chain positions of CL are, in general, independently and similarly remodeled in normal mouse brain and in brain tumors. The acyl composition of CL in these samples can be well-fit by a linear combination of the pool of acyl chains in phosphatidylcholine (PC), phosphatidylethanolaimine (PE), phosphatidylglycerol (PG), and acyl CoA, with weighted coefficients describing their relative contributions. These findings suggest that the CL remodeling enzymes in brain are mostly selective in regard to donor lipid class and have little preference between acyl chain types, implying a simple remodeling process. Additional mass spectrometric data from mouse heart, lung, and liver samples suggest that heart and lung have additional remodeling mechanisms allowing the precise regulation of CL content and molecular species composition.

[#] *Authors contributed equal effort.*

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12. Lysophosphatidic acid (LPA) signaling as an initiating cause of fetal hydrocephalus

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Fetal hydrocephalus (FH) is the most common neurological disorder of newborns. It is characterized by cerebrospinal fluid (CSF) accumulation, enlarged heads, histological defects, and neurological dysfunction. Its etiology is unclear; however FH has been strongly associated with intracranial hemorrhage. Here we report that LPA, a blood-borne lipid that signals through defined receptors, provides a molecular explanation for FH associated with hemorrhage. An *in vivo* model for intracranial hemorrhage, whereby embryonic brains were overexposed to blood fractions or LPA, produced FH and associated histological defects, which depended predominantly on the receptor subtype $LPA_{1,}$ expressed on neural progenitor cells (NPCs). Administration of a LPA_1 antagonist prevented induced FH. These results identify LPA receptor mechanisms in FH and LPA signaling pathways as targets for potential pharmacological intervention.

13. Characterization of CC1736, a conserved START domain protein and putative coenzyme Q-binding protein

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In *Saccharomyces cerevisiae* there are currently ten known *COQ* genes required for the biosynthesis and activity of coenzyme Q. Unlike the other nine *COQ* genes *COQ10* is not required for the biosynthesis of Q but rather its function in the mitochondria, as the yeast *coq10* null mutant retains wild type levels of Q_6 but fails to respire. While Coq10p does not have homology to any proteins of known function, it is widely distributed in both prokaryotes and eukaryotes. The structure of a Coq10p homologue from *Caulobacter crescentus*, CC1736, was recently solved by NMR, revealing the protein to be a member of the START domain superfamily. Proteins containing START domains typically have hydrophobic cavities that bind lipophilic substrates such as cholesterol and polyketides. Given this structural homology it was hypothesized that CC1736 is coenzyme Q-binding protein. In this study we demonstrate that expression of CC1736 in a yeast *coq10* null mutant restores respiratory competency and this rescue depends on the presence of a mitochondrial leader sequence. Binding assays were also performed using purified CC1736 with Q_2 and Q_{10} and the results indicate that this protein binds these lipids with a 1:1 protein-ligand stochiometry. Taken together these results suggest that CC1736 and by extension Coq10p may serve a role in the proper localization of coenzyme Q from its site of synthesis to its location in the electron transport chain.

This work was supported by NIH GM45952.

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14. Stabilization of catalytic Coq polypeptide complex in *Saccharomyces cerevisiae* by over-expression of the Coq8 protein kinase

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Coenzyme Q (ubiquinone or Q) is a mitochondrial lipid that is essential in cellular energy metabolism, but its biosynthesis is not fully understood. Biosynthesis of Q in yeast requires nine proteins, Coq1p-Coq9p, and deletion of any one of the yeast *COQ* genes leads to the decreased steady state of several other Coq polypeptides. Recent results show that when the Coq8 polypeptide is over-expressed in some of the yeast *coq* null mutants, steady state levels of Coq polypeptides were restored to near wild-type levels. We hypothesize that over-expression of Coq8p may stabilize the Coq polypeptides by stabilizing the catalytic complex composed of Coq3p-Coq7p and Coq9p. To test this hypothesis, we isolated mitochondria from yeast *coq* null mutant cells with and without expression of multi-copy *COQ8*. Digitonin-extracts of mitochondria were separated on non-denaturing 2-dimensional blue native gels, and the Coq polypeptides were observed by immuno-blot. We confirmed that over-expression of *COQ8* restored steady state levels of Coq4p, Coq7p, and Coq9p in *coq5* and *coq6* null mutant strains. We found that the Coq polypeptide complex dissociated upon the deletion of *COQ* gene, but that over-expression of *COQ8* rescued high molecular mass Coq polypeptide complex. These results support our hypothesis that multi-copy Coq8p stabilizes the Coq polypeptide Q-biosynthetic complex.

This work was supported by NIH GM45952 and Ruth L. Kirschstein National Research Service Award GM007185.

15. Isotope-reinforced polyunsaturated fatty acids profoundly slow lipid chain autoxidation in yeast

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The facile abstraction of bis-allylic hydrogens from polyunsaturated fatty acids (PUFAs) is the hallmark chemistry responsible for initiation and propagation of autoxidation reactions. PUFA autoxidation has deleterious effects on the biological system and has been linked to lipid-based disease, age-related degenerative diseases and associated with apoptosis. The products of these autoxidation reactions can form cross-links to other membrane components, damage proteins and nucleic acids. We demonstrate that replacement of the bis-allylic hydrogen atoms with its heavier isotope deuterium atoms protects PUFAs against lipid autoxidation, due to the isotope effect. The coenzyme Q-deficient *Saccharomyces cerevisiae coq* mutants with defects in biosynthesis of coenzyme Q (Q) provide a sensitive in vivo model for toxicity exerted by PUFA autoxidation. Q is an essential lipid redox molecule that functions in energy metabolism and as a chain terminating antioxidant in biological membranes. We show that Q-less yeast treated with linoleic or, α-linolenic acid, for ten hours show a dramatic loss in viability. In contrast, Q-less yeast treated with either of the bis-allylic deuterated PUFAs: 11,11-D_2-linoleic or 11,11,14,14-D_4-α-linolenic retain viability similar to wild-type yeast. Reinforcement at the bis-allylic position is essential for this protection, since treatment with mono-allylic 8,8-D_2-linoleic acid failed to protect. We investigate the effects of different mixtures of isotope-reinforced PUFAs and natural PUFAs on Q-less yeast viability. Surprisingly, only a small fraction of isotope-reinforced PUFAs is required for rescue. Q-less yeast treated with a mixture of 20%:80% isotope-reinforced PUFA: natural PUFA are profoundly protected from lipid autoxidation-mediated cell killing. These findings suggest bis-allylic isotope-reinforced PUFAs delay the initiation of lipid peroxidation and may also be able to block propagation to neighboring lipids. These findings suggest that relatively small amounts of isotope-reinforced PUFAs can be utilized to slow lipid autoxidation in vivo.

This work was supported by NIH GM45952.

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16. Aromatic precursors in the *S. cerevisiae* coenzyme Q biosynthetic pathway

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Coenzyme Q (also known as ubiquinone or CoQ) is a complex organic molecule essential for mitochondrial energy production. CoQ is also a crucial antioxidant that can protect membrane lipids from oxidative damage. The CoQ molecule has two parts: (1) a long "tail" that anchors it in the membrane, and (2) an aromatic ring structure (benzoquinone) with properties that allow it to ferry electrons and protons essential to ATP formation. Cells are generally thought to synthesize the benzoquinone ring from the precursor 4-hydroxybenzoic acid (4-HB). Although nine genes essential for CoQ biosynthesis have been identified in yeast, plants and animals, none are required for 4-HB synthesis. Therefore, major questions remain unanswered regarding the generation of the aromatic benzoquinone component of CoQ. 4-HB has been speculated as produced via coumarate from the phenylpropanoid biosynthesis pathway by using the amino acid tyrosine. Recent observations indicate that yeast can also use 4-aminobenzoic acid (pABA) as a ring precursor in CoQ biosynthesis. This research utilizes yeast to define how pABA is converted to CoQ, and how tyrosine and coumarate are converted to 4-HB. Candidate yeast mutants wereselected as having likely defects in phenylpropanoid metabolism, and were characterized for their ability to incorporate $^{13}C_6$-ring labeled forms of pABA, 4HB, phenylalanine, and tyrosine into $^{13}C_6$-CoQ. An established CoA analysis method was applied to aqueous yeast extracts and the resulting $^{13}C_6$-CoA intermediates were compared to $^{13}C_6$-CoQ intermediates present in lipid extracts of duplicate samples.

This work was supported by NSF-CMB0919609 and NIH GM45952.

17. Yeast *coq* null mutants harboring multi-copy *COQ8* accumulate novel intermediates in coenzyme Q biosynthesis

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Coenzyme Q (ubiquinone or Q) is a lipid electron carrier in the electron transport chain. In yeast *Saccharomyces cerevisiae* nine genes, designated *COQ1* through *COQ9*, have been identified as being required for Q biosynthesis. Deletion of any one of the nine *COQ* genes leads to decreased steady state levels of other Coq polypeptides, including Coq4, Coq6, Coq7, and Coq9 (Hsieh *et al.,* 2007). One of these polypeptides, Coq8, is now thought to function as a kinase (Tauche *et al*., 2008). In this study, we find overexpression of Coq8p in certain *coq* null mutants restores steady state level of Coq4p, Coq7p and Coq9p; suggesting Coq8p facilitates the stabilization of the high molecular weight Coq polypeptide complex. Six of the *coq* null mutants (*coq3* – *coq9*) accumulate early Q-intermediates such as 3-hexaprenyl-4-amino-benzoic acid (prenyl-PABA) and 3-hexaprenyl-4-hydroxy-benzoic acid (HHB) (Marbois et al., 2010). Here, we utilize liquid chromatography and mass spectrometry (LC-MS/MS), and show that certain *coq* null mutants harboring multi-copy *COQ8* now accumulate late stage Q-intermediates. For example, demethyl-demethoxy-Q_6 accumulates in a yeast *coq5* null mutant harboring multi-copy *COQ8*, and imino-demethoxy-Q_6 and demothoxy-Q_6 accumulate in *coq9* null mutant harboring multi-copy *COQ8*. We suggest that over-expression of Coq8 facilitates the assembly of and/or stabilizes the Coq polypeptide complex, allowing the synthesis of Q-intermediates that can be diagnostic of the blocked step in the corresponding *coq* null) mutant.

This work was supported by National Institutes of Health (NIH) Grant GM45952 and Ruth L. Kirschstein National Research Service Award GM007185. The LC-MS/MS determination of Q content was supported by Grant Number S10RR024605 from the National Center for Research Resources. CNRS grant PEPS 2008 and Region Rhone-Alpes program CIBLE 2009 supported the work of F.P.

18. The use of stable isotopic labeled glycerol and oleic acid to differentiate the hepatic functions of diacylglycerol acyltransferase-1 and -2 *

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Intrahepatic triglyceride (IHTG) content is a major contributing factor to hepatic and skeletal muscle insulin resistance in type 2 diabetes. Diacylglycerol acyltransferase (DGAT), catalyzes the final step in triglyceride (TG) synthesis. There are two isoforms (DGAT1 and DGAT2) with distinct protein sequences and different biochemical, cellular, and physiological functions. DGAT2 is predominantly expressed in the liver and is involved in the bulk of TG synthesis and VLDL-TG secretion. The specific role of DGAT1 in hepatic TG synthesis and steatosis was recent reported using live-specific DGAT1 knockout mice and hepatic DGAT1 ASO knockdown. DGAT1 was required for hepatic steatosis induced by a high-fat diet and prolonged fasting, which are both characterized by delivery of exogenous fatty acids to the liver. In the current study, we explored the acute metabolic tracing of TG synthesis in hepatic cells and in mice with the use of stable isotopic labeled oleic acid and glycerol.

We used a stable isotopic labeled glycerol to specifically measure DGAT2-mediated TG synthesis in hepatic cells. The use of stable isotopic labeled glycerol allows selectively monitoring the newly synthesized TG products of endogenous fatty acid incorporation to stable isotopic labeled glycerol. We treated endogenous HepG2 hepatic cells with stable isotopic-labeled $^{13}C_3$-D_5-glycerol and the major $^{13}C_3$-D_5-glycerol TG species were profiled by LC/MS/MS. Potent and selective DGAT1 and DGAT2 inhibitors demonstrated that the $^{13}C_3$-D_5-glycerol incorporated TG synthesis is mediated by DGAT2, not DGAT1. Conversely, when HepG2 cells were incubated with $^{13}C_{18}$-oleic acid, the $^{13}C_{18}$-oleoyl-incorporated TG synthesis was predominantly mediated by DGAT1. Furthermore, we developed a novel LC/MS/MS method to trace hepatic TG synthesis and VLDL-TG secretion in vivo by administering D_5-glycerol to mice. Using DGAT2 antisense oligonucleotides as a pharmacological tool, we measured changes in D_5-glycerol incorporated TG and demonstrated that hepatic DGAT2 inhibition significantly reduced D_5-glycerol incorporated VLDL-TG. In contrast, DGAT2 antisense oligonucleotides had no effect on exogenous $^{13}C_{18}$-oleic acid administrated and incorporated VLDL-TG. Thus, our results indicate that DGAT1 and DGAT2 mediate distinct hepatic functions; DGAT2 is primarily responsible for incorporating endogenously synthesized fatty acid into TG, whereas DGAT1 is involved in esterifying exogenous fatty acids.

19. Identification of 1-deoxydihydroceramide biosynthesis by mammalian cells

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A novel sphingoid base, 1-deoxysphinganine, and its *N*-acyl-metabolites were recently discovered in studies of mammalian cells in culture and animals (Zitomer *et al*., *J. Biol. Chem.* 284:4786, 2009) and represent a novel new category of sphingolipid. 1-Deoxysphinganine was shown to arise from condensation of *L*-alanine with palmitoyl-CoA by cells in culture, and most is metabolized to 1-deoxydihydroceramides rather than accumulating as the free sphingoid base. In anticipation of interest in these compounds, such as their possible elevation in clinically relevant samples, we have now developed a high-throughput and structure-specific method for analysis of 1-deoxysphingolipids by high performance liquid chromatography coupled to an electrospray ionization (ESI) QSTAR Pulsar *i* time-of-flight (TOF) mass spectrometer which combines, speed, sensitivity, high mass accuracy and high resolution. 1-Deoxydihydroceramides were extracted from a Hek293 cell line stably overexpressing serine palmitoyltransferase (SPT1/2 cells) which were incubated with different stable isotope labeled amino acids, such as [3-13C]-*L*-Serine, [2,3-13C2]-*L*-Alanine, and [3-D3]-*L*-Alanine, and examined by LC-ESI MS/MS. These amino acid supplementations shows that novel 1-deoxysphingolipid arises from condensation of alanine with palmitoyl-CoA as evidenced by incorporation of [2,3-13C2]-*L*-alanine and [3-D3]-*L*-Alanine into 13C2-1-deoxydihydroceramides and D3-1-deoxydihydroceramides, respectively, by cells in culture. The results to be presented show that these underappreciated categories of bioactive sphingoid bases and “ceramides” that are likely to play important roles in cell regulation can be separated and detected by developed LC MS/MS methods.

These investigations are supported by NIH grants P41 RR10888 (CEC) and GM69338 (Lipid Maps) (AHM).

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20. A protective role for LXR in diabetic nephropathy

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Purpose: Diabetes is the leading cause of end stage renal disease and affects 25-40% of diabetics. Diabetic nephropathy is highly correlated with glucose control but the progression has also been linked to high serum cholesterol. Liver X receptors (LXRα and LXRβ) are cholesterol sensors. LXR gene expression is decreased in mouse models of diabetes and numerous target genes of LXR are altered in diabetic nephropathy. Thus, we hypothesized that LXR activation would be beneficial for improving renal function and that the loss of LXR would accelerate the progression of diabetic nephropathy.

Methods: db/db and db/m control mice were treated with vehicle or GW3965 (LXR agonist) in the diet for 12 weeks. In a separate experiment, diabetes was induced in WT and LXRα/β-/- mice with streptozotocin and a high fat/high cholesterol diet for 14 weeks. Proteinuria was assessed at the end of the study and the kidneys excised for histology and molecular analysis. Gene expression was measured by QPCR.

Results: GW3965 treatment significantly decreased proteinuria in db/db mice. LXRα/β-/- mice had significantly elevated proteinuria compared to the control mice. Histological analysis showed an accumulation of lipids in the kidney of diabetic LXRα/β-/- mice. Kidney gene expression analysis showed decreases in cholesterol efflux transporters and increases in profibrotic growth factors, inflammatory, macrophage and oxidative stress markers in the LXRα/β-/- mice and the db/db mice. Inflammatory and oxidative stress markers were reduced with the LXR agonist in db/db mice.

Conclusions: These data support a role for LXR in decreasing inflammation and increasing cholesterol efflux in the kidney and is consistent with a beneficial role for LXR activation in diabetic nephropathy. Furthermore, diabetic LXRα/β-/- mice showed elevated proteinuria, increased lipid accumulation in the kidney and detrimental changes in gene expression supporting an accelerated progression to diabetic nephropathy.

21. Essential role of ELOVL4 in very long chain fatty acid synthesis and retinal function

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Very long chain polyunsaturated fatty acids (VLC-PUFAs) are relatively enriched in the retina. However, retinal VLC-PUFA biosynthesis and function have not been precisely determined. *ELOVL4* has been identified as a fatty acid elongase involved in the synthesis of VLC-PUFAs. Mutations in *ELOVL4* have also been found to cause an autosomal dominant form of Stargardt disease (STGD3), a type of juvenile macular degeneration. We have generated photoreceptor specific conditional knockout mice and have used liquid chromatography-mass spectrometry (LC-MS) to examine and analyze retinal fatty acid composition. We also used immunofluorescent staining and histology, coupled with electrophysiological data, to assess retinal morphology and visual responses. The conditional knockout mice showed a significant decrease in retinal VLC-PUFA species, implicating the role of *ELOVL4* in their synthesis. Conditional knockout mice were also found to have normal retinal morphology, while demonstrating photoreceptor-specific abnormalities in visual response, indicating the critical role of *ELOVL4* for proper rod or cone photoreceptor function. Together, this study demonstrates the essential role of *ELOVL4* in VLC-PUFA synthesis and retinal function.

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22. Specificity of eicosanoid production depends on the TLR-4 stimulated macrophage phenotype

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Eicosanoid metabolism differs in profile and quantity between macrophages of different tissue origin and method of elicitation, as well as between primary and immortalized macrophages after activation with inflammatory stimuli. Using a lipidomic approach, we comprehensively analyzed the eicosanoids made by murine resident peritoneal macrophages, thioglycollate-elicited peritoneal macrophages, bone marrow-derived macrophages, and the macrophage-like cell line RAW264.7 after stimulation with the TLR-4 specific agonist Kdo_2-lipid A. Direct correlation between total COX metabolites, COX side products (11-HETE, 15-HETE), COX-2 mRNA and protein at 8 hours was found when comparing each cell type. Comprehensive qPCR analysis was used to compare relative transcript levels between the terminal prostanoid synthases themselves as well as between each cell type. Levels of PGE_2, PGD_2, and TxB_2 generally correlated with enzyme transcript expression of PGES, PGDS, and TBXS providing evidence of comparable enzyme activities. PGIS transcript was expressed only in RPM and TGEM macrophages and at an exceptionally low level despite high metabolite production compared to other synthases. Presence of PGIS in RPM and TGEM also lowered the production of PGE_2 vs. PGD_2 by ~10 fold relative to BMDM and RAW cells which lacked this enzyme. Our results demonstrate that delayed prostaglandin production depends on the maximal level of COX-2 expression in different macrophages after TLR-4 stimulation. Also, the same enzymes in each cell largely dictate the profile of eicosanoids produced depending on the ratios of expression between them, with the exception of PGIS which appears to have much greater synthetic capacity and competes selectively with mPGES-1.

23. Identification of oxidative modifications in phosphatidylserine polar head using lipidomic approach

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Phosphatidylserine (PS) is a phospholipid distributed among all cells of mammalians, playing important roles in diverse biological processes, including blood clotting, apoptosis and related diseases, as cancer, chronic autoimmunity, and infections. Non-oxidized phosphatidylserine is recognized to have a key role in apoptosis, but recently there is evidence of the involvement of oxidized PS in this event, leading to the increasing interest in studying PS oxidative modifications [1,2]. Despite the importance of this group of phospholipids, the knowledge about the role of the serine head group in oxidative conditions is still limited. The purpose of this study is to evaluate, in detail, the molecular changes induced to phosphatidylserine molecules when subjected to oxidative stress conditions.

In this work, different PS (1-palmitoyl-2-linoleoyl-sn-glycero-3-phospho-L-serine (PLPS), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS) and 1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine (DPPS) were oxidized by the hydroxyl radical, generated under Fenton reaction conditions, and the reactions were monitored by ESI-MS in negative mode. Oxidation products were then fractionated by TLC and characterized by tandem mass spectrometry (MS/MS). This approach allowed identifying oxidative modifications in polar head and in fatty acyl chains. The oxidation products with modifications in polar head were identified as $[M-29-H]^-$ (terminal acetic acid), $[M-30-H]^-$ (terminal acetamide), $[M-13-H]^-$ (terminal hydroperoxyacetaldehyde), and $[M-13-H]^-$ (terminal hydroxyacetaldehyde plus hydroxy fatty acyl chain). Due to oxidation of unsaturated fatty acyl chains were identified the hydroxyl, peroxy and keto derivatives. Phosphatidic acid is also formed in these conditions. These findings confirm the oxidation of the serine polar head induced by the hydroxyl radical. The identification of these modifications may be a valuable tool to evaluate phosphatidylserine alteration under physiopathological conditions and also to help understanding the biological role of phosphatidylserine oxidation in the apoptotic process and other biological functions.

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Thanks are due to Fundação para a Ciência e a Tecnologia and COMPTE for funding to projects PTDC/QUI-BIQ/104968/2008 and REDE/1504/REM/2005 (that concerns the Portuguese Mass Spectrometry Network).and to PhD grants to Elisabete Maciel (SFRH/BD/73203/2010) and to Cláudia Simões (PhD grant, SFRH/BD/46293/2008).

24. The influence of PE glycation, oxidation and glycoxidation on monocyte stimulation

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In the last years, the interest in the role of phospholipids oxidation in inflammation and signaling is growing. Several studies indicate that oxidized phospholipids can induce monocyte adhesion to endothelial cells that accumulate in atherosclerotic lesions, and play a role in inflammation and signaling inflammatory processes. However, it is also been found that bioactive phospholipid oxidation products can present anti-inflammatory and protective properties. Besides, PE glycation in LDL has been related with atherosclerosis complications of diabetic patients with hyperglycemia, as result of an increase of oxidative stress modifications. The objective of this work is to determine the effect of PE oxidation, glycation and glycoxidation in monocytes stimulation. Fresh blood samples collected with heparin were initially incubated with mixtures of oxidized PE, glycated PE and glycoxidated PE and monocyte stimulation was measured using flow cytometric analysis. The expression of two cytokines, namely tumor necrosis factor α (TNFα) and interleukin-6 (IL-6) (with an important role in the sustained inflammation verified in type 2 diabetes mellitus) was accessed by flow cytometry and compared with those of unstimulated cells. Preliminary results, showed that monocytes can be stimulated with LPS, oxidized PE, glycated PE or glycoxidized PE, leading to an increase in the production of both TNFα and IL-6. However the number of stimulated cells and the amount of cytokines production was different depending on the nature of modified PE. Comparison of the results showed that glycated and glycoxidized PE stimulated more monocytes and produce an higher amount of TNFα and IL-6 than oxidized PE, and this effect is more evident for glycoxidized PE. Therefore, it is possible to assume that glycation and glycoxidation have a positive effect on monocyte stimulation and TNFα and IL-6 production, which could have an important role in the increased atherosclerosis in diabetic patients.

Thanks are due to Fundação para a Ciência e a Tecnologia and COMPTE for funding to projects PTDC/QUI-BIQ/104968/2008 and REDE/1504/REM/2005 (that concerns the Portuguese Mass Spectrometry Network) and to PhD grant to Cláudia Simões (SFRH/BD/46293/2008).

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25. Novel serum albumin-bound lipids correlate with newly diagnosed type II diabetes

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Our lab found numerous protein correlates to type II diabetes (T2D) in human plasma after depleting abundant proteins (Laffoon, et al, in preparation). Unexpectedly, many of these protein correlates to T2D are normally found bound to human serum albumin (HSA), even though the HSA had been removed before the proteomic analysis. We investigated fatty acids and esters bound to HSA, which are known to alter other HSA cargos. Due to limited plasma availability, fatty acid analysis of DCM:MeOH extracts of the abundant protein fractions (largely composed of HSA) were conducted using NCI GCMS analysis of highly sensitive PFB esters. Enzymatic analysis of the total NEFA in plasma showed increases in T2D, as expected, whereas the total fatty acids in the abundant protein fractions were significantly down in T2D. Surprisingly, other prominent PFB ester peaks were present in NCI GCMS that were greatly elevated in T2D (2 +/- 0.3, $p < 0.005$), but these peaks could not be identified by mass or subsequent EI analysis (NIST database). High resolution QTOF MS (Bruker MaXis) identified the prominent PFB ester peaks as di- and tri-PFB glycine. Doping plasma with C13-glycine confirmed that free glycine was not extracted into the DCM:MeOH phase. Experiments on model compounds showed that glycine amides were not cleaved by the PFBBr derivatization, but glycine esters were cleaved. Subsequent immuno-affinity purification of HSA found > 98% of the PFB glycine-generating compounds were carried on HSA in plasma. Seeking compounds that yielded glycine by PFB derivatization, RP fractionation of the underivatized hydrophobic extracts showed substantially different compounds in T2D and controls. Accurate mass (~1 ppm) MS and MS/MS of RP fractions that yielded glycine fragments led to a small number of molecular formulas, but no hits in the Lipid Maps or Metlin databases. We are scaling up the preparations for NMR analysis.

26. Contribution of H3K4 methylation by SET-1A to interleukin-1–induced cyclooxygenase 2 and inducible nitric oxide synthase expression in human osteoarthritis chondrocytes

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Objective: To investigate the role of histone H3 lysine 4 (H3K4) methylation in interleukin-1_ (IL-1_)– induced cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS) expression in human osteoarthritic (OA) chondrocytes.

Methods: Chondrocytes were stimulated with IL-1, and the expression of iNOS and COX-2 messenger RNA and proteins was evaluated by real-time reverse transcriptase–polymerase chain reaction analysis and Western blotting, respectively. H3K4 methylation and the recruitment of the histone methyltransferases SET-1A and MLL-1 to the iNOS and COX-2 promoters were evaluated using chromatin immunoprecipitation assays. The role of SET-1A was further evaluated using the methyltransferase inhibitor 5_-deoxy-5_-(methylthio)adenosine (MTA) and gene silencing experiments. SET-1A level in cartilage was determined using immunohistochemistry.

Results: The induction of iNOS and COX-2 expression by IL-1 was associated with H3K4 di- and trimethylation at the iNOS and COX-2 promoters. These changes were temporally correlated with the recruitment of the histone methyltransferase SET-1A, suggesting an implication of SET-1A in these modifications. Treatment with MTA inhibited IL-1–induced H3K4 methylation as well as IL-1–induced iNOS and COX-2 expression. Similarly, SET-1A gene silencing with small interfering RNA prevented IL-1–induced H3K4 methylation at the iNOS and COX-2 promoters as well as iNOS and COX-2 expression. Finally, we showed that the level of SET-1A expression was elevated in OA cartilage as compared with normal cartilage.

Conclusion: These results indicate that H3K4 methylation by SET-1A contributes to IL-1–induced iNOS and COX-2 expression and suggest that this pathway could be a potential target for pharmacologic intervention in the treatment of OA and possibly other arthritic diseases.

27. Valproic acid suppresses interleukin-1β-induced microsomal prostaglandin E2 synthase-1 expression in chondrocytes through upregulation of NAB1

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Objective: Microsomal prostaglandin E(2) synthase-1 (mPGES-1) catalyzes the terminal step in the biosynthesis of PGE(2). Early growth response factor-1 (Egr-1) is a key transcription factor in the regulation of mPGES-1, and its activity is negatively regulated by the corepressor NGF1-A-binding protein-1 (NAB1). We examined the effects of valproic acid (VA), a histone deacetylase inhibitor, on interleukin 1ß (IL-1ß)-induced mPGES-1 expression in human chondrocytes, and evaluated the roles of Egr-1 and NAB1 in these effects.

Methods: Chondrocytes were stimulated with IL-1 in the absence or presence of VA, and the level of mPGES-1 protein and mRNA expression were evaluated using Western blotting and real-time reverse-transcription polymerase chain reaction (PCR), respectively. mPGES-1 promoter activity was analyzed in transient transfection experiments. Egr-1 and NAB1 recruitment to the mPGES-1 promoter was evaluated using chromatin immunoprecipitation assays. Small interfering RNA (siRNA) approaches were used to silence NAB1 expression.

Results: VA dose-dependently suppressed IL-1-induced mPGES-1 protein and mRNA expression as well as its promoter activation. Treatment with VA did not alter IL-1-induced Egr-1 expression, or its recruitment to the mPGES-1 promoter, but prevented its transcriptional activity. The suppressive effect of VA requires de novo protein synthesis. VA induced the expression of NAB1, and its recruitment to the mPGES-1 promoter, suggesting that NAB1 may mediate the suppressive effect of VA. Indeed, NAB1 silencing with siRNA blocked VA-mediated suppression of IL-1-induced mPGES-1 expression.

Conclusion: VA inhibited IL-1-induced mPGES-1 expression in chondrocytes. The suppressive effect of VA was not due to reduced expression or recruitment of Egr-1 to the mPGES-1 promoter and involved upregulation of NAB1.

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28. Detection of breast cancer-associated lipids in hair

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In 1999, a correlation between the incidence of breast cancer and an observed change in the synchrotron generated X-ray diffraction (XRD) pattern of hair from afflicted individuals was reported [1]. The association between an altered XRD pattern and breast cancer has since been shown in several publications by different groups, and on average XRD-based assays detect around 80% of breast cancer patients in blinded studies [2]. To date however, the molecular mechanism(s) leading to this alteration are largely unknown. We have experimental evidence that the alteration seen in an XRD pattern of hair from cancer patients is due to a breast-cancer associated lipid(s), in particular phosphatidylcholine. A series of experiments using solvent extraction confirmed the lipid nature of the feature in the hair. Subsequent analysis of the solvent-extracted lipids using liquid chromatography mass spectrometry demonstrated that there was significantly more phosphatidylcholine in hair samples from breast cancer patients. There is also evidence that other phospholipid species are increased in hair samples from breast cancer patients compared to disease-free controls. Further characterization of the phospholipids associated with breast cancer could be used to develop a novel sensitive and specific diagnostic screening test for breast cancer, based on hair initially, and potentially extendable to other biological samples.

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29. The putative *N*-acyl PE synthase from *Arabidopsis thaliana* is a lyso-glycerophospholipid acyltransferase *

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N-acylphosphatidylethanolamine (*N*-acyl PE) is a minor membrane phospholipid found in plants, animals, and most recently in *Escherichia coli*. In eukaryotes *N*-acyl PE is thought to be required for the production of bioactive N-acylethanolamines that are involved in a variety of physiological processes including stress responses in plants and control of appetite in mammals. At1g78690, a gene isolated from *Arabidopsis thaliana*, has been reported as an *N*-acyltransferase that transfers an acyl chain from acyl-CoA to the headgroup of PE, making *N*-acyl PE. Our investigation suggests that At1g78690 is *not* a PE-dependent *N*-acyltransferase, but is instead a lyso-glycerophospholipid acyltransferase. We overexpressed At1g78690 in *E. coli*, extracted the cellular lipids, and identified the accumulating phospholipid. Electrospray ionization quadrupole time-of-flight mass spectrometry analysis yields $[M-H]^-$ ions corresponding by exact mass to acylphosphatidylglycerol (acyl PG) rather than *N*-acyl PE. Collision-induced dissociation mass spectrometry (MS/MS) corroborates this finding. In addition, we assayed the activity of both crude extracts and the partially-purified acyltransferase *in vitro* using both ^{32}P and ^{14}C radiolabeled substrates and show that At1g78690 acylates lyso-PE and lyso-PG, but not PE or PG, in an acyl-CoA-dependent manner. We analyzed the diacylated product formed by At1g78690p using arachidonyl-CoA as the acyl-donor and 1-acyl PE or 1-acyl PG as the acyl acceptor using MS/MS. This revealed that At1g78690p predominately acylates the *sn*-2 position of the glycerol to yield products consistent with PE or PG. We believe that At1g78690 from *A. thaliana* is a lyso-glycerophospholipid acyltransferase, rather than an *N*-acyl PE synthase. Future work will focus on determining why overexpression of At1g78690p in *E. coli* leads to the accumulation of a tri-acylated acyl-PG.

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30. Down-regulation of sterol biosynthesis network by interferon

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Little is known about inflammatory mediators in modulating lipid metabolism in infection. In particular, the interrelation between sterol metabolic genes, infection and subsequent immune reactions that alter cellular metabolism on a functional level are poorly understood. This work focuses on studying the coordinate down-regulation of multiple sterol metabolic genes upon infection by viruses and interferon treatment. Drug and interference RNA Inhibition of the pathway confers protection, indicating that the down-regulation of the sterol network has a cellular countermeasure role to infection. Genetic knock-out studies elucidate an inflammatory regulatory loop mechanism linking the innate immune response activation of type 1 interferon and signalling of its receptor to the down-regulation of the pathway upon infection. Coordinate transcriptional control of the sterol pathway is governed by the SREBP2 transcription factor, which is reduced at both the protein and gene transcription level upon infection or interferon action in a type 1 receptor dependent manner. Thus this study adds a new perspective to the current understanding of the structural requirements of virus replication and the role of lipids by revealing a mechanism of molecular dependency to an interferon regulatory loop in down modulating the sterol pathway upon infection. Moreover, this work underscores a new physiological role for the sterol pathway as part of a host protection pathway that acts against infection and it highlights the potential for pharmacological targeting of host metabolic regulatory networks as an anti-viral strategy.

31. New pool of brain esterified prostanoids

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To address a mechanism for a rapid (within seconds) increase in brain PG levels upon stimulation, we hypothesized a presence of esterified prostanoid pool that undergoes a rapid mobilization upon stimulation. To test this hypothesis, mouse brains were fixed using head focused microwave irradiation to heat denature proteins involved in PG metabolism. PG levels were measured in control mice, or upon treatment with LPS or global brain ischemia. Using chemical hydrolysis, a significant pool of PG was found esterified in brain tissue. The esterified PG pool was 30-fold greater than the levels of free prostanoids found in brain, demonstrating a large, releasable pool of prostanoids. These esterified PG were rapidly released in vivo by 30 sec of global ischemia or upon LPS treatment, and contributed to the 30-fold increase in free prostanoid levels under these conditions. To address the possibility that free PG might undergo re-esterification back onto esters, mice were infused (i.c.v.) with PG labeled with O18 and H2. The kinetic analysis indicates a very rapid (within minutes) PG re-esterification rate in vivo. This data were confirmed in the ex-vivo and in-vitro experiments. To address the chemical structure of esterified PG, we analyzed isoPG composition of phospholipids and found that PG esterified onto phospholipids does not contribute to the PG increase upon brain stimulation. In summary, these results confirm our hypothesis that a novel esterified PG pool accounts for the rapid increase in brain PG mass via the rapid (<1 min) mobilization of a releasable pool of preformed PG found in brain tissue.

Supported by NIH Grant 1R21NS064480-01A1 (MYG), ND EPSCoR grant EPS-0814442 (MYG), UND New Faculty Scholar Award (MYG), and by the NIH-funded COBRE Mass Spec Core Facility Grant 5P20RR017699.

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32. Cholestenoic acids and motor neuron development?

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Bile acids are usually thought of as liver derived metabolic end products of cholesterol. However, the expression of enzymes of the bile acid biosynthetic pathways in organs such as brain and lung suggests an extrahepatic role for bile acids or their precursors. To investigate this we have profiled human plasma using LC-ESI-MS for the presence of bile acid precursors biosynthesised extrahepatically. We have concentrated attention on members of the acidic pathway of bile acid biosynthesis extending from 26-hydroxycholesterol[1] to 7α-hydroxy-3,24-*bis*oxocholest-4-en-26-oic acid, the final C_{27} metabolite of the pathway. The levels of some of these metabolites are low (<ng/mL), hence we have utilised a “charge-tagging” strategy to enhance sensitivity of LC-ESI-MS analysis. Once identified, the biological activity of the metabolites have been tested as ligands to LXR, NURR1, VDR and FXR nuclear receptors and for effects on Islet1+ cells in zebrafish embryos. Interestingly, 3β,7α-dihydroxycholest-5-en-26-oic acid is both an LXR ligand and increases expression of Islet 1 protein in embryos. Islet 1 is a protein expressed at the earliest stage of neural differentiation in motor neurons and is highly conserved throughout vertebrate evolution. These results suggest a link between cholesterol metabolism, LXR and motor neuron development.

[1]*We use the nomenclature where 26-hydroxycholesterol refers to the isomer with a 25R configuration.*

33. Oxysterols in newborn mouse brain

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Oxysterols are produced from cholesterol in enzymatic conversions catalysed by CYP enzymes or by cholesterol 25-hydroxylase. Alternatively, they can be formed by interaction with endogenous ROS or by autoxidation during sample preparation. In the past oxysterols were regarded as intermediates in steroid hormone and bile acid biosynthesis, or as transport forms of cholesterol. Newer findings point to a role for oxysterols as ligands to nuclear receptors e.g. LXR, FXR, PXR, and also to INSIG. More recently, oxysterols were shown to modulate immune response and neurogenesis.

In the present study we profiled the sterol and oxysterol content of newborn mouse brain. Prior to MS analysis, the sterol/oxysterol content of brain was treated with cholesterol oxidase and derivatised with Girard P hydrazine then separated by LC. By inclusion of an isotope-labelled standard this allowed the quantification of sterols/oxysterols with 3β-hydroxy-5-ene or 3β-hydroxy-5α-hydrogen structure. With this protocol we quantified a wide range of oxysterols including 22R-hydroxycholesterol, 24S-hydroxycholesterol, 7α-hydroxycholesterol, 7β-hydroxycholesterol, 6α-hydroxycholesterol, 24S,25-epoxycholesterol, 22-oxocholesterol, and 24-oxocholesterol. The most abundant oxysterols were 24S-hydroxycholesterol and 24S,25-epoxycholesterol. 24S-Hydroxylation is the major pathway of cholesterol exertion from CNS and reflects cholesterol metabolism in brain tissue.

High levels of 24S,25-epoxycholesterol in foetal brain were reported previously. Unlike other oxysterols, 24S,25-epoxycholesterol is formed via a shunt of the mevalonate pathway, not by enzymatic hydroxylation of cholesterol. 24S,25-Epoxycholesterol can affect cholesterol homeostasis in different ways: as an agonist to the LXRs and also by suppressing the processing of SREBP-2 to its active form as a transcription factor. The presence of 22-oxocholesterol is a surprising result. Brain localization of this steroid has not been previously reported in the literature and needs a further investigation.

34. HSP70/70B' induced by lipoprotein immune complexes sequester lipids in the endosomal compartment: impact on oxidative stress and macrophage survival

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Background and objective: Oxidized low-density lipoproteins (oxLDL) and oxLDL-containing immune complexes (oxLDL-IC) contribute to formation of lipid-laden macrophages. We have previously shown that knockdown of HSP70B' protects cells from cytotoxic effects of oxLDL. Oxidative and nitrosative stresses were shown to be induced by oxLDL in macrophages. We have recently shown that mitochondrial membrane potential was decreased and generation of reactive oxygen and nitrogen species was increased in U937 cells treated with oxLDL compared to oxLDL-IC. We hypothesized that HSP70/70B' through co-localization with internalized lipids in the endosomal compartment could influence the differential intracellular trafficking of oxLDL and oxLDL-IC. This study examines whether HSP70/70B' are necessary for the differential oxidative and nitrosative stresses in response to oxLDL and oxLDL-IC.

Methods and results: U937 cells were transfected with HSP70/70B' siRNAs then treated with oxLDL, oxLDL-IC, or keyhole-limpet hemocyanin immune complex (KLH-IC), or vehicle control in combination with DCF-DA for H_2O_2, DAF-FM plus l-arginine for nitric oxide (NO), and Mito Tracker for mitochondrial membrane potential and visualized using confocal microscopy. Knockdown of HSP70B' significantly decreased NO production in response to oxLDL compared to control-transfected cells; knockdown of HSP70 resulted in an intermediate reduction in NO generation. Knockdown of HSP70 or HSP70B' did not significantly decrease H_2O_2 production in response to oxLDL. The knockdown of HSP70 and HSP70B' did not change either H_2O_2 or NO production in response to oxLDL-IC compared to control-transfected cells. The mitochondrial membrane potential was not affected by oxLDL-IC in cells transfected with either HSP70 siRNA or HSP70B' siRNA compared to control-transfected cells. However, mitochondrial membrane potential was significantly decreased in response to oxLDL compared to oxLDL-IC regardless to HSP70 siRNA or HSP70B' siRNA transfection.

Conclusion: The findings suggest that HSP70 and HSP70B' expression regulates the nitrosative but not oxidative stress response induced by oxLDL in macrophages. Immune complexes containing oxLDL, however, appear to have no effect on H_2O_2 or NO production.

Funding: NIH HL079274, NIH (ARRA) grant R01 HL079274-04S1, and the South Carolina COBRE in Lipidomics and Pathobiology (P20 RR17677 from NCRR) to SMH; NIH P01 HL55782, R01 DK081352 and R01 DK081352-02S1 (ARRA) to MLV; and the Department of Veterans Affairs to MLV.

35. Oxidized low density lipoprotein immune-complexes protect monocytes from apoptosis and stimulate increased cytokine release through acid sphingomyelinase activation

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Background and objective: Oxidized low-density lipoproteins (oxLDL) and oxLDL-containing immune complexes (oxLDL-IC) contribute to formation of lipid-laden macrophages. Fc-□RI mediates the uptake of oxLDL-IC, whereas several scavenger receptors bind to oxLDL. We have previously determined that oxLDL-IC activates macrophages and prolongs their survival compared to oxLDL alone. Sphingomyelin is a major constituent of lipoproteins, and acid sphingomyelinase (ASMase) can hydrolyze sphingomyelin to generate the bioactive lipid, ceramide. ASMase exists in two forms: lysosomal (L-ASMase) and secretory (S-ASMase). In this study we examined whether oxLDL and oxLDL-IC regulate ASMase activity differently.

Methods and results: U937 monocytic cells were treated with LDL, oxLDL, oxLDL-IC or keyhole-limpet hemocyanin immune complex (KLH-IC) (50-100μg/ml) for 30 min up to 18 hr. ASMase protein levels were decreased in LDL-treated cells compared to oxLDL and oxLDL-IC. Treatment with oxLDL produced a short, transient L-ASMase activation at 30 min before falling below the baseline (Time 0), while L-ASMase activity gradually increased with oxLDL-IC over 6 hr, accompanied by pronounced redistribution of ASMase into the insoluble extracellular oxLDL-IC aggregates. Treatment with immune-complexes (oxLDL-IC and KLH-IC) resulted in no change in S-ASMase levels compared to non-stimulated control at 18 hr. However, treatment with oxLDL as well as LDL induced a dose-dependent attenuation of S-ASMase activity. Interestingly, exposure to oxLDL-IC induced the highest L-ASMase activity compared to all other treatments at 18 hr. Desipramine inhibited oxLDL and oxLDL-IC induced IL-1β release. Apoptosis was induced by oxLDL in monocytes from both ASMase wild-type (WT) and knock-out mice (KO), while oxLDL-IC did not promote survival of ASMase WT monocytes or of ASMase siRNA-treated U937 cells. Apoptosis was quantified 48 hr post-treatment by staining nuclei with *bis*-benzimide.

Conclusion: The data suggest that a brief burst of ASMase-mediated ceramide generation is sufficient for IL-1β release after oxLDL treatment. Additionally, prolonged activity of ASMase may be responsible for the prolonged survival effect of oxLDL-IC.

Funding: NIH HL079274 to SMH; South Carolina COBRE in lipodomics and pathobiology (P20RR17677 from NCRR) to SMH, KJS and YAH; College of Graduate Studies/Summer Under Graduate Research Program to CMP; Department of Veterans Affairs to MLV.

36. Lipid metabolome of fibroblasts with peroxisomal diseases *

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Very long chain fatty acids (VLCFAs) which are usually degraded in peroxisomes by beta-oxidation accumulate in various tissues and cells in peroxisomal diseases, such as adrenoleukodystrophy and Zellweger syndrome. A ratio of fatty acid content, C26:0/C22:0, has been used as the diagnostic index of those diseases. The most part of VLCFAs are contained in complex lipids, such as phospholipids, glycosphingolipids, cholesterylesters etc. In these clinical analyses, however, fatty acid content has been measured as the fatty acid composition after hydrolysis of those lipids. The purpose of this study is elucidation of the molecular structures of the VLCFA-containing lipids. We analyzed the lipid metabolome of fibroblasts with peroxisomal diseases using LC/MS/MS technique. The total lipid fraction was separated by a reversed-phase column, injected to a mass spectrometer and analyzed. Several extra spots with longer retention time and larger m/z were found in both of the peroxisomal diseases compared to the control. The structural analysis of these lipids using MS/MS will be presented.

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37. Eicosanoid profiling reveals shunting towards PGD_2 pathway in MPGES1 knock-out mice

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Background: Microsomal prostaglandin E synthase-1 (MPGES1) is the terminal enzyme in the induced PGE_2 production at the sites of inflammation and well-recognized target for the development of novel anti-inflammatory drugs. Genetic deletion of MPGES1 in arthritic mice reduces inflammation and protects them from pain and joint destruction. However, molecular mechanisms involved in anti-inflammatory effects of MPGES1 inhibition/deletion at sites of inflammation have not been explored.

Objective: To develop a method for eicosanoid profiling and study effects of MPGES1 deletion on the eicosanoid profile upon LPS-induced macrophage activation.

Methods: Peritoneal macrophages (PM) from wild type (WT) and knock-out (MPGES1-/-, KO) mice were induced with LPS for 16 h and supernatants were harvested for eicosanoid analysis. Eicosanoid profiling of approximately 30 compounds was performed using LC-MS/MS. The fatty acid composition of total lipids in spleen of WT and KO mice was determined using GC/MS.

Results: Compared to WT, MPGES1 deficient PM displayed a markedly attenuated increase in PGE_2 production upon LPS stimulation, and exhibited significantly increased levels of 15-deoxy-PGD_2 and PGF_{2a} ($p<0.01$). There were no significant differences in the fatty acid composition of total lipids from spleen of KO and WT mice, suggesting that the change occurs downstream.

Conclusion: Data reveals that mPGES-1 deficient PMs displayed shunting towards production of PGD_2 metabolites upon LPS stimulation compared to WT. These effects of inducible PGE_2 have important implications regarding potential consequences of pharmacologic mPGES-1 inhibition. It is of importance to measure as many relevant eicosanoids as possible in a lipidomics approach to obtain a more holistic picture of the studied system.

38. The metabolic fate of fatty acids in yeast: to store or not to store, that is the question

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Fatty acids (FAs) serve essential functions in a cell as nutrients, signalling molecules and parts of membrane-forming phospholipids. On the other hand, dysregulation of FA metabolism may also be detrimental for a cell, leading to lipotoxicity and ultimately cell death. To understand the molecular mechanisms that control the metabolic flow of FA from de novo synthesis or external supply into phospholipids or storage lipids (triacylglycerol and steryl esters) we are making use of defined yeast mutants in combination with UPLC-qTOF mass spectrometry for the analysis of lipid molecular species. Of particular interest is to unravel the partitioning of FAs between triacylglycerols and phospholipids, which share common precursors, during cellular growth. Time course experiments show that externally supplied deuterated palmitic acid (C16:0 d_{31}) is subject to rapid elongation and desaturation and incorporated in cellular phospholipids and triacylglycerols. These efficient modifications also explain why palmitic acid is not toxic to yeast cells, in contrast to mammalian cells. After 3 hours of incubation with C16:0 d_{31} almost every lipid class is labelled albeit with different efficiency, ranging from 35 percent up to 60 percent. This observation indicates a selective channelling of the labelled acyl-CoA species into cellular lipids. The method is currently applied to various yeast mutants defective in FA synthesis and acyl-transfer, to uncover the mechanisms that control the specific intracellular flux of FAs.

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39. Glucose induced lipid stress and inflammation, drivers of insulin resistance development

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Worldwide, chronic diseases contributed to about 60 % of all deaths in 2001, and the incidence is expected to increase to 75 % by the year 2020. Diet is a major modifiable risk factor. Changing quality and quantity of fats has received considerable attention, whereas the role of carbohydrates has been less studied.

We have compared the effects of intake of two iso- and normocaloric diets differing in relative macronutrient quantity, 65:15:20 (AHC) and 27:30:43 (BMC) energy percent (E %) of carbohydrates, proteins and fats, respectively, on blood markers and leukocyte gene expression. This was performed in a randomized cross-over study where thirty-two young, healthy, but modestly overweight women and men, where they were subjected to a two times six-day diet intervention with a washout period of 8 days between the diets. Fasting blood samples were collected before and after each diet period. Six days consumption of the balanced diet (BMC) improved the atherogenic index, main blood cytokines/adipokines, and HOMA indices significantly. The diet interventions resulted in diet-specific changes in leukocyte gene expression profiles suggesting differential effects on apoptosis, proliferation/cell cycle regulation, and stress/immunity, including activation of genes for transcription factors NF-κB; STAT3/5; CTNN□, LEF/TCF; and CEBPA on the AHC diet, and inhibition of gene for NF-κB and activation of FOXO3 during the BMC diet. Insulin resistance is an independent predictor of several lifestyle diseases, including T2DM. Considering the complex genetics of susceptibility to insulin resistance, individual responses to a dietary challenge are expected to vary among individuals. Based on results above combined with genotype we suggested a method to identify QTL markers associated with insulin resistance, and used these markers to explain individual physiological responses to dietary glycemic load. In conclusion, a reduction in diet carbohydrate quantity seems to be an important single factor in reducing postprandial hyperglycemia-induced systemic low-grade inflammation, associated with lifestyle disease development.

40. Five-lipoxygenase/leukotriene pathway inhibition by caffeic acid phenethyl ester and propolis

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Caffeic acid phenethyl ester (CAPE) is a natural polyphenol abundant in honey bee propolis. Here, we report on characterization of the human 5-LO inhibitory profile by CAPE, as compared with two other naturally occurring phenols, namely caffeic acid (already recognized as a 5-LO inhibitor) and 5-O-caffeoylquinic acid (chlorogenic acid), both found in various edible plants. Thus, enzyme activity assays carried out with human recombinant 5-LO revealed that CAPE effectively and dose-dependently inhibited the formation of 5-LO derivatives (IC_{50} ≈ 0.6 μM). Under the same experimental conditions, the caffeic acid IC_{50} was ≈ 70-100 μM, whereas that of chlorogenic acid, obtained from artichoke (*Cynara scolymus*), was ≈ 100 μM. Also, CAPE readily inhibited leukotriene (LT) biosynthesis in intact human cells, namely healthy donor polymorphonuclear leukocytes (PMNL; IC_{50} ≈ 0.1 – 0.3 μM) and the mast cell line HMC-1. In both cell types the inhibition occurred in the nanomolar range (PMNL: IC_{50} ≈ 0.1 – 0.3 μM; HMC-1 cells: IC_{50} ≈ 0.5 μM). Moreover, CAPE appeared to be equally effective in inhibiting rat 5-LO, in the rat mast cell model represented by the RBL-1 cell line (IC_{50} ≈ 0.4 μM). Finally, CAPE also inhibited the activity of the human platelet-type 12-LO, although at slightly higher concentrations (IC_{50} ≈ 15 μM), as assessed in human platelets. In contrast, the inhibition of LT biosynthesis in intact cell models by caffeic acid and chlorogenic acid occurred only at concentrations 20- to 100-fold higher (e.g., IC_{50} ≈ 10 μM and IC_{50} ≈ 80 μM, respectively, in the RBL-1 cell model). Further experiments revealed that propolis itself (as an ethanolic extract) inhibited 5-LO activity and LT biosynthesis at extremely high dilutions. Thus, inhibition of recombinant 5-LO activity was still substantial in the presence of 10^7-fold diluted propolis (or 0.1 ppm). On the other hand, in the PMNL model, 10^5-fold diluted propolis extracts (10 ppm) determined a 50% decline in generation of 5-LO derivatives.

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41. Expression of $cPLA_2$ in macrophages is critical for lung cancer metastasis

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Lung cancer is the leading cause of cancer death in both men and women. Despite extensive research, five year survival has not significantly improved, largely due to the presence of advanced disease and metastasis at the time of presentation. While cancer initiation is predominantly determined by genetic changes in tumor cells, progression and metastasis involves critical contributions by the tumor microenvironment (TME). To determine critical pathways in the TME regulating lung cancer metastasis, we developed an orthotopic immunocompetent mouse model in which murine lung cancer cells are injected into the lungs of syngeneic mice. These cells form primary tumors and metatasize to the lymph nodes, and distant organs including the liver and brain. $cPLA_2$ represents the rate limiting enzyme in eicosanoid production. While studies have implicated this enzyme in tumor cells, less is known regarding the role of these pathways in the TME. In our model, tumors injected into $cPLA_2$ knock out mice had a marked impairment of metastasis resulting in a survival advantage (Weiser-Evans et al, Cancer Research 2009). Examination of tissues from these mice showed decreased number of macrophages associated with tumors growing in $cPLA_2$ knockout mice. Using in vitro co-cultures of murine cancer cells with bone marrow-derived macrophages we demonstrated that loss of $cPLA_2$ in macrophages resulted in impaired migration and reductions in cytokine production. This defect was reversed by adding exogenous arachidonic acid. Eicosanoid profiling of tumors growing in either WT or $cPLA_2$ knockout mice was performed using LC/MS/MS. Levels of multiple eicosanoid products were significantly decreased in both tumors and surrounding tissues isolated from the knockout mice, including both cyclooxygenases and lipoxygenase products. These data indicate that eicosanoid production in tumor associated macrophages is critical for macrophage migration to tumors and promotion of lung cancer metastasis.

42. Steady-state and dynamic analytical approaches for identifying lipid biomarkers and understanding the pharmacology of lipid-modifying drug targets

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From first in man to registration, lack of efficacy is the primary reason for attrition of targets in drug discovery. Biomarkers help to address this issue by serving as indicators of target engagement and change in disease state with genetic or pharmacological treatment (i.e. LDL-cholesterol in nonhuman primates with statin treatment). Lipid biomarkers are often translatable from preclinical species to humans because lipid analytical techniques are usually applicable to plasma from any species. A combination of static (i.e. free cholesterol) and dynamic (i.e. cholesterol synthesis rate) biomarkers are often necessary to interrogate biological mechanisms. In this presentation, several MS-based analytical approaches used to discover and develop lipid biomarkers will be presented, including: profiling, quantitation, stable isotope tracing, and metabolic flux. Case studies for mitochondrial triglyceride transfer protein (MTTP) and Apolipoprotein B SiRNA knockdown will be presented where biomarkers are discovered using lipid profiling (LC-QTOF) and assessed using lipid quantitation (LC-QQQ). With some biomarkers like triglycerides, where the concentration is high and the turnover rate is relatively low, measuring a change in steady state concentration after treatment is challenging. To address this, tracing with a stable-isotope labeled fatty acid quantifies newly made triglycerides, resulting in a more robust target engagement assay. The fatty acid tracer assay is also translatable to humans where basal triglyceride levels vary, making the method a powerful alternative to the traditional lipid tolerance test. An alternative method to fatty acid tracing is flux analysis in which a ubiquitously distributed tracer, like deuterium oxide (^2H_2O) is dosed, and deuterium is covalently incorporated into many newly synthesized lipids, proteins, and metabolites in the same animal experiment. Examples of fatty acid, bile acid, cholesterol, and apolipoprotein flux using ^2H_2O are discussed as dynamic biomarkers for lipid and lipoprotein metabolism.

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43. Sensitive detection of nonpolar lipids in human meibomian gland secretions via shotgun electrospray ionization-mass spectrometry

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Recently, the major intact lipid components in human meibomian gland secretions (meibum) were characterized via direct infusion electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (MS/MS).[1] These lipid species are mainly nonpolar (detected in positive mode) including wax esters, cholesteryl esters, diesters and triacylglycerols, along with some polar lipids including free fatty acids and (O-acyl)-omega-hydroxy fatty acids (detected in negative mode). In this present work, the effect of the type and the concentration of additive, cone voltage, and concentration of meibum lipid on the detection of the nonpolar lipids in positive mode were systematically studied. The addition of ammonium acetate (NH_4Ac) to the sample solution yielded relatively high intensity ammoniated lipid peaks at low cone voltage. Collision-induced dissociation (CID) of these ammoniated lipid species facilitated the identification of the lipids. However, the addition of NH_4Ac led to multi-forms of adducts (+ NH_4^+, + Na^+ and/or + H^+), due to the ubiquitous sodium ions, high affinity and high stability of sodiated lipid ions, and labile ammonia loss from ammoniated ions. These multi-forms of adducts complicated spectra and decreased detection sensitivity. The addition of an appropriate concentration of sodium iodide (NaI) instead to the sample solution significantly increased the detection sensitivity and simplified the spectra by forming only one stable form of adducted ions, i.e. sodiated form. Typically, at least 150 isobaric lipid species were detected within 1 minute for a sample of ~ 64 ng (~16 µg/mL sample at the flow rate 40 µL/min) at a higher cone voltage. The addition of an appropriate concentration of NaI may be beneficial for further qualitative and quantitative analysis of the intact lipids in a complex sample once the major components in the sample are identified.

1. Chen, J.; Green-Church, K. B.; Nichols, K. K. *Invest Ophthalmol Vis Sci* 2010, **51**, 6220-6231.

44. Investigating the effects of lipid peroxidation on the dynamic properties of model membrane system

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Membranes, in the form of phospholipid bilayers, are vital part of all forms of life. Lipid peroxidation in membranes plays a central role in many pathologic processes, including cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, diabetes, and other chronic inflammatory diseases. Lipid peroxidation is of great importance because it modifies the structural and dynamic properties of the membranes, which in turn, influences the membranes' function. One remarkable feature of all biological membranes is their dynamic properties or flexibility (fluidity). This study is focused on examining the dynamic properties of model membrane system, as well as, oxidized model membrane system utilizing Fluorescence Polarization (FP) Spectroscopy. The model membrane system consists of 1,2-dilinoleoyl-3-sn-glycero phosphatidylcholine (DLPC) phospholipid bilayers.

The effects of cholesterol and lipid saturation on the dynamic properties of DLPC phospholipid bilayers are examined. In addition, the local anesthetic (Bupivacaine) interaction with the membrane system is studied. The attained results agree well with other biological and model membrane systems. These data suggest that the DLPC model membrane system can be employed to study lipid peroxidation. Therefore, DLPC phospholipid bilayer samples are subjected to autooxidation. The lipid peroxidation assay is based on the oxidation of iodide (I^-) to triiodide anion (I_3^-) by hydroperoxides. The current research work is focused on investigating (1) the effects of lipid peroxidation on the dynamic properties of cell membrane, and (2) how different factors, including antioxidant (vitamin E) and Bupivacaine drug-membrane interaction, can influence lipid peroxidation process.

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45. Monitoring the dynamics of oxygen and free radicals within neuronal membranes by EPR techniques

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Excessive levels of reactive oxygen species (ROS) cause dysfunctional oxidative modulations of DNA, proteins and membranes. Techniques to monitor oxidized proteins and DNA are established but those to probe oxidative membrane damage in real time are not developed. In the present work we demonstrate the ability of the spin-trapping/labeling electron paramagnetic resonance (EPR) techniques to study sources and dynamics of ROS in synaptosomes and neuronal membranes in young as well as old mice. Moreover, a spin-silencing approach is employed to probe levels of free radical flux within synaptosomal membranes while detecting phospholipids-derived radicals and simultaneously monitoring changes in membrane fluidity. We show that aging is associated with a sustained increase in oxidative stress due to activities of mitochondria and the superoxide-producing enzyme NADPH-oxidase (Nox). The sustained production of superoxide caused an age-dependent decrease in synaptosomal membrane order parameter, which implicates membranes as targets for Nox-induced neuronal damage and eventually dysfunction. Furthermore, EPR lineshape analysis indicated that membrane oxygen contents are depleted following Nox-activation in old, but not young brains. These data suggest that Nox(s) along with mitochondrial ROS-generating activity may mediate age-dependent increase in oxidative damages and concomitant oxygen depletion in old neuronal membranes.

46. Discovery and biological characterization of novel non-cytidine liponucleotides

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CDP-diacylglycerol (CDP-DAG) and deoxy-CDP-diacylglycerol (dCDP-DAG) are requisite phosphatidyl donor intermediates in the biosynthesis of all phospholipids of *E. coli* and anionic phospholipids of most eukaryotes. In addition to cytidine liponucleotides, we have been able to detect novel non-cytidine liponucleotides in organisms from all three biological domains using HPLC-MS. *In vitro* characterization of *E. coli* CDP-DAG synthetase (cdsA) shows enzymatic specificity for phosphatidyl transfer to (d)CTP nucleotides; thus non-cytidine liponucleotides are enigmatic with respect to biosynthesis and biological function.

To elucidate the biological role of non-cytidine liponucleotides we have focused attention on *S. cerevisiae* and *E. coli* model organisms. In lipid extracts from a *S. cerevisiae* cardiolipin synthase knockout (Δcrd1), we detect dramatic accumulations of ADP-DAG and UDP-DAG relative to their WT steady-state levels. Accumulation appears to be growth phase dependent; observed only in lipid extracts from stationary phase cultures. Alternatively, a *S. cerevisiae* phosphatidylglycerophosphate synthetase knockout (Δpgs1) accumulates ADP-DAG and UDP-DAG only during logarithmic growth. In both cases, the magnitude of non-cytidine liponucleotide accumulation varies independently from CDP-DAG accumulation. These observations suggest ADP-DAG and UDP-DAG likely participate in mitochondrial phospholipid metabolism, but their precise roles (i.e. precursor or regulator) may differ from CDP-DAG and await further characterization.

To determine the role of non-cytidine liponucleotides in *E.coli* we rationally screened over 50 mutants and observed changes in steady-state liponucleotide levels only when genes involved in phospholipid or nucleic acid metabolism are disrupted. A physiological survey of wild-type *E. coli* revealed liponucleotide levels are dependent on growth phase, and compartmentalize with the inner membrane. Pulse-chase experiments with *E. coli* strain BB20-14, an auxotroph dependent on glycerol or glycerol-3-phosphate supplementation for glycerophospholipid biosynthesis, indicate ADP-DAG is synthesized/ incorporated at a slower rate than CDP-DAG, hinting at distinct synthetic pathways for these two liponucleotides.

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47. A pathway for lipid A biosynthesis in *Arabidopsis thaliana* resembling that of *Escherichia coli*

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The lipid A moiety of *Escherichia coli* lipopolysaccharide is a hexa-acylated disaccharide of glucosamine that makes up the outer monolayer of the outer membrane. Structurally related lipid A molecules are found in most other Gram-negative bacteria. Lipid A and its precursors have not been detected in plants. However, *Arabidopsis thaliana* and many higher plants contain nuclear genes encoding significant orthologs of key enzymes of bacterial lipid A biosynthesis, including LpxA, LpxC, LpxD, LpxB, LpxK and KdtA. Homozygous insertional knock-out mutations or RNAi knock-down constructs of these genes in *A. thaliana* did not reveal any obvious growth phenotypes compared with wild type. However, analysis of the total lipids extracted from 10-day-old seedlings of *Arabidopsis lpx* and *kdtA* mutants by liquid chromatography/electrospray ionization mass spectrometry revealed accumulation (or disappearance) of the expected monosaccharide or disaccharide lipid A precursors. GFP-gene fusions in transgenic *Arabidopsis* suggest that the Lpx and KdtA proteins are targeted to mitochondria. The structures of the end-products generated by this pathway in plants are unknown, but given their apparently low levels, they might function in signal transduction or regulation. Our work demonstrates that plants synthesize lipid A precursor molecules by essentially the same pathway that is present in *E. coli*.

48. Sterol analysis in humans: the Dallas Heart Study

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Novel and semi high-throughput methods for the analysis of 60 sterols and related compounds from human serum have been developed. These methods combine several steps of classic analytical methods, reducing time and sample processing. In contrast to other solvent precipitations, which cause loss of sterol-containing lipoproteins, and classic two-phase extractions, which result in gelatinous interfaces, this method utilizes a combined extraction-precipitation-hydrolysis where lipids are extracted into solution, proteins and insoluble material precipitated, and mild hydrolysis performed in a solution of methanol and dichloromethane at 37° C. Newly developed SPE procedures simplify sterol isolation and the use of aminopropyl columns, instead of silica columns, may reduce formation of oxidation products. We implemented recently developed core-shell HPLC technology to afford a unique combination of resolution and efficiency that separates many isobaric positional isomers while maintaining standard operating HPLC pressures. Additionally, incorporating scheduled MRM allows older MS instruments to acquire sufficient data points across narrow chromatographic peaks. We have determined that APCI is a superior ionization technique for sterols and ESI with ammonium adducts is sufficient for oxysterols. We are currently evaluating APCI for oxysterols.

Results from the analysis of serum sterol levels in >3,000 subjects from the Dallas Heart Study, a clinically well-characterized patient cohort will be presented. Inter-individual variation in sterols levels will be described and the genetic bases of this variation considered.

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49. Reversal of foam cell infiltration with non-macrophage targeted rescue of Niemann-Pick C disease

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Foam cells (FCs), a common pathological feature of genetic lipid storage disorders and atherosclerosis, form when lipid accumulates in inflammatory macrophages. FCs are believed to form in response to nearby damaged cells, but this has not been directly demonstrated. In the lipid storage disease Niemann-Pick type C (NPC), infiltrating FCs appear to exacerbate lethal liver and pulmonary failure. We have examined two ideas about FC formation: FCs may form in response to loss of NPC1 function within macrophages, or macrophages could turn into FCs in response to genetically damaged surrounding cells. The *ROSA26-rtTA-M2; tetO-Npc1-YFP; Npc1*$^{-/-}$ mouse line used in this study, fed doxycycline (Dox), produced NPC1-YFP protein in the liver and other visceral tissues but not in CD68+ macrophages. Within days of Dox administration to a late stage diseased mouse, non-macrophage NPC1-YFP induction reduced liver damage substantially as assessed by blood alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. The prevalence of FCs in the liver was reduced and remaining FCs clustered in non-rescued cholesterol-rich areas. With prolonged NPC1-YFP production, the majority of macrophages exhibited morphology similar to that of resident Kupffer cells in non-diseased mice. Thus, FC formation can be prevented and reversed by correcting the genetic disorder exclusively in liver cells other than macrophages. We conclude that macrophage FC infiltration in NPC disease is secondary to loss of NPC1 in other cell types. This non-autonomous control of FC formation indicates that a therapy targeting the liver cells primarily affected by the disease will correct macrophage FC formation as well.

50. A role for DGKθ in cAMP-dependent steroidogenic gene transcription in the adrenal cortex

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In H295R human adrenocortical cells, adrenocorticotropin (ACTH) signaling activates the cAMP-dependent protein kinase (PKA), which phosphorylates downstream protein and promotes the transcription of genes required for glucocorticoid and adrenal androgen biosynthesis. Steroidogenic factor 1 (SF1) is a nuclear receptor that regulates the transcription of most of the steroidogenic genes and we have previously identified phosphatidic acid (PA) as an agonist for this receptor. DGK (diacylglycerol kinase) θ is an intracellular lipid kinase that phosphorylates diacylglycerol to form PA. Because the activity of DGK is rapid increased by ACTH/cAMP signaling in the nuclei of H295R cells, we sought to investigate the role of DGKθ in regulating steroidogenic gene expression. We determined the effect of cAMP signaling on DGKθ expression and found that activation of the ACTH signaling pathway induces DGKθ transcription. Reporter gene analysis indicated that SF1 and the glucocorticoid receptor regulate the activity of the DGKθ promoter. We used a (TetR)-inducible H295R stable cell line to express small hairpin RNA (shRNA) against DGKθ. Genome-wide DNA microarray analysis revealed that silencing DGKθ expression leads to global change in gene expression. DGKθ knockdown significantly attenuated cAMP-dependent mRNA expression of multiple genes involved in steroid hormone production, including CYP11A1, CYP17 and steroidogenic acute regulatory protein. mRNA expression studies were supported by western blotting and ELISA analysis of cortisol and dehydroepiandrosterone secretion in wild type versus DGKθ-knockdown cells. Taken together, our data identified a role for DGKθ in cAMP-dependent steroidogenic gene expression and cortisol production.

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51. Acid ceramidase is a novel transcriptional coregulator of steroidogenic genes in human H295R adrenocortical cells

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Sphingolipids, such as sphingosine (SPH) and sphingosine-1-phosphate (S1P), play multiple roles in steroidogenesis including regulation gene transcription and steroid hormone secretion, primarily by acting as second messengers and modulating the protein kinase A (PKA)/cAMP-dependent pathway. In H295R cells, adrenocorticotropin (ACTH) signaling rapidly activates sphingomyelin and ceramide (cer) turnover, with a concomitant increase in S1P secretion. Acid ceramidase (ASAH1) regulates the cellular concentrations of SPH, a ligand for the nuclear receptor steroidogenic factor 1 (SF-1), by catalyzing the degradation of cer to SPH and a free fatty acid. The aim of these studies was to define the mechanism by which ASAH1 regulates steroidogenic gene expression and cortisol production. We show that ASAH1 is expressed in the nuclei of H295R cells, binds directly to SF-1, and cAMP signaling regulates the temporal binding of ASAH1 and SF-1 to the CYP17 promoter. Further, ASAH1 suppression leads to global changes in gene expression including increased transcription of CYP11B, CYP21, and steroidogenic acute regulatory protein (StAR). ASAH1 silencing results in a higher capacity to secrete cortisol and DHEA and increases ACTH responsiveness. Depletion of ASAH1 alters the transcriptional levels of many sphingolipid-metabolizing genes, including both sphingosine kinase isoforms and multiple members of the ceramide synthase family. Intriguingly, mRNA levels of various cAMP-responsive genes were altered in ASAH1-depleted cells. NR4A1, NR4A2, and NR4A3 were induced by 2.1-, 2-, and 20-fold, respectively. The induction of these nuclear receptors was concomitant with suppression of dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1 (DAX-1), a repressor of SF-1 and steroidogenic gene expression. Finally, we demonstrate that ASAH1 suppression decreased cell proliferation and the expression of multiple cell cycle-related proteins including cyclin-dependent kinase 2 (CDK2). Collectively, these data establish a key role for ASAH1 as a nuclear coregulatory protein in modulating gene expression in adrenocortical cells.

52. An automated workflow for rapid processing and analysis of lipidomic datasets *

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The advent of automated shotgun lipidomic analysis allows for the high throughput quantitative characterization of lipidomes yielding comprehensive and extensive datasets. However, large amounts of data from hundreds of samples (e.g. from clinical trials) require automated data processing and also analysis. Therefore, we developed a lipidomic database that enables the automated generation of graphical outputs of various lipidomic features, e.g. lipid class composition, unsaturation indices, fatty acid length distributions, lipid species composition *etc*. Furthermore, hierarchical clustering is performed in order to group samples according to common lipidomic signatures, allowing for a fast classification of lipid composition-based phenotypes, simplifying further analysis. This workflow was applied to lipidomic datasets obtained from total cell extracts of the yeast *Saccharomyces cerevisiae*. Yeast is a valuable and widely used experimental system for studying fundamental cellular processes. More specifically, recent insights into neutral lipid metabolism, sphingolipid homeostasis, physiological sphingolipid-sterol interactions and integration of lipid metabolism with cell cycle signals have been gained in yeast by the application of lipidomic methods. However, a comprehensive description of “basal” factors influencing the yeast lipidome is missing. Therefore, by applying shotgun lipidomics, we determined the lipidomes (covering neutral lipids, glycerophospholipids, (glyco-) sphingolipids and sterols) of yeast cells under various standard growth conditions. These include: different carbon sources, growth phases and temperatures, protein over-expression as well as different commonly used selective drugs (~90 samples in total). Strikingly, most of the variables had a significant impact on the lipidome of wildtype yeast cells. The obtained results have important implications for the design of lipid-related experiments in yeast and probably also in mammalian cell culture systems. Taken together, our automated data processing and analysis workflow has proven to be suitable for handling large lipidomic datasets and is tremendously simplifying the process of data interpretation, thus making it interesting for large scale clinical studies.

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53. Development of a kinetic model for eicosanoid metabolism in bone-marrow derived macrophages

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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 unsaturated fatty acid. Eicosanoids play important roles in pain, fever, inflammation and related disorders such as multiple sclerosis. Eicosanoids are not stored, but are locally produced from the biological membrane on demand, and then promptly released into the extracellular space. Therefore, quantification of these metabolites and modeling of the eicosanoids network using temporal data is imperative for an understanding of eicosanoids biology. The Lipid Metabolites and Pathways Strategy (LIPID MAPS) consortium has identified and quantified lipid species using sophisticated MS systems in murine macrophage. Previously, we have developed a mathematical model of AA metabolic network in RAW264.7 cell, a murine macrophage cell line. However, our model was lacking the gene expression data of eicosanoid-synthesizing enzymes and the LOX products of AA. Additionally, it is unclear whether the model developed in the RAW cell line is acceptable for the primary macrophages. In the present study, bone marrow-derived macrophage cells were stimulated with ATP. Using both metabolomic and transcriptomic data, we have developed a quantitative model of the COX and LOX branches of AA metabolism through a two-step matrix-based approach to estimate the rate constants. 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 an independent data set in which the cells were treated with both Kdo_2-Lipid A and ATP, by allowing the parameters to vary within only 30% (obtained from uncertainty analysis) of the estimated values.

54. Kinetic modeling of eicosanoid and sphingolipid pathways

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There is increasing recognition of the role of lipids in signaling and disease pathways. The LIPID MAPS consortium (www.lipidmaps.org) has developed methods to quantitatively measure the composition of lipids in RAW 264.7 macrophage cells. Time-course data in response to treatment with KDO2 lipid A (a lipopolysaccharide analogue) has been collected for several lipids. Towards systems modeling and analysis, we have developed a framework for kinetic modeling of various lipid pathways. First we generate the reaction network using information from KEGG pathways and literature. Then we develop the mathematical model and estimate the rate parameters using experimental data through a two-step approach. The system is modeled as a set of ordinary differential equations. The flux expressions are based on law of mass action kinetics. Thus, the flux expressions are linear in rate parameters and nonlinear in metabolite concentrations. In the first step, a matrix-approach uses Matlab's optimization function *lsqlin* (constrained least squares-based optimization). These initial estimates serve as the starting point for the second step in which they are further refined using a general constrained nonlinear optimization (Matlab function *fmincon*). This makes the overall process computationally efficient. Further, in the second step, numerical integration is used to compute the time-courses to eliminate discretization errors. We have applied this approach to eicosanoid pathway and sphingolipid pathway [1,2]. For both of the pathways, the resulting models fitted the experimental data well and demonstrated that the integrated metabolic and signaling networks and the experimental data are consistent with each other. Further, the estimated activities for main enzymes were similar to their literature values. These quantitative models can be used for making predictions and designing further experimental studies utilizing genetic and pharmacological perturbations to probe fluxes in lipid pathways.

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2. Gupta, S., M. Maurya, A. Merrill, C. Glass and S. Subramaniam, 'Integration of lipidomics and transcriptomics data towards a systems biology model of sphingolipid metabolism', *BMC Systems Biology*, **5**, 26, 2011.

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55. Blood lymphocytes lysophosphatidylcholine homeostasis in acute lymphoblastic leukemia

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We investigated the processes of different exogenous [^{14}C]fatty acids (FA) early (5 *sec*) incorporation into the lysophosphocholine (LPC) fractions in human peripheral blood lymphocytes (HPBL) obtained from patients with acute lymphoblastic leukemia (ALL) in comparison with the healthy people. Alterations in the endogenous activities of enzyme systems involved in LPC generation/utilization mechanisms in purified plasma membrane (PM) fraction of HPBL were also investigated. The data obtained provide evidence for elevated level of diverse FA-containing LPC in ALL compared to norm. We have shown that Ca^{2+}-dependent PM-associated activities of phospholipases A_1 and A_2 detected in normal HPBL were completely inhibited in ALL. Notably, abnormally high lysophospholipase activity, distinctly individual for each patient, was observed in ALL, but not in normal controls. In malignancy it was also shown that LPC-acyltransferase and glycerophosphorylcholine–acyltransferase activities remain unchanged and increased, correspondingly. To summarize, elevated levels of different (by FA content) LPCs as well as disorders in enzyme activities responsible for deacylation/reacylation reactions indicated deregulation of LPC homeostasis in crude HPBL of ALL patients. Some of alterations observed are common characteristics of different types of cancer studied, and can lead to the new personalized treatment modes of disease.

56. Changes in blood limphocyte lipids fatty acid content modification by arachidonic acid in breast cancer

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The importance of diverse lipids in the regulation of various cellular functions is well established but their role in malignancy is not studied in detail. Particularly, there are no data concerning the possible alterations in plasma membrane diverse lipids fatty acid (FA) content modification processes in breast cancer (BC).

The main objective of this study was the comparative investigation of regularities of exogenous [^{14}C]arachidonic acid (AA) early (5 sec) incorporation into the phospholipid (PL) and neutral lipid (NL) different fractions in human peripheral blood lymphocytes (HPBL) and it's relatively sustained (60min) interfractional redistribution in norm and BC.

The data obtained indicate that in BC there are significant and regular disturbances in the mechanisms of crude HPBL membrane lipids FA content modification by AA. Incorporation of AA into the diverse NL fractions of HPBL was significantly altered, especially at the early (5sec) stage of lipids modification. Notably, alterations revealed were regular but individual for different BC patients. It is also important that the similar pattern of disturbances in HPBL lipids acylation was observed in ovarian cancer and three different forms of leukemia. Some differences for each type of disease were observed at the relatively sustained (60min) stages of lipid modification processes.

Thus, we conclude that the regularities revealed are common characteristics for all types of malignancy studied and can be used in clinic for detection, state value of disease and individual correction of chemotherapy for cancer treatment.

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57. Lipid second messengers generation by stimulated blood lymphocytes in ovarian cancer

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Generation of diverse lipid second messenger (LSM) molecules by human peripheral blood lymphocytes (HPBL) at different (5, 10, 30 and 60 sec) time points of cell co-stimulation by anti-CD3 and anti-CD28 monoclonal antibodies have been investigated in norm and ovarian cancer (OC).

It was revealed that some mechanisms of LSM molecules generation/utilization in stimulated crude HPBL were significantly altered in OC compared to norm. Particularly, the reliable generation of arachidonyl-1,2-diacylglycerol at the initial step (5 sec) of cell stimulation in norm was depressed in OC and reached below the basal level in unstimulated cells. It's important that some disturbances in LSM molecules formation in HPBL obtained from patients with OC were identical with those observed in breast cancer and three different forms of leukemia.

Experimental results indicate that in solid tumors as well as in leukemia there are significant and similar disturbances in cell stimulation mechanisms, which are more obvious at the initial, membrane-associated step of signal transduction. We propose that regular disorders revealed in OC and other forms of cancer can be resulted from changes in T-cell subpopulations in human peripheral blood detected over the past decade. Data obtained can be used as additional testing parameters for further definition and individual correction of chemotherapy programs in cancer treatment.

58. A multiplexed lipid biomarker for non-invasive diagnosis of endometriosis

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Endometriosis is a common chronic gynaecological disorder afflicting more than 10% of women. It manifests clinically with cyclical debilitating pain, blood-filled endometrotic ovarian cysts, pelvic adhesions and have a complex interaction with infertility. In current practice, definitive diagnosis of endometriosis requires invasive laparoscopies and tissue biopsies. Herein, we report the unbiased quantitative analysis of potential lipid biomarkers from patient serum. The study population consisted of 21 patients (with endometriosis, EM+ = 13, without endometriosis, EM- = 8). All patients were carefully stratified into cases versus controls after a careful laparoscopic assessment. We developed a quantitative automated liquid chromatography-Multiple Response Monitoring (MRM) to rapidly and specifically screen more than 200 sera lipids *en masse* in an unbiased fashion so that every lipid will have equal chance in being selected as a biomarker. A throughput of 15 samples/module/day was attained with the current setup. We used Support Vector Machine (SVM) to statistically select lipids that optimally distinguishes EM+ and EM-. SVM selected five serum lipids when computed in combination, accurately discriminates EM+ and EM-. This composite biomarker panel greatly increased accuracy of EM prediction over individual lipids. By Leave One Out-Cross Validation, the performance of the composite biomarker panel was assessed. SVM selected five serum lipids when computed in combination, accurately discriminates EM+ and EM-. This composite biomarker panel greatly increased accuracy of EM prediction over individual lipids. Validation of the performance of the five serum lipids showed that they were able to achieve good classification (sensitivity = 100%, specificity = 87.5%). Our findings suggest that the composite lipid biomarker panel is effective in predicting EM in patients. A provisional patent has been filed for this invention. An expansion of sample size is in progress, and if validated, the biomarker panel may obviate the need for invasive procedures to confirm the diagnosis of endometriosis.

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59. Characterizing the enzymatic function of Pxt, the *Drosophila* prostaglandin G/H synthase 1

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Drosophila *pxt* encodes for the prostaglandin (PG) G/H synthase 1 or cyclooxygenase 1 (COX1) homolog. Pxt is required for female fertility and regulates many aspects of follicle development; treatment of follicles *ex vivo* with PG synthesis inhibitors recapitulates defects seen in *pxt* mutants. Importantly, mouse COX1 can rescue *pxt* mutants. While we have a genetic handle on PG signaling in Drosophila, very little PG biochemistry has been performed with flies. In 1986, Pagés *et al.* demonstrated that PGE2 and several potential arachidonic acid precursors could be detected in *Drosophila melanogaster*. To date, however, the PG species present within the fruit fly, their functions, and their synthesis/signaling pathways remain poorly studied. Therefore, we are interested in addressing two key questions: (1) What PGs are present within the fruit fly and at what quantities? And (2) What are the key residues necessary for Pxt function? In order to identify and quantify the PGs present within *Drosophila*, we are currently developing methodologies to extract/purify PGs from whole flies, both wild-type and *pxt* mutants, as well as from specific tissues for analysis using HPLC-MS/MS. To explore the enzymatic function of *pxt* we are taking two genetic approaches. First, we are testing whether Pxt can functionally replace zebrafish COX1 (zCOX1), which is required for gastrulation. Utilizing this system, we will then determine whether point mutations in the putative catalytic residues of Pxt impair its ability to rescue zCOX1 morphants. Secondly, mutations that reduce or ablate Pxt enzymatic activity in zebrafish will be validated in the fly based on their ability/inability to rescue *pxt* mutant phenotypes. By profiling the PGs present and characterizing the enzymatic activity of Pxt we will advance our understanding of PG synthesis and signaling in Drosophila, providing a more defined genetic system to elucidate the molecular mechanisms underlying PG action.

60. Lipidomics in studies lifestyle associated diseases

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Changing concentrations of different lipid classes in biofluids and tissue are important markers of lifestyle-associated diseases such as obesity, diabetes type II, liver steatosis, systemic inflammation, atherosclerosis. Semi-quantitative profiling of major lipid classes is therefore an important area in metabolomics and metabolite profiling. In addition, multi-targeted quantitative analysis of low abundant lipid classes, like N-acyl amides (endocannabinoids) and eicosanoids, is also becoming increasingly important.

All these types of lipids classes can be analyzed by LC-MS based methods in biological matrices although different requirements are needed with respect to sample work-up, sensitivity and ionization.

This poster will give an overview of the different LC-MS methods that were developed for the lipid classes described earlier. All methods were validated[1,2] and already have been used in both human and animal studies[3-6].

1. Patent pending: P89944EP00 Improved method for the detection of polyunsaturated fatty acids
2. Balvers et al., J. Chromatogr. B 877 (2009) 1583-1590.
3. Kleemann et al., Genome Biology 8 (2007) R200
4. Kleemann et al., PLoS One 5 (2010) e8817.
5. Bakker et al., Am. J. Clin. Nutr. 91 (2010) 1044-1059.
6. Joosten et al., Nutrition & Metabolism 7 (2010) 49.

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61. Sulfoglucuronosyl paragloboside expression mediates apoptosis of human cerebromicrovascular endothelial cells in inflammation

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Glucuronosyl paragloboside (SGPG), a minor glycosphingolipid (GSL) of the nervous system, is characterized by the presence of a unique HNK-1 carbohydrate epitope and it has the following structure: Sulfate-3GlcA(β1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')ceramide. In addition, it has been shown to be present in cerebrovascular endothelial cells. The HNK-1 epitope, whose minimal requirement consists of the terminal sulfated dissacharide residue, is a ligand for L-selectin for T-cell adhesion and has been implicated in neuroinflammatory diseases, such as Guillian-Barré Syndrome (GBS). Inflammatory cytokines, such as TNFα and IL-1β, up-regulate SGPG expression by stimulating gene expression for glucuronosyltransferases, both the P and S forms (GlcATp and GlcATs), and the HNK-1 sulfotransferase (HNK-1 ST). The precise mechanism of SGPG elevation, however, and its role in maintaining blood-brain or blood-nerve barrier (BBB or BNB) function in inflammation has not been elucidated. Transfection of a human cerebromicrovascular endothelial cell line (SV-HCEC) with HNK-1 ST siRNA down-regulated SGPG expression, inhibited cytokine-stimulated T cell adhesion, and offered protection against endothelial cell death (apoptosis). Blocking SGPG expression also inhibited cytokine-mediated stimulation of NF-κB activity but stimulated MAP kinase (ERK) activity. Furthermore, transfection of SV-HCECs using EGFP-GlcATp and EGFP-GlcATs elevated SGPG expression and triggered cell apoptosis, the S form being more potent than the P form. SGPG-mediated cell apoptosis preceded by inhibiting the intracellular NF-κB activity, interfering with Akt (PI3 kinase) and ERK (MAP kinase) activation, and stimulating caspase 3 in SV-HCECs. Our data indicate that SGPG is a critical regulatory molecule for maintaining cell permeability and survival, through which the BBB/BNB barrier is regulated. (Supported by NIH NS11853 and NS26994.)

62. *In situ* glycerophospholipid imaging with C_{60} SIMS

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Mass spectrometry imaging has been used to map lipid distributions across the surface of tissues and single cells. The goal is to utilize the high spatial resolution of SIMS to improve our understanding of the lipid heterogeneity within cells. Molecular sensitivity is a critical issue at high spatial resolution since there are a limited number of molecules in the analysis volume. To overcome this constraint, here we examine sample preparation in an attempt to improve molecular sensitivity with C_{60}-SIMS. Cooling the sample to LN_2 temperatures offers a two-fold advantage for SIMS sensitivity; it enhances protonated molecular ion yields and reduces the damage cross section during depth profiling. Single component and muti-component lipid films were interrogated at room and cryogenic temperature and ion signal enhancement was evaluated. A cryogenic-compatible sample stage for the QSTAR XL mass spectrometer is currently in the preliminary stages of development and preliminary data is presented.

In accordance with the LIPID MAPS agenda, lipid distributions obtained from RAW 264.7 cells stimulated with Kdo_2-Lipid A were analyzed using C_{60}-SIMS. High mass resolution and tandem MS capabilities of the modified QSTAR instrument were utilized for *in situ* lipid identification efforts. To evaluate the ability of C_{60}-SIMS to detect and identify glycerophospholipids *in situ*, lipid extracts were analyzed without separation and compared to HPLC data obtained from the consortium. Lipid distributions obtained from a lawn of stimulated raw cell was also interrogated with limited spatial resolution. In this investigation, adequate molecular sensitivity to a range of lipids was achieved with as little as 500 cells, demonstrating a promising start towards single cell analyses.

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63. Spinal 12-lipoxygenases (12-LOX) contribute to inflammatory hyperalgesia

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Peripheral inflammation initiates changes in spinal nociceptive processing leading to enhanced sensitivity to light touch and thermal stimulation, or hyperalgesia. Using systematic quantitative LC-MS/MS analysis, we demonstrate that of the 174 lipid species detectable by our methodology, 102 were detected in the rat lumbar spinal cord. In addition to the predicted elevation in levels of cyclooxygenase (COX) metabolites such as Prostaglandin E_2 (PGE_2) after peripheral inflammation with intraplantar (IPLT) carrageenan, the most notable increases were observed with the arachidonic acid (AA)-derived metabolites of 12-lipoxygenases (12-LOX), in particular the hepoxilins (HXB_3). In contrast, spinal 5-LOX products remained unchanged after peripheral inflammation. Based on these results, we examined involvement of spinal LOX enzymes in inflammatory hyperalgesia. We found that intrathecal (IT) delivery of the general LOX inhibitor NDGA blocked the carrageenan-evoked increase in spinal HXB_3 at a dose that attenuated the associated tactile allodynia and thermal hyperalgesia. Furthermore, IT delivery of 12-LOX-preferring inhibitors (CDC, Baicalein), but not those targeting 5-LOX (Zileuton) or 12/15-LOX (PD146176), dose-dependently attenuated carrageenan-induced tactile allodynia. Likewise, IT delivery of 12-LOX metabolites of AA (12(S)-HpETE, 12(S)-HETE, HXA_3 or HXB_3) each triggered profound, persistent tactile allodynia after a single injection. In contrast, 12(S)-HpETE and HXA_3 produced modest, transient thermal hyperalgesia only at the highest dose tested. These data indicate that spinal 12-LOX products of AA are increased by peripheral inflammation and promote initiation of facilitated spinal nociceptive processing. The spinal cell types in which these metabolites are generated and the mechanism(s) by which they contribute to inflammatory pain hypersensitivity are currently under investigation.

Supported by NIH NS16541 and NIH GM064611.