

## POSTER SESSIONS

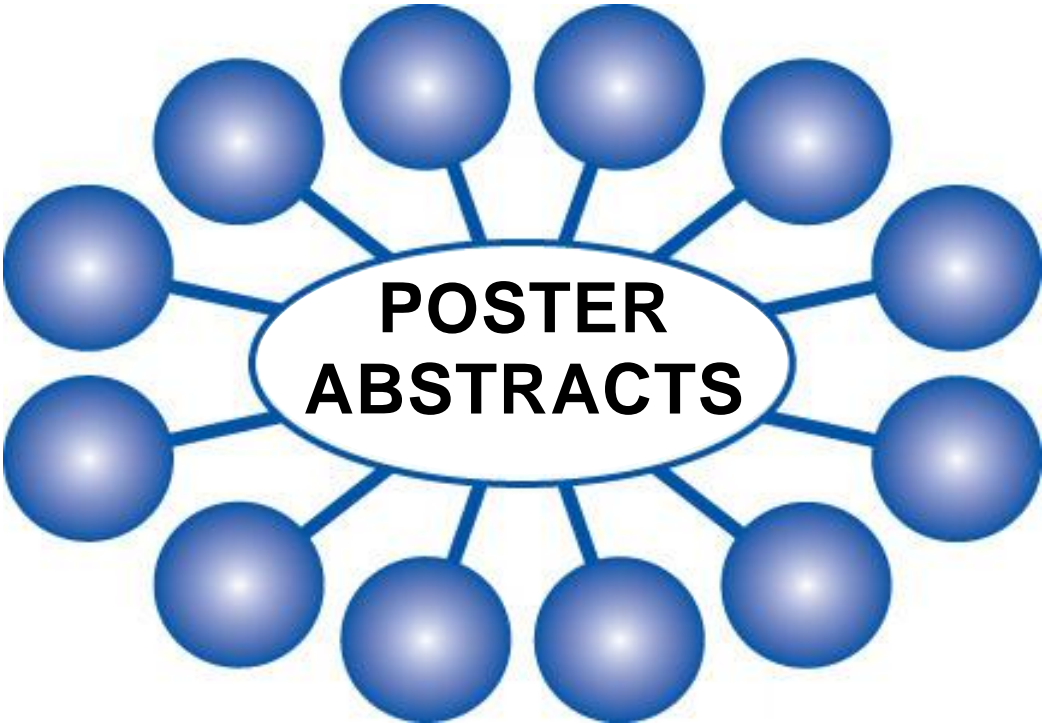
The LIPID MAPS meeting features two poster sessions with 44 posters. Of these, twelve were selected for lightning talks (see program) by the review committee, consisting of Susan A. Henry, Alfred H. Merrill, and Sarah Spiegel, and those selected are marked by an asterisk (\*) below. We received so many high-quality, relevant abstracts that making these selections was difficult. The poster presenters are also cross-referenced in the attendee list.

- 1. Site-directed mutagenesis studies of h15-LOX-1 and their effects on inhibitor potency**  
*Michelle Armstrong, Thomas Horn, Josh Deschamps, Chris van Hoorebeke, Cody Freedman, Theodore Holman (University of California, Santa Cruz, CA)*
- 2. A lipid-mediated proinflammatory response to ionizing radiation**  
*Evagelia C. Laiakis, Katrin Strassburg, Steven Lai, Rob J. Vreeken, Thomas Hankemeier, Rob Plumb, Albert J. Fornace Jr., Giuseppe Astarita (Georgetown University, Washington, DC)*
- 3. Supercritical fluid chromatography-mass spectrometry approaches for lipidomics profiling**  
*Anne E. Blackwell, Joseph L. Hedrick (Agilent Technologies, DE)*
- 4. Untargeted lipidomics reveals loss of key phospholipids and several lysophospholipids from vitamin E-deficient zebrafish brains \***  
*Jaewoo Choj, Scott W. Leonard, Katherine Kasper, Melissa McDougall, Jan F. Stevens, Robert L. Tanguay, Maret G. Traber (Linus Pauling Institute, OR)*
- 5. Elucidating the metabolism of omega-3 and omega-6 endocannabinoids by cardiac CYP2J2 epoxigenase in Nanodiscs \***  
*Daniel R. McDougale, William R. Arnold, Aditi Das (University of Illinois Urbana-Champaign, IL)*
- 6. The mammalian antimicrobial metabolite itaconate drives inflammation by inhibiting succinate dehydrogenase (SDH)**  
*Thekla Cordes, Ajit S. Divakaruni, Anne N. Murphy, Karsten Hiller, Christian M. Metallo (University of California, San Diego, CA)*
- 7. Post-sampling release of free fatty acids - effects of heat stabilization and methods of euthanasia**  
*Fredrik Jernerén, Jörg Hanrieder, Ylva Elias, Marcus Söderquist, Oskar Karlsson (Denator AB, Sweden)*
- 8. Establishment of a high throughput functional lipidomics platform for urine analysis in population health and precision medicine applications**  
*Fei Gao, Hannah Rockwell, Emily Chen, Justice McDaniel, Rangaprasad Sarangarajan, Niven Narain, Michael Kiebish (Berg LLC, MA)*
- 9. Mooreamide A: A cannabinomimetic lipid from the marine cyanobacterium *Moorea bouillonii* \***  
*Emily Mevers, Teatulohi Matainaho, Marco Allara, Vincenzo Di Marzo, William H. Gerwick (University of California, San Diego, CA)*
- 10. Tissue metabolomic analysis of bioactive lipids requires tissue fixation with focused microwave irradiation \***  
*Stephen A. Brose, Svetlana A. Golovko, Mikhail Y. Golovko (University of North Dakota, ND)*
- 11. Synthesis of  $\alpha$ -cholesteryl glycosides associated with *H. pylori*/host immune modulation**  
*Jacquelyn Gervay-Hague, Ryan Davis, Huy Nguyen (University of California, Davis, CA)*
- 12. Mass spectrometry imaging reveals lipid biochemistry aberrations in Myc-dependent lung tumours \***  
*Zoe Hall, Catherine Wilson, Trevor Littlewood, Gerard I. Evan, Julian L. Griffin (MRC Human Nutrition Research, UK)*

- 13. Synthesis and structure-function relationships of glycolipid surfactants**  
*Dillon J. Hanrahan, Ricardo Palos Pacheco, Laurel L. Kegel, Lajos Szabó, Evan M. Jones, Raina M. Maier, Jeanne E. Pemberton, Robin Polt (University of Arizona, AZ)*
- 14. Metabolomic changes associated with obesity and weight loss**  
*Angelina Hernández-Carretero, Natalie Weber, Johannes Fahrman, Michael La Frano, John Newman, Oliver Fiehn, Olivia Osborn (University of California, San Diego, CA)*
- 15. Ultrahigh-performance supercritical fluid chromatography/mass spectrometry - New approach for high-throughput lipidomic analysis**  
*Michal Holčápek, Miroslav Lída (University of Pardubice, Czech Republic)*
- 16. Mutations in *MBOAT7* links cortical polymicrogyria to lipid remodeling**  
*Anide Johansen, Damir Musaev, Hiroyuki Arai, Maha Zaki, Rami Abou Jamra, Matloob Azam, Tipu Sultan, Joseph G. Gleeson (The Rockefeller University, NY)*
- 17. Novel inhibitor of cytosolic group IVA phospholipase A2 (cPLA2 $\alpha$ ) ameliorate mesangial inflammation and kidney fibrosis development in diabetic nephropathy \***  
*Berit Johansen, Andrea Huwiler, Astrid J. Feuerherm, Mari Sæther (Norwegian University of Science and Technology, Norway)*
- 18. TMEM16F plays a crucial role in membrane lipid scrambling, hemostasis/thrombosis and lipid metabolism \***  
*Andrew Kim, Huanghe Yang, Tovo David, Amin Khalifeh-Soltani, Kamran Atabai, Shaun Coughlin, Lily Jan (University of California, San Francisco, CA)*
- 19. The endocannabinoid *N*-arachidonoyl dopamine (NADA) regulates endothelial prostanoid metabolism\***  
*Samira Khakpour, Alphonso Tran, Judith Hellman, Kevin Wilhelmsen (University of California, San Francisco, CA)*
- 20. Role of TNF signaling in de novo lipogenesis in the liver upon hypernutrition**  
*Ju Youn Kim, Michael Karin (University of California, San Diego, CA)*
- 21. Large scale lipid profiling of a human serum lipidome using a high resolution accurate mass LC/MS/MS approach**  
*Reiko Kiyonami, David A Peake, Xiaodong Liu, Yingying Huang (Thermo Fisher Scientific, CA)*
- 22. Involvement of sphingosine 1-phosphate on palmitate-induced insulin resistance of hepatocytes via the S1P<sub>2</sub> receptor subtype**  
*Lukasz Japtok, Susann Fayyaz, Thomas Homann, Stephanie Krämer, Burkhard Kleuser (University of Potsdam, Germany)*
- 23. Comprehensive separation and profiling method of phospholipids and neutral lipids using UPLC-Normal phase with binary gradient and MALDI-Q-ToF-MS/MS**  
*Adriana Lebkuchen, Fabio Mitsuo Lima, Luciano Ferreira Drager, Valdemir Melechco Carvalho, Karina Helena Morais Cardozo (University of Sao Paulo Medical School, Brazil)*
- 24. Targeting of heparin binding EGF-like growth factor (HB-EGF) inhibits atherosclerosis**  
*Sangderk Lee, Richard Lee, Mark J. Graham, Seon-gu Kim, Lihua Yang, Debra Rateri, Aldons J. Lusis, Judith A. Berliner (University of Kentucky, KY)*
- 25. Robust inhibitory effects of conjugated octadecatrienoic acids on a cyclooxygenase-related linoleate 10S-dioxygenase: Comparison with COX-1 and COX-2**  
*Zahra Mashhadi, William E. Boeglin, Alan R. Brash (Vanderbilt University, TN)*
- 26. Identification of 14,20-dihydroxy-docosaheptaenoic acid as a novel anti-inflammatory metabolite**  
*Shinnosuke Matsueda, Yoshiyuki Yokokura, Yosuke Isobe, Daisuke Urabe, Masayuki Inoue, Hiroyuki Arai, Makoto Arita (RIKEN Center for Integrative Medical Sciences (IMS) and University of Tokyo, Japan)*

- 27. Isotopomer spectral analysis of cholesterol biosynthesis *in vivo* reveals multiple tissue-specific, flux-dependent pathways \***  
*Matthew A. Mitsche, Jeffrey G. McDonald, Helen H. Hobbs, Jonathan C. Cohen (UT Southwestern Medical Center, TX)*
- 28. B4GALT6 regulates astrocyte activation during CNS inflammation \***  
*Lior Mayo, Sunia A. Trauger, Manon Blain, Meghan Nadeau, Bonny Patel, Jorge I. Alvarez, Ivan D. Mascanfroni, Ada Yeste, Pia Kivisäkk, Keith Kallas, Benjamin Ellezam, Rohit Bakshi, Alexandre Prat, Jack P. Antel, Howard L. Weiner, Francisco J. Quintana (Brigham and Women's Hospital, MA)*
- 29. Regulatory mechanisms of platelet-activating factor (PAF) production in macrophages and identification of a specific inhibitor for PAF synthetic enzyme (LPCAT2) by high-throughput screening \***  
*Ryo Morimoto, Hideo Shindou, Megumi Tarui, Takao Shimizu (National Center for Global Health and Medicine and University of Tokyo, Japan)*
- 30. Processing of a Complex Lipid Dataset for the NIST Inter-laboratory Comparison Exercise for Lipidomics Measurements in Human Serum and Plasma**  
*David A. Peake, Reiko Kiyonami, Yasuto Yokoi, Yukihiko Fukamachi, Yingying Huang (Thermo Fisher Scientific, CA)*
- 31. Bridging a gap between cytochrome *bc*<sub>1</sub> complex structure and function**  
*Pekka A. Postila, Oana Cramariuc, Sanja Pöyry, Karol Kaszuba, Ilpo Vattulainen, Marcin Sarewicz, Artur Osyczka, Tomasz Róg (Tampere University of Technology, Finland)*
- 32. Unsaturated phosphatidylinositols promote cotton fiber cell growth**  
*Gao-Jun Liu, Guang-Hui Xiao, Yong-Mei Qin, Yu-Xian Zhu (Peking University, China)*
- 33. Synthesis of analogs of *myo*-inositol to enable chemical biology studies**  
*Tanei Ricks, Michael D. Best (University of Tennessee-Knoxville, TN)*
- 34. Programming macrophage inflammation-resolution: The role of  $\omega$ -3 polyunsaturated fatty acids**  
*Ramil Sapinoro, Boris Krasnov, Mathew Lombardozi, Maria Caraballo (St. John Fisher College, NY)*
- 35. Apolipoprotein A-I Binding Protein regulates macrophage polarization in the tumor microenvironment**  
*Dina A. Schneider, Longhou Fang, Jack D. Bui, Yury I. Miller (University of California, San Diego, CA)*
- 36. Oxidized phospholipids regulate macrophage bioenergetics through a TLR2-dependent ceramide accumulation.**  
*Vlad Serbulea, Akshaya Meher, Badr Alsayab, Norbert Leitinger (University of Virginia, VA)*
- 37. Lipidomics profiles reveal altered sphingolipids in Fanconi Anemia cells**  
*Xueheng Zhao, Wujuan Zhang, Marion Brusadell, Paul F. Lambert, Susanne I. Wells, Kenneth D.R. Setchell (Cincinnati Children's Hospital Medical Center, OH)*
- 38. Regulation of the inflammatory and monocyte recruitment pathways in HAECs by PEIPC and EI**  
*Piao Jian Tan, Judith Berliner, James R. Springstead (Western Michigan University, MI)*
- 39. Alterations in lipid metabolism due to gravitational effects**  
*Magda Latorre-Esteves (University of Puerto Rico-Mayagüez Campus, Puerto Rico)*
- 40. Nano-LC-MS/MS for the quantitation of prostanoids in immune cells**  
*Dominique Thomas, Jing Suo, Thomas Ulshöfer, Klaus Scholich, Holger Jordan, Nastasja de Bruin, Gerd Geisslinger, Nerea Ferreirós (Goethe-University, Germany)*

- 41. Lipid Profiling in Rheumatoid Arthritis Patients Indicates Response to Rixuimab Treatment**  
*Shannon R. Sweeney, Alessia Lodi, Bo Wang, Surendar Tadi, Arthur Kavanaugh, David Boyle, Monica Guma, Stefano Tiziani (UT Austin, Texas)*
- 42. Lipidomic “deep profiling” reveals uncommon phosphorylated sphingolipids in different organisms**  
*Federico Torta, Pradeep Narayanaswamy, Markus R Wenk (National University of Singapore, Singapore)*
- 43. Identification of small subunit of serine palmitoyltransferase a as a lysophosphatidylinositol acyltransferase 1-interacting protein**  
*Natsumi Yamamori, Yusuke Hirata, Nozomu Kono, Hyeon-Cheol Lee, Takao Inoue, Hiroyuki Arai (University of Tokyo, Japan)*
- 44. Aldehyde dehydrogenases as a source of fatty acids in cancer stem cells \***  
*Rushdia Z. Yusuf, Borja Saez, Sanket Acharya, Dana S’aulis, Vionnie Yu, Dongjun Lee, Shrikanta Chattopadhyay, David B. Sykes, Ninib Baryawno, Francois Mercier, Colleen Cook, Jacqueline Bachand, Julien Cobert, Mildred Duvet, Elizabeth Scadden, Gohar Warraich, Hao Liu, John Doench, Michael Churchill, Sidharta Mukherjee, William Rizzo, David T. Scadden (Harvard Stem Cell Institute, MA)*



## 1. Site-directed mutagenesis studies of h15-LOX-1 and their effects on inhibitor potency

Michelle Armstrong, Thomas Horn, Josh Deschamps, Chris van Hoorebeke, Cody Freedman, Theodore Holman

Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA.

Mammalian lipoxygenases have been linked to inflammation, immune response, cardiovascular disease, asthma, different cancers, arthritis and stroke. Human reticulocyte 15-lipoxygenase-1, which catalyzes the dioxygenation of various polyunsaturated fatty acids, has been implicated in all of these important human diseases and is therefore a relevant target for investigation. Molecular modeling studies of human 15-lipoxygenase-1, based on the X-ray crystal structure of rabbit lipoxygenase, suggest specific residues in the active site that may be critical for inhibitor binding. We have generated six mutants (R402L, R404L, F414I, F414W, E356Q, Q547L) of h15-LOX-1 through site-directed mutagenesis to test this model. Steady state kinetic experiments confirmed that the mutations did not affect the catalytic activity of the isozymes. To test whether the active site mutations altered inhibitor binding, 3-pt IC<sub>50</sub> screens were conducted on all mutants against two previously published potent and selective h15-LOX-1 inhibitors varying in length: ML351 (short) and an ML094 derivative (long). The steady state inhibition kinetics were investigated only on the mutants that exhibited significant differences relative to wild type h15-LOX-1 in their IC<sub>50</sub> potencies. The mutations that affected inhibitor potency relative to wild type h15-LOX-1 were F414I, F414W and E356Q for both inhibitors, ML351 and ML094 derivative. The data implies that both inhibitors interact with the same residues in the active site. Structure activity relationships will be presented.

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## 2. A lipid-mediated proinflammatory response to ionizing radiation

Evagelia C. Laiakis<sup>1</sup>, Katrin Strassburg<sup>2</sup>, Steven Lai<sup>3</sup>, Rob J. Vreeken<sup>2</sup>, Thomas Hankemeier<sup>2</sup>, Rob Plumb<sup>3</sup>, Albert J. Fornace Jr.<sup>1</sup>, Giuseppe Astarita<sup>1,3</sup>

<sup>1</sup>Department of Biochemistry and Molecular & Cellular Biology, Georgetown University, Washington, DC; <sup>2</sup>Netherlands Metabolomics Centre, Leiden University, Leiden, the Netherlands; <sup>3</sup>Waters Corporation, Health Sciences, Milford, MA.

Exposure to ionizing radiation has dramatically increased in modern society, raising serious health concerns. The deleterious consequences of ionizing radiation exposure can result in cancer and non-cancer-related diseases, including cardiovascular diseases and cognitive decline. The molecular response to ionizing radiation, however, is still not completely understood. Here we screened mouse serum for metabolic alterations following an acute exposure to gamma radiation using a multi-platform, mass-spectrometry-based strategy. A global, molecular profiling revealed that mouse serum undergoes a series of significant molecular alterations following radiation exposure. We identified and quantified bioactive metabolites belonging to key biochemical pathways and low-abundance, oxygenated, polyunsaturated, fatty acids (PUFAs) in the two groups of animals. Exposure to gamma radiation induced a significant increase in the serum levels of ether phosphatidylcholines (PCs) while decreasing the levels of diacyl PCs carrying PUFAs. In exposed mice, levels of pro-inflammatory, oxygenated metabolites of arachidonic acid increased, whereas levels of anti-inflammatory metabolites of omega-3 PUFAs decreased. Our results indicate a specific serum lipidomic biosignature, which could be utilized as an indicator of radiation exposure and as novel target for therapeutic intervention. Monitoring such a molecular response to radiation exposure might have implications not only for radiation pathology but also for countermeasures and personalized medicine.

*For all poster abstracts:*

*Presenting author is underlined*

*# indicates equal effort contributed by first authors*

*\* indicates lightning talk selection*

### 3. Supercritical fluid chromatography-mass spectrometry approaches for lipidomics profiling

Anne E. Blackwell, Joseph L. Hedrick

Agilent Technologies, Wilmington, DE.

Given the complexity of biological matrices, long chromatography is often needed for lipidomics and metabolomics profiling experiments, resulting in extremely low throughput. When combined with the large numbers of samples needed for good statistics, profiling is time consuming and expensive. The use of supercritical fluid chromatography (SFC) offers significantly shorter runs than liquid chromatography while preserving or improving chromatographic separation for certain classes of lipids.

An SFC coupled to a QTOF mass spectrometer, with full flow sent from the SFC to the ionization source, was used for this work. Complementary chromatographic approaches were investigated. Samples analyzed include the lipid fractions of plasma and liver, cooking oils, and numerous pure chemical standards.

SFC-MS was found to be an excellent platform for lipidomics profiling experiments. The chromatographic reproducibility was good, and the approach had several unique strengths, the most obvious of which was speed. Triglycerides (TG) are a lipid class where SFC is particularly advantageous. A mixture of five TG standards (C:8-C:16) were analyzed in liver matrix by reverse-phase C18 LC and C18 SFC with a CO<sub>2</sub>-MeOH gradient. Using a 38 minute H<sub>2</sub>O-IPA LC gradient, the five TG standards elute over a 35 minute period. With a 13 minute SFC method, the same standards elute completely resolved within 5 minutes. Using the same two LC and SFC methods, liver lipids were annotated using the METLIN database. The majority of TGs eluted at high IPA in a 2 minute window by LC. With the 13 minute SFC method, annotated TGs elute over a 10 minute window – an improvement in both separation and speed. SFC with a C18 column shows excellent separation of TGs based on chain length and degrees of saturation, giving baseline separation of TGs that differ by a single double bond. Trends for other lipid classes will also be discussed.

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### 4. Untargeted lipidomics reveals loss of key phospholipids and several lysophospholipids from vitamin E-deficient zebrafish brains \*

Jaewoo Choi<sup>1,2</sup>, Scott W. Leonard<sup>1</sup>, Katherine Kasper<sup>1,4</sup>, Melissa McDougall<sup>1,4</sup>, Jan F. Stevens<sup>1,2,5</sup>, Robert L. Tanguay<sup>3,5</sup>, Maret G. Traber<sup>\*1,4,5</sup>

<sup>1</sup>Linus Pauling Institute; <sup>2</sup>College of Pharmacy; <sup>3</sup>Environmental and Molecular Toxicology; <sup>4</sup>College of Public Health and Human Sciences; <sup>5</sup>Environmental Health Sciences Center; Oregon State University, Corvallis, OR.

We hypothesized that brains from vitamin E-deficient (E-) zebrafish (*Danio rerio*) would undergo increased lipid peroxidation and loss of specific, highly polyunsaturated fatty acids. To test this hypothesis, brains from zebrafish fed for 9 months defined diets without (E-) or with added vitamin E (E+, 500 mg RRR- $\alpha$ -tocopheryl acetate/kg diet) were studied. Using an untargeted lipidomic analysis (MS/MSALL), 1-hexadecanoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (DHA-PC 38:6, PC 16:0/22:6) was the lipid that showed the most significant and greatest fold-differences between groups. Using LC-MS/MS for quantification, we found that DHA-PC 38:6 concentrations in extracts from dissected brains were lower in E- ( $4.3 \pm 0.6$   $\mu$ g/mg brain, mean  $\pm$  SEM) compared with E+ fish ( $6.5 \pm 0.9$ ,  $P=0.0413$ ). Using UPLC-TOF-MS/MS and lipidomics, 155 lipids in brain extracts were identified. Several lipid species, including four phospholipids (PLs) containing DHA, were lower ( $p < 0.05$ ) in E- zebrafish brains. Notably, DHA-PC 38:6 had the largest intensities of any of the lipids that were significantly different between groups. Moreover, hydroxy-DHA-PC was increased in E- brains ( $P=0.0341$ ) supporting the hypothesis of greater DHA peroxidation. More striking was the depletion in E- brains of nearly 60% of 19 different lyso-PL (combined  $P=0.0003$ ), which are critical for membrane PL remodeling. Thus, E-brains contained less DHA-PL, more hydroxy-DHA-PC and fewer lyso-PLs, suggesting that lipid peroxidation depletes membrane DHA-PC and homeostatic mechanisms to repair the damage result in lyso-PL depletion. DHA-PC 38:6 is a plasma biomarker of increased Alzheimer's disease risk in humans. Thus, inadequate vitamin E status in zebrafish results in a specific depletion in a critical brain PL that is associated with increased Alzheimer's disease risk in humans.

## 5. Elucidating the metabolism of omega-3 and omega-6 endocannabinoids by cardiac CYP2J2 epoxygenase in Nanodiscs \*

Daniel R. McDougle<sup>†,1</sup>, William R. Arnold<sup>‡</sup>, Aditi Das<sup>†,‡,2</sup>

<sup>†</sup>Department of Comparative Biosciences; <sup>‡</sup>Department of Biochemistry; <sup>1</sup>Medical Scholars Program; <sup>2</sup>Beckman Institute for Advanced Science and Technology and Department of Bioengineering, University of Illinois Urbana-Champaign, Urbana, IL.

The human body contains endocannabinoids that elicit similar effects as  $\Delta^9$ -tetrahydrocannabinol (THC), the principal component of cannabis, which produces similar psychoactive and anti-nociceptive effects. The two most well studied endocannabinoids include anandamide (AEA) and 2-arachidonoylglycerol (2-AG), which are derived from the omega-6 arachidonic acid (AA). Previously, we showed that both of these omega-6 endocannabinoids are substrates for metabolism by human heart cytochrome P450 2J2 epoxygenase (CYP2J2) to form novel AEA and 2-AG epoxides with distinct biological activity. Recently, the structural diversity of the endocannabinoid family has expanded with the discovery that the omega-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) also form endocannabinoids eicoasapentaenoic ethanolamide (EPEA) and docosahexaenoic ethanolamide (DHEA), respectively. Using LC-MS we demonstrate that EPEA and DHEA are metabolized by the epoxygenases such as CYP2J2 to produce novel EPEA and DHEA epoxides with unknown biological functions. In order to accomplish the detailed mechanistic studies, we use the model membranes of Nanodiscs to solubilize and stabilize membrane bound CYP2J2. Furthermore we elucidate the nuances of ligand-protein interactions using spectral titrations, small molecule ligand egress with stopped-flow spectroscopy, molecular dynamics simulations and LC-MS/MS. We further evaluate the biological effects on vasodilation using porcine coronary artery and angiogenesis using human microvascular endothelial cells (HMVEC). In summary, we successfully show that cardiovascular CYP2J2 epoxygenase can bind and metabolize the omega-6 and omega-3 endocannabinoids- AEA and 2-AG, DHEA and EPEA to form novel epoxides that elicit distinct biological activity.

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## 6. The mammalian antimicrobial metabolite itaconate drives inflammation by inhibiting succinate dehydrogenase (SDH)

Thekla Cordes<sup>1</sup>, Ajit S. Divakaruni<sup>2</sup>, Anne N. Murphy<sup>2</sup>, Karsten Hiller<sup>3</sup>, Christian M. Metallo<sup>1</sup>

<sup>1</sup>Department of Bioengineering, University of California, San Diego, La Jolla, CA; <sup>2</sup>Department of Pharmacology, University of California, San Diego, La Jolla, CA; <sup>3</sup>Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Luxembourg.

To remove and to protect itself against harmful stimuli, e.g. bacteria, cells activate their innate immune system resulting in drastic changes of cellular metabolism. Recently, the antimicrobial metabolite itaconate was identified in immune cells synthesized by *cis*-aconitate decarboxylase (CAD/IRG1) only under inflammatory conditions. Another recent study found succinate to be an important metabolite in the inflammatory response enhancing interleukin (IL)-1 $\beta$  production through stabilization of HIF. But how this metabolite accumulates during inflammation is poorly understood. Here, we demonstrate a new molecular mechanism for succinate accumulation: Under inflammatory conditions, macrophages produce itaconate to inhibit succinate dehydrogenase (SDH) resulting in increased succinate and IL-1 $\beta$  levels. Accumulation of succinate and itaconate in LPS-activated macrophages is well known, but a mechanistic link connecting accumulation of these metabolites has not been shown before. Performing *Irg1* gain- and loss- of function experiments to affect itaconate concentrations in immune and non-immune cells, we show for the first time a direct correlation between itaconate and succinate levels. We exposed cells to increasing extracellular itaconate concentrations resulting in increasing intracellular itaconate and succinate levels confirming the correlation between these metabolites. Although the inhibitory effect of itaconate on SDH has already been shown in-vitro in 1964 its role for succinate accumulation in mammals under inflammatory conditions has not yet been addressed and is unknown. To elucidate if itaconate inhibits SDH activity, we determined the influence of this metabolite on mitochondrial respiration and confirmed that itaconate specifically inhibits SDH while having no effect on other mitochondrial respiratory chain complexes. Since it has been proposed that succinate stabilizes HIF1 enhancing IL-1 $\beta$  production, we exposed macrophages to extracellular itaconate resulting in increased IL-1 $\beta$  levels suggesting a regulatory role of itaconate for IL-1 $\beta$  production. Our results clearly demonstrate the importance of itaconate as SDH inhibitor for intracellular succinate accumulation leading to increased IL-1 $\beta$  expression.



## 7. Post-sampling release of free fatty acids - effects of heat stabilization and methods of euthanasia

Fredrik Jernerén<sup>1</sup>, Jörg Hanrieder<sup>2,3</sup>, Ylva Elias<sup>4</sup>, Marcus Söderquist<sup>4</sup>, Oskar Karlsson<sup>5</sup>

<sup>1</sup>Department of Pharmacology, University of Oxford, Oxford, UK; <sup>2</sup>National Center for Imaging Mass Spectrometry, Gothenburg University and Chalmers University of Technology, Sweden; <sup>3</sup>Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg Sweden; <sup>4</sup>Denator AB, Uppsala Science Park, Uppsala, Sweden; <sup>5</sup>Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden.

**Introduction:** The quality of lipidomics research critically depends on recognition that the lipid pool is labile. Postmortem changes can cause accumulation of tissue free fatty acids (FFA). Here, we investigate the effects of heat stabilization, as well as the effects of different euthanasia methods, on FFA in the brain and liver using liquid chromatography tandem mass spectrometry. Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) activity was measured using the fluorometric EnzCheck PLA<sub>2</sub> Assay kit. **Methods:** Rat brains and livers were treated using different protocols. Animals were either instantly dissected from fresh tissue, or dissected after enzyme inactivation by heat stabilization of the intact tissues using Stabilizer T1 (Denator AB, Sweden). Tissue from other animals were kept at room temperature for 10 and 20 min before heat stabilization. Last group was directly heat stabilized and left at room temperature for 20 min. To compare the impact of euthanasia methods on FFA, rats were killed using three different protocols (decapitation, CO<sub>2</sub> inhalation, and pentobarbital injection). **Results:** Brain homogenates clearly demonstrated PLA<sub>2</sub> activity and time dependent post-sampling changes in the lipid pool of snap frozen non-stabilized tissue with a significant increase already at 2 min. Heat stabilization was shown to reduce PLA<sub>2</sub> activity and ex vivo lipolysis. Post-sampling effects induced a massive release of FFAs (up to 3700%). The ToF-SIMS imaging of brain sections further demonstrated that the heat stabilization inhibited postmortem lipolysis. The study shows that heat inactivation of enzymes responsible for the release of fatty acids can be utilized to minimize unintended consequences from post-sampling effects on experimental outcomes. Finally, our results show that it is important to take the method of euthanasia in consideration when conducting and comparing lipidomic studies. The studied FFA were decreased significantly group euthanized by pentobarbital injection compared with CO<sub>2</sub> inhalation or decapitation.

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## 8. Establishment of a high throughput functional lipidomics platform for urine analysis in population health and precision medicine applications

Fei Gao, Hannah Rockwell, Emily Chen, Justice McDaniel, Rangaprasad Sarangarajan, Niven Narain, Michael Kiebish

Berg LLC, Framingham, MA.

The development of robust enabling mass spectrometry platforms for the quantitation of diverse lipid species in human urine is of paramount importance to understanding metabolic homeostasis in normal and pathophysiological conditions. Urine represents a non-invasive biofluid for collection, which can capture distinct differences in a person's physiologic state, however, there currently is a lack of quantitative workflows or understanding to engage high throughput lipidomic analysis. Here in, we developed an MS/MS<sup>ALL</sup> shotgun lipidomic workflow as well as a High Resolution MS/MS microLC workflow for structural and oxidized lipid analysis, respectively, on the SCIEX 5600+ TripleTOF platform for urine analysis. This workflow has been employed to understand biofluid sample handling and collection, matrix stability, natural human variation over time, as well as to support molecular adaptive clinical trials at BERG. Utilization of 0.5 mL of urine for structural lipidomic analysis reproducibly quantified 700+ lipid molecular species from 20+ lipid classes. Analysis of 1 mL of urine routinely quantified 60+ oxidized lipid metabolites comprised of octadecanoids, eicosanoids, and docosanoids generated by lipoxygenase, cyclooxygenase, and Cytochrome P450 activities. In summary, this high throughput workflow has demonstrated impressive robustness and reproducibility for engagement of functional lipidomics analysis for population health and precision medicine strategies for future applications.

## 9. Mooreamide A: A cannabinomimetic lipid from the marine cyanobacterium *Moorea bouillonii* \*

Emily Mevers<sup>1,2</sup>, Teatulohi Matainaho<sup>3</sup>, Marco Allara<sup>4</sup>, Vincenzo Di Marzo<sup>4</sup>, William H. Gerwick<sup>2,5</sup>

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Marine life forms have been a rich source of structurally novel and highly bioactive lipid species, including prostaglandins, diverse oxylipins, and more recently, acyl amides structurally resembling anandamide. In this regard, bioassay-guided fractionation of a collection of *Moorea bouillonii* from Papua New Guinea led to the isolation of a new alkyl amide, mooreamide A, along with the cytotoxic apratoxins A-C and E. The planar structure of mooreamide A was elucidated by NMR spectroscopy and mass spectrometry analysis. Structural homology between mooreamide A and the endogenous cannabinoid ligands, anandamide and 2-arachidonoyl glycerol, inspired its evaluation against the neuroreceptors CB<sub>1</sub> and CB<sub>2</sub>. Mooreamide was found to possess relatively potent and selective agonist activity to CB<sub>1</sub> ( $K_1 = 0.47 \mu\text{M}$  and  $K_1 > 25 \mu\text{M}$ , respectively). This represents the most potent marine-derived CB<sub>1</sub> agonist described to date and adds to the growing family of marine metabolites that exhibit cannabinomimetic activity.

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## 10. Tissue metabolomic analysis of bioactive lipids requires tissue fixation with focused microwave irradiation \*

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A number of tissue bioactive lipids, including eicosanoids and endocannabinoids, are dramatically and instantly increased during tissue handling, thus altering quantification results for their basal levels. We, and others, have demonstrated that high energy focused microwave irradiation (MW) is a safe method to prevent a rapid, 30-fold increase in brain and 150-fold increase in kidney eicosanoid mass within seconds upon brain extraction from the body. In addition, MW is also required to measure endocannabinoid N-arachidonylethanolamine concentration in rat brain. However, this technique has not been previously validated and/or applied to measure endogenous levels of another bioactive endocannabinoid, 2-arachidonoylglycerol (2-AG), and other monoacylglycerols.

In the present study, we demonstrated a dramatic 110 fold increase in brain 2-AG levels within 30s after rat decapitation. MW prevented post-mortem 2-AG increase and didn't affect stability of endogenous and exogenous 2-AG in brain tissue. Importantly, a fast freezing of the animal in liquid nitrogen didn't prevent post-mortem 2-AG increase, probably because of 2-AG induction during tissue extraction. We also demonstrated that MW is required to measure 2-AG alterations under brain ischemia/injury. In addition to a significant, a ~100 fold difference in the basal 2-AG levels between MW and not-MW brains, 5 min of global brain ischemia resulted in a 2.2-fold increase in 2-AG when analyzed with MW fixation, while not-MW brains demonstrated a 2.0-fold decrease upon ischemia. Similar results were obtained for other monoacylglycerols. In summary, MW is a safe and required technique to quantify tissue levels of a number of bioactive lipids including eicosanoids and endocannabinoids. *Grant support: NIH Grants 5R01AG042819-03, 5P30GM103329-03*

## 11. Synthesis of $\alpha$ -cholesteryl glycosides associated with *H. pylori*/host immune modulation

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Three classes of  $\alpha$ -cholesterylglucosides (CG) have recently been isolated from *H. pylori*. The simplest form is designated  $\alpha$ CG, the analogue acylated with tetradecanoic acid at the C6-hydroxyl of glucose is referred to as  $\alpha$ CAG, and derivatives having a phosphatidyl group at the C6-hydroxyl position are named  $\alpha$ CPG. The lipid portion on the phosphatidyl glycerol varies in composition as identified from lyso-CPG analogues isolated from *H. pylori*. Together, these  $\alpha$ -sterolglycosides make up approximately 25% of the total lipid content of *H. pylori* and are a distinguishing feature of these bacteria. These compounds have also been shown to modulate host immunity suggesting a symbiotic relationship between microbe and man. Up until now, biological studies have mostly relied upon natural sources of these compounds, which are not easily separable making it difficult to study their roles independently. Recently, the Gervay-Hague lab has succeeded in making  $\alpha$ CG,  $\alpha$ CAG, and three  $\alpha$ CPG analogs through implementation of step-economical syntheses. Using a transient protecting group strategy along with chemoenzymatic approaches these important glycolipids are now available to the biological community for further study.

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## 12. Mass spectrometry imaging reveals lipid biochemistry aberrations in Myc-dependent lung tumours \*

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Myc is a highly pleiotropic transcription factor, responsible for the regulation of cell cycle control and whose expression is frequently elevated in human cancers. Whilst Myc is responsible for controlling a wide variety of functions in healthy cells, its oncogenic activation results in aberrations of these processes, leading to uncontrolled cell proliferation. We use a novel transgenic mouse model of K-Ras<sup>G12D</sup> driven lung adenocarcinoma with reversible activation of Myc, to study the lipid changes occurring during tumorigenesis. We characterised the spatial and temporal changes in lipid biochemistry occurring in lung with matrix assisted laser desorption mass spectrometry imaging (MALDI-MSI). We find that healthy lung tissue is characterised predominantly by phosphocholine (PC) 32:0 and PC 32:1, the major lipid components of pulmonary surfactant fluid. In contrast, tumour regions were characterised by increased signalling molecules including phosphoinositols (PI), arachidonic acid and arachidonic acid-containing PCs and PIs, with depleted palmitic acid and phosphatidylglycerols relative to non-tumour tissue. Both arachidonic acid and PIs are thought to be involved in cancer metabolism, with the former involved in the COX-2 pathway and inflammation response, and the latter a class of important secondary messengers. Interestingly, differences were observed for distributions of salt adducts, with a higher proportion of sodiated lipids present in healthy tissue, and potassiated lipids in tumour regions. This may be a consequence of dysregulation of potassium channels in cancerous cells. Switching off Myc resulted in reduced proliferation and apoptosis, and a decreased arachidonic acid content was observed by MSI and liquid extraction surface analysis mass spectrometry (LESA-MS). Our results highlight the tremendous potential of mass spectrometry for imaging tumours whilst simultaneously probing their metabolic features.

### 13. Synthesis and structure-function relationships of glycolipid surfactants

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Recently developed gram-scale synthetic preparations of bacterial rhamnolipids has allowed for structural modifications of the native structures, incorporating a wide range of carbohydrate headgroups and lipid chains. This enables the development of a glycolipid library designed to better understand the effects of structural changes on bulk surfactant properties. Indium(III) and Bismuth(III) Lewis acid catalysts were applied to provide glycosides in a “green” approach to multigram glycolipid synthesis. These catalysts have reduced waste, environmental impact, and reaction times using relatively inexpensive materials in a variety of glycoside syntheses. Catalytic glycosylation of lipophilic alcohols and thiols with various mono- and disaccharide peracetates, followed by Zemplén deacylation, provided structurally diverse glycolipids in moderate to high yield. In synthetic two-tailed rhamnolipids, variations in length of the lipid tail distal to the glycoside show relationships to surface and aggregation behavior. Du Noüy ring tensiometry, dynamic light scattering (DLS), steady state fluorescence quenching, and diffusion-ordered spectroscopy (DOSY) have been used to determine surface activity and aggregation properties such as critical micelle concentration (CMC), micelle size, and aggregation number of glycolipid variants. Here we describe the synthesis of structurally diverse glycolipids and their characterization as biosurfactants.

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### 14. Metabolomic changes associated with obesity and weight loss

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**Introduction:** A chronic imbalance between energy intake and energy expenditure results in excess fat deposition and the development of obesity. Obesity induces changes at the gene expression, protein and metabolite levels in peripheral tissues as well as the central nervous system that drive the development of associated co-morbidities including insulin resistance, diabetes and cardiovascular disease. In these studies we investigated the metabolic changes induced in mice by obesity after chronic high fat diet feeding and after weight loss. We performed both untargeted and targeted (endocannabinoids/ oxylipins/ceramide) metabolomic analysis of insulin target tissues from lean, obese and lean mice that were previously obese. **Methods:** Male C57BL6 mice were divided into three groups. One group was fed a high fat (HF) diet (60% calories from fat, Research Diets) for 18 weeks to induce obesity. A lean group was fed a low fat (LF) diet (10% calories from fat, Research diets) for 18 weeks. A third group was fed the HF diet for 9 weeks and then switched (SW) to the LF diet for a further 9 weeks to induce weight loss. At the end of the study mice were sacrificed and tissues (liver, adipose, muscle, hypothalamus and plasma) collected for metabolomic studies. **Preliminary Results:** We have identified changes in metabolites that occur in the key insulin target tissues, including adipose, liver, muscle, hypothalamus in the obese state that are reversed upon weight loss. These reversible metabolites are likely associated with insulin resistance in the obese state and insulin sensitivity in the lean state. Furthermore, we have identified metabolites that are induced by obesity that do not reverse upon weight loss. We hypothesize that metabolites that are irreversibly changed in obesity may play a role in driving weight regain and may provide important targets for therapeutic intervention in maintenance of weight loss.

## 15. Ultrahigh-performance supercritical fluid chromatography/mass spectrometry - New approach for high-throughput lipidomic analysis

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New analytical approach for high-throughput and comprehensive lipidomic analysis of biological samples using ultrahigh-performance supercritical fluid chromatography (UHPSFC) with electrospray ionization-mass spectrometry (ESI-MS) is presented as an alternative approach to established shotgun MS or HPLC/MS. The lipid class separation is performed using UHPSFC method based on 1.7  $\mu\text{m}$  particle bridged ethylene hybrid silica column with the gradient of methanol – water – ammonium acetate mixture as a modifier. All parameters of UHPSFC conditions are carefully optimized and their influence on the chromatographic behavior of lipids is discussed. The final UHPSFC/ESI-MS method enables a fast separation of 30 nonpolar and polar lipid classes within 6 minute analysis covering 6 main lipid categories including fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterols and prenols. Individual lipid species within lipid classes are identified based on positive and negative-ion full-scan and tandem mass spectra measured with high mass accuracy and high resolving power. Applications of UHPSFC/ESI-MS for the analysis of various biological samples including human cancer tissues will be presented with the quantitation of >20 lipid classes containing >400 lipid species. The method is fully validated for the quantitative analysis of lipid species in biological samples using internal standards for each lipid class. This high-throughput, comprehensive and accurate UHPSFC/ESI-MS method is suitable for the lipidomic analysis of large sample sets in the biomarker discovery research.

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## 16. Mutations in *MBOAT7* links cortical polymicrogyria to lipid remodeling

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The activity of LPIAT1 was first proposed in phospholipid remodeling as a part of the *Lands' cycle*. The *MBOAT7* gene was identified by Lee et al. in 2007 with the help of a *C. elegans* screening system to identify genes required for use of exogenous polyunsaturated fatty acids. The membrane-bound O-acyltransferase (MBOA) domain-containing enzyme facilitates the transfer of fatty acyls to lysophosphatidylinositol. LPIAT1 is specific for arachidonyl-containing acyl-donors and regulates the arachidonic acid content in phosphatidylinositol (PI). In mice LPIAT1 deficiency results in altered neuronal migration, differentiation and neurite outgrowth, producing neuronal atrophy and failed cortical lamination. LPIAT1 is required for maintaining physiological levels of phosphoinositides (PtdIns) and PtdInsP<sub>2</sub> in mice. Calcium stimulation accompanied by LPIAT1 and LPCAT3 inhibition causes a large increase in eicosanoid production in zebrafish myeloid cells. We performed whole exome sequencing (WES) of families displaying presumed autosomal recessive intellectual disability (AD), epilepsy and cortical polymicrogyria (PMG), which revealed inframe and frameshift deletions in *MBOAT7* in four consanguineous families. *MBOAT7* encodes a lysophosphatidylinositol acyltransferase (LPIAT1) and has 8 exons (7 of which are coding), where alternative splicing results in 4 transcripts. Families 2497 and 2920 had a common founder deletion of 22 bp located in exon 3, which affects one of the 5 transmembrane domains. Family 2631 had an inframe deletion of 21 bp located in exon 6, which affects the acyltransferase domain. The last family had a single nucleotide deletion in exon 5. Exons 3, 5 and 6 are spliced into all 4 transcripts. We propose that LPIAT1 enzyme deficiency leads to decreased or delayed removal of free arachidonic acid (AA), or a decreased production of AA-containing PI (AA-PI). An accumulation of free AA may also cause cellular accumulation of the pro-inflammatory eicosanoids, metabolites of AA. Accumulation of pro-inflammatory metabolites early in development might lead to inflammation in the human embryonic brain. LPIAT1 deficiency might also lead to deficiency of AA-PI, and possibly PtdIns, deficiency.

## 17. Novel inhibitor of cytosolic group IVA phospholipase A2 (cPLA2 $\alpha$ ) ameliorate mesangial inflammation and kidney fibrosis development in diabetic nephropathy \*

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**Background:** Diabetic nephropathy (DN) remains the most common cause of end-stage renal disease worldwide. Current standard of DN therapy involves stringent blood pressure control via blockade of the renin-angiotensin system and control of hyperglycemia, but with no effect on kidney fibrosis. Despite these strategies, DN is still seen to progress relentlessly. The complex series of pathological events occurring in DN is largely regulated via glomerulonephritis and excessive production of pro-inflammatory cytokines, the most prominent being interleukin 1beta (IL-1 $\beta$ ). Upon activation by IL-1 $\beta$ , mesangial cells respond with glomerulonephritis: (i) increased proliferation; (ii) increased mediator production, including cytokines, chemokines, NO, superoxide radicals and PGs; and (iii) increased extracellular matrix production. IL-1 $\beta$  is a potent inducer of mesangial cell cPLA2 $\alpha$  [1]. **Objectives:** To determine if the cPLA2 $\alpha$  inhibitor AVX002 reduces parameters of DN progression and severity in a rat model of streptozotocin (STZ) induced DN. **Methods:** STZ-induced DN in rats. Oral daily administration of Losartan (30 mg/kg) was the positive therapeutic comparator. Intraperitoneal injection of AVX002. **Results:** DN was evident in STZ-treated animals, and disease severity was significantly reduced with Losartan. Treatment with cPLA2 $\alpha$  inhibitor AVX002 significantly reduced DN in dose dependent manner as measured by urine protein secretion. As opposed to Losartan, which had no effect on kidney fibrosis development, AVX002 potently and significantly also reduced fibrosis development. Thus, suggesting that the anti-inflammatory effect of AVX002 is beneficial on kidney fibrosis.

**Conclusion:** The cPLA2 $\alpha$  inhibitor AVX002 significantly and potently improved the clinical course of DN, both measured as urine protein secretion and kidney fibrosis development. These data suggest that cPLA2 $\alpha$  inhibitors, such as AVX002, are potent disease modifying therapeutics and have the potential to be developed as novel, specific, small molecule, anti-DN human therapeutics.

<sup>1</sup>Huwiler, A. et al. *Br. J. Pharmacol.* 2012; **167**:1691.

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## 18. TMEM16F plays a crucial role in membrane lipid scrambling, hemostasis/thrombosis and lipid metabolism \*

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The asymmetric lipid composition at the membrane bilayer is maintained by the activity of flippases and floppases. A third category of membrane lipid transporters called lipid scramblases collapse this lipid asymmetry, which is the initiating trigger for several intercellular processes, for example blood coagulation and apoptotic clearance. TMEM16F of the TMEM16 family of Ca<sup>2+</sup>-activated Cl<sup>-</sup> channels (CaCCs) has been linked to a rare human bleeding disorder called Scott syndrome that is associated with deficiencies in Ca<sup>2+</sup>-dependent membrane lipid scrambling. We previously reported that TMEM16F knockout mice exhibit bleeding defects and protection in an arterial thrombosis model. These defects were associated with a platelet deficiency in Ca<sup>2+</sup>-dependent phosphatidylserine exposure leading to decreased recruitment and activation of clotting factors and reduction in thrombin generation. Notwithstanding TMEM16F's important platelet procoagulant function, its role throughout the body where it is ubiquitously expressed is unclear. Here, we report a role of membrane lipid scramblase in lipid metabolism as TMEM16F knockout mice show reduced ability to take up free fatty acids by white adipocytes, which correlates to a reduction in total body fat percentage and total body weight compared to wild-type mice. Although the exact mechanism of TMEM16F activation remains unknown, this study thus identifies TMEM16F scramblase activity as a potential target for antithrombotic therapy as well as obesity and other metabolic diseases.

## 19. The endocannabinoid *N*-arachidonoyl dopamine (NADA) regulates endothelial prostanoid metabolism \*

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**Background:** Endocannabinoids are endogenous, arachidonic acid-derived lipid mediators with immunomodulatory activities. We have previously shown that the endocannabinoid *N*-arachidonoyl dopamine (NADA) reduces endothelial cell (EC) activation induced by inflammatory agonists (Wilhelmsen, *JBC* 2014), however the precise mechanism of this activity remains unclear. Endothelial cells (ECs) are centrally involved in the pathogenesis of organ injury in acute inflammatory disorders since they regulate leukocyte trafficking and vascular barrier function. We hypothesize that NADA's immunomodulatory activity in the endothelium is dependent upon its regulation of other arachidonic acid-derived lipids, including the prostanoids. **Methods:** To determine the effects of endocannabinoids on arachidonic acid metabolism, a qPCR array was performed using cDNA derived from primary human lung microvascular ECs (HMVEC-Lung) treated with vehicle or LPS in the presence or absence of the endocannabinoids anandamide, 2-arachidonoylglycerol, and NADA. Genes showing significant changes in expression (>3-fold) were verified by immunoblot. Prostanoid levels were quantified in supernatants and cell culture lysates by ELISA after similar treatments. To determine if prostanoids contribute to the immunomodulatory activity of NADA, HMVEC-Lung were treated with NADA and LPS in the presence of the COX-1/2 inhibitor indomethacin, and cytokine levels were quantified in culture supernatants by ELISA. **Results:** In contrast to other endocannabinoids, NADA reduces LPS-induced prostacyclin (PGI<sub>2</sub>), thromboxane A<sub>2</sub> (TXA<sub>2</sub>), and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production by HMVEC-Lung. In addition, NADA is sufficient to dramatically upregulate prostaglandin D<sub>2</sub> (PGD<sub>2</sub>). Furthermore, NADA induces a robust upregulation of COX-2 expression, while inhibition of the COX enzymes reversed the NADA-mediated decrease in cytokine secretion. **Conclusion:** Our results suggest that the immunomodulatory activity of NADA depends upon its regulation of COX-2-derived lipid mediators. By elucidating the mechanism by which endocannabinoids modulate prostanoid metabolism in ECs, we hope to better understand the therapeutic potential of manipulating the endocannabinoid system in acute inflammatory disorders characterized by endothelial activation.

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## 20. Role of TNF signaling in *de novo* lipogenesis in the liver upon hypernutrition

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Obesity-associated tissue inflammation is thought to be an important cause of decreased insulin sensitivity and glucose intolerance that are the key pathophysiological characteristics of type 2 diabetes and non-alcoholic steatohepatitis (NASH).

In this study, we will investigate the hypothesis that TNF activates SREBP1 through caspase2 (Casp2) and this can lead to elevated *de novo* lipogenesis in liver. Casp2 is highly activated in HFD-fed *MUP-uPA* mice, which develop NASH, and its activation is largely reduced upon TNFR1-ablation (i.e. in *Tnfr1*<sup>-/-</sup>*MUP-uPA* mice). Moreover, activation of SREBP1 in HFD-fed *MUP-uPA* mice is completely blocked in Casp2-ablated *MUP-uPA* mice, suggesting that Casp2 cleaves/activates SREBP1 in response to TNFR1 signaling in HFD-fed *MUP-uPA* mice. Noteworthy, we found that Casp2 is also activated in livers of patients with NASH but not in those with milder non-alcoholic fatty liver disease (NAFLD), suggesting that Casp2 plays a significant role in the pathogenesis of inflammation-associated metabolic diseases. To better understand Casp2 function, we will use genetically engineered mice, molecular biological studies, and metabolic studies to investigate the mechanism of SREBP1 activation by Casp2 and evaluate the mechanism and pathogenic impact of Casp2-mediated SREBP1 activation in NASH progression and exacerbation of liver metabolic complications.

This study will provide a new insights to the molecular mechanisms by which TNF signaling stimulates SREBP1 processing via Casp2, leading to aberrant lipid accumulation during NASH progression and offer new opportunities for development of drugs that ameliorate the pathogenesis of NASH, one of the most severe outcomes of the metabolic syndrome and type 2 diabetes.

## 21. Large scale lipid profiling of a human serum lipidome using a high resolution accurate mass LC/MS/MS approach

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HPLC-MS platforms are increasingly used for large scale lipid profiling experiments aimed to discover new biomarkers for early disease diagnose. It is critical that the adapted LC/MS platform offers the capability to separate and identify the isobars and isomers from the biological lipid extracts. C30 HPLC columns with small particle size uniquely offer high shape selectivity for separation of structurally related isomers and provide improved lipid isomer separation efficiency compared to C18 columns. Here we report that thousands of lipid molecules from human serum sample were simultaneously identified and quantified using a new C30 column and a quadrupole Orbitrap HR/AM MS platform.

Human serum samples provided by NIST were collected from three types of donors with different controlled diets. The total lipids were extracted from the human serum samples by using organic solvents of chloroform, methanol and water. Multiple internal lipid standards which represent typical lipid classes were spiked into the serum samples prior to the lipid extraction. The profiling of total lipid extracts from the human serums was performed by UHPLC-MS using a C30 prototype column (2.1x250mm, 1.9 $\mu$ m) and the Thermo Scientific™ Q Exactive™ HF mass spectrometer. MS and MS/MS data were obtained at 120,000 and 30,000 mass resolving power, respectively. Each serum sample was run in triplicate with positive mode and negative mode ionization. The HPLC gradient was 60:40 acetonitrile/water to 90:10 IPA/acetonitrile (0.1% formic acid, 10mM ammonium formate) in 58 min. The flow rate was 200 $\mu$ L/min. Thermo Scientific™ LipidSearch™ version 4.1 software was used for lipid identification and quantitation.

The combination of increased lipid isomer separation offered by the C30 column and faster scan speed and high resolving power offered by the Q Exactive HF MS enabled very high lipid identification coverage to be achieved from the total lipid extract of the human serum samples.

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## 22. Involvement of sphingosine 1-phosphate on palmitate-induced insulin resistance of hepatocytes via the S1P<sub>2</sub> receptor subtype

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Glucolipotoxic stress has been identified as a key player in the progression of pancreatic  $\beta$ -cell dysfunction contributing to insulin resistance and the development of type 2 diabetes mellitus (T2D). It has been suggested that bioactive lipid intermediates, formed under lipotoxic conditions, are involved in these processes. Here, we show that sphingosine 1-phosphate (S1P) levels are not only increased in palmitate-stimulated pancreatic  $\beta$ -cells but also regulate  $\beta$ -cell homeostasis in a divergent manner. Although S1P possesses a pro-survival effect in  $\beta$ -cells, an enhanced level of the sphingolipid antagonizes insulin-mediated cell growth and survival via the S1P<sub>2</sub> receptor subtype followed by an inhibition of Akt-signalling.

In an attempt to investigate the role of the S1P/S1P<sub>2</sub> axis *in vivo*, the NZO diabetic mouse model, characterized by  $\beta$ -cell loss under high fat diet conditions, was used. The occurrence of T2D was accompanied by an increase of plasma S1P levels. To examine whether S1P contributes to the morphological changes of islets via S1P<sub>2</sub>, the receptor antagonist JTE-013 was administered. Most interestingly, JTE-013 rescued  $\beta$ -cell damage clearly indicating an important role of the S1P<sub>2</sub> receptor subtype in  $\beta$ -cell homeostasis. Therefore, the present study provide a new therapeutic strategy to diminish  $\beta$ -cell dysfunction and the development of T2D.



## 23. Comprehensive separation and profiling method of phospholipids and neutral lipids using UPLC-Normal phase with binary gradient and MALDI-Q-ToF-MS/MS

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Lipids play many important roles in cell, tissue and organ physiology, and recent works linked specific lipids with diseases such as cancer, Alzheimer's disease, inflammation, cardiovascular diseases, among many others. Lipid analysis is challenging, but promising, and there is much to be uncovered about the human lipidome. The full range of lipids and lipid classes cannot be separated easily in a single run, requiring extensive work in method development. Aiming this issue, we have developed a protocol for phospholipid class and neutral lipid separation looking for an easier method than those reported in the literature until the present day. The method was conducted using standards and organic extracts from typical biological samples including, bovine brain, heart and liver for method development, optimization and reproducibility, and human plasma (MTBE extraction) for biological application. The lipid classes were separated using normal-phase chromatography with a binary gradient on the AQUITY UPLC I-Class (Waters) and collected using Gilson 215 Liquid Handler. The collected fractions were analyzed by MALDI-Q-ToF instrument (Synapt G1, Waters) by data-dependent acquisition and MS/MS mode. Almost all lipid classes were separated using this normal-phase UPLC method and they include TG, Ceramides, PA, PE, LPE, PI, PC, SM, LPC. However, PS and Cardiolipins were not detected in single fraction and coelute with PI and PA. The feature's number detected in each plasma fraction, were: 191 (Fraction-1), 22 (Fraction-2), 308 (Fraction-3), 562 (Fraction-4), 543 (Fraction-5), 240 (Fraction-6), 641 (Fraction-7), 1448 (Fraction-8) and 443 (Fraction-9). This number of features is greater than reported in the literature when analyzed with ESI-MS/MS. The use of normal-phase chromatography and MALDI-Q-ToF-MS/MS analyses resulted in a simple, reproducible and fast method for lipids separation and identification.

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## 24. Targeting of heparin binding EGF-like growth factor (HB-EGF) inhibits atherosclerosis

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**Objective:** Heparin-binding EGF-like growth factor (HB-EGF) is a ligand for EGF receptor (EGFR) that is involved in inflammatory gene expression in various cells. HB-EGF levels in the plasma or lesion area were associated with lesion progress in the vessel wall. In this study, we examined the effects of HB-EGF targeting using antisense oligonucleotide (ASO) in the development of atherosclerosis. **Methods and Results:** With or without pretreatment of 8 week high fat diet feeding (HFD) feeding, LDLR null mice were injected with control and HB-EGF ASOs by weekly for 12 weeks under fed a fat-enriched diet (21% fat; 0.2% cholesterol). Compared with the control ASO injected group, the HB-EGF ASO injected group showed significant repression of the initiation of lesion and progress of preformed atherosclerosis. The mice injected with HB-EGF ASO showed significant reduction of plasma very low-density lipoprotein (VLDL) and cholesterol levels. In the liver tissue, the HB-EGF ASO injection downregulated inflammatory gene expressions but enhanced accumulation of lipid droplets compared with control ASO injected mice group. **Conclusions:** This study indicates that HB-EGF is a key mediator for the development and progress of atherosclerosis and targeting if HBEGF is an efficient approach to prevent atherosclerosis. Downregulation of inflammatory gene expression in the liver and decrease of VLDL level in the blood stream appear to contribute to the protective effects of HB-EGF ASO in in vivo condition.

## 25. Robust inhibitory effects of conjugated octadecatrienoic acids on a cyclooxygenase-related linoleate 10S-dioxygenase: Comparison with COX-1 and COX-2

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There are numerous investigations on the bio-activity of conjugated fatty acids with many reports of their anti-inflammatory, anti-cancer, and anti-atherosclerotic activities. The conjugated linolenic acids (CLNA) constitute a small percentage of fatty acids in the typical human diet, although up to 80% of the fatty acids in certain fruits such as pomegranate. In the course of studying a bacterial fatty acid dioxygenase (*Nostoc* linoleate 10S-DOX), we detected strong inhibitory activity in a commercial sample of linoleic acid that we could attribute to specific CLNA isomers. The *Nostoc* 10S-DOX is an ancient relative of the mammalian fatty acid dioxygenases, the cyclooxygenases COX-1 and COX-2. It has relatively low sequence identity to mammalian cyclooxygenases (~27%), yet it retains the key catalytic residues. Our investigation reveals that two conjugated triene isomers,  $\beta$ -eleostearic (9*E*,11*E*,13*E*-18:3) and  $\beta$ -calendic acid (8*E*,10*E*,12*E*-18:3), were responsible for that striking inhibition with  $K_i$  of ~49 nM and ~125 nM, respectively, the most potent among eight CLNA tested. We also examined the effects of all eight CLNA on the activity of COX-1 and COX-2. In case of COX-1 only three of the eight at 10  $\mu$ M concentrations produced 50% or more inhibition; Jacaric acid (8*Z*,10*E*,12*Z*-18:3) and its 12*E* isomer, 8*Z*,10*E*,12*E*-18:3, strongly inhibit the activity of cyclooxygenase-1 with  $K_i$  of ~1.69 and ~1.11  $\mu$ M, respectively. By contrast, COX-2 was  $\leq$  30% inhibited at 10  $\mu$ M concentrations of the conjugated linolenic acids. Identifying the activities of the naturally occurring fatty acids is of interest in terms of understanding their interaction with the enzymes, and for explaining the mechanistic basis of their biological effects.

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## 26. Identification of 14,20-dihydroxy-docosahexaenoic acid as a novel anti-inflammatory metabolite

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Docosahexaenoic acid (DHA) exhibits anti-inflammatory activity related to some of its oxygenated metabolites, such as D-series resolvins, protectin and maresin. Here, we analysed the lipids in inflammatory exudates using liquid chromatography-tandem mass spectrometry and identified a novel DHA metabolite, 14,20-dihydroxy-DHA (14,20-diHDHA) and showed that it is biosynthesized by eosinophils through the 12/15-lipoxygenase pathway. The stereochemical structure of the major 14,20-diHDHA isomer, which was endogenously biosynthesized by eosinophils, was assigned as 14*S*,20*R*-diHDHA using chemically synthesized stereoisomers. Nanogram doses of 14,20-diHDHA displayed a potent anti-inflammatory action by limiting neutrophil infiltration in zymosan-induced peritonitis. The *in vivo* formation and anti-inflammatory activity of 14,20-diHDHA may contribute to the anti-inflammatory and tissue-protective effects of DHA.

## 27. Isotopomer spectral analysis of cholesterol biosynthesis *in vivo* reveals multiple tissue-specific, flux-dependent pathways \*

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The final stage of cholesterol biosynthesis involves the conversion of lanosterol to cholesterol. Two alternative pathways, known as the Bloch and Kandutsch-Russell (K-R) pathways, have been proposed for this conversion. To assess the relative utilization of these pathways *in vivo* we used LC-MS/MS methods developed by the Lipid MAPS consortium, deuterium water (D<sub>2</sub>O) incorporation, and isotopic spectral analysis to measure flux of post-squalene cholesterol biosynthetic intermediates in mouse tissues and cultured cells. Surprisingly, no tissue utilized the K-R pathway. Rather, we identified a hybrid between the two pathways that we called the modified K-R (MK-R) pathway. Proportional flux through the Bloch pathway varied widely - from 8% (preputial gland) to 97% (testes). Tissues with a higher biosynthesis rate had a greater utilization of the Bloch pathway, with the exception of ectodermal tissues (skin and preputial). The tissue-specific pathways utilized *in vivo* were retained in cells culture. Sterol depletion in cultured cells increased flux through the Bloch pathway, without affecting the flux through the MK-R pathway. Overexpression of desmosterol reductase (DHCR24) enhanced usage of the MK-R pathway, however DHCR24 protein or expression levels only loosely correlated with utilization of the Bloch pathway in mouse tissues. We also provide evidence that DHCR24 is the rate limiting enzyme for the conversion of zymosterol → cholesterol, but not lanosterol → cholesterol. Thus, relative use of the Bloch and MK-R pathways is highly variable, tissue-specific, flux dependent, and epigenetically fixed. Maintenance of two intersecting pathways permits production of diverse bioactive sterols that can be regulated independently of cholesterol.

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## 28. B4GALT6 regulates astrocyte activation during CNS inflammation \*

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Astrocytes play complex roles in the response to trauma, infection or inflammation in the central nervous system (CNS). Thus, it is important to characterize the mechanisms regulating astrocyte function, as well as potential targets for the therapeutic modulation of astrocyte activity. Here we report that during the chronic-progressive phase of experimental autoimmune encephalomyelitis (EAE), astrocytes promote disease pathogenesis by a lipid-dependent signaling pathway.

In line with previous studies, depletion of reactive astrocytes during the acute phase of chronic-progressive EAE exacerbated disease. However, depletion of astrocytes in the chronic phase ameliorated the disease. Hence, to understand this dichotomy we compared gene expression profiles of astrocytes from mice in acute and chronic stages of EAE. One gene associated with the chronic stage was B4GALT6, which codes for the β-1,4-galactosyltransferase 6 that catalyzes the synthesis of lactosylceramide (LacCer).

We observed that LacCer levels are up-regulated in the CNS during chronic EAE, and that LacCer synthesized by the astrocytes acts in an autocrine manner to trigger transcriptional programs that promote the recruitment and activation of CNS-infiltrating monocytes and microglia, and neurodegeneration. We also detected increased B4GALT6 expression and LacCer levels in CNS MS lesions. Finally, the inhibition of LacCer synthesis suppressed local CNS innate immunity and neurodegeneration in EAE, and interfered with the activation of human astrocytes *in vitro*. Thus, B4GALT6 is a potential therapeutic target for MS and other neuroinflammatory disorders.

## 29. Regulatory mechanisms of platelet-activating factor (PAF) production in macrophages and identification of a specific inhibitor for PAF synthetic enzyme (LPCAT2) by high-throughput screening \*

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PAF is a lipid mediator that has a variety of pathophysiological roles in inflammatory diseases. However, regulatory mechanisms of PAF production were to be elucidated. The rapid production and degradation of PAF are closely related to glycerophospholipid metabolism of biomembranes. Among the phospholipid remodeling enzymes, lysophosphatidylcholine acyltransferase 2 (LPCAT2) was identified as the enzyme that synthesizes PAF in response to extracellular stimuli. We have characterized three independent signaling pathways to increase PAF production in macrophages. First, endotoxin stimulation for 16 h increased mRNA of LPCAT2. Second, endotoxin stimulation for 30 min activated LPCAT2 by Ser34 phosphorylation via the p38 MAPK/MAPK-activated protein kinase2 (MK2) dependent pathway. Third, LPCAT2 is activated by Ser34 phosphorylation in response to mcPAF or ATP stimulation for 30 sec. In addition, we also report LPCAT2-specific inhibitors, N-phenylmaleimide derivatives, selected from a 174,000-compound library by fluorescence-based high-throughput screening and LC-MS. Although PAF receptor (PAFR) antagonists are not available for clinical use, these compounds can be used as a lead to develop novel class modulator of PAF-related diseases.

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## 30. Processing of a complex lipid dataset for the NIST inter-laboratory comparison exercise for lipidomics measurements in human serum and plasma

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Identification of lipids by untargeted lipidomics requires sophisticated software with an extensive lipid database. In addition, the mass spectrometer employed must be capable of separating many overlapping isobaric and isomeric lipid ions. We present here the details and challenges of the data processing of the NIST plasma and serum extracts using the latest version of LipidSearch software. New algorithms were introduced specifically to reduce false positives and to help automate the data review.

LipidSearch software (Thermo Scientific) was used for lipid identification through a database search of the accurate masses of precursors and their predicted fragment ions. Each lipid identification is ranked by mass tolerance, match to the theoretical fragmentation and the fraction of total MS-MS intensity. The number of lipid species identified in each different experiment were assessed at the sum composition (MS) and isomer (MS-MS) levels. For each LC-dd-MS<sup>2</sup> run potential lipid species were identified using the predicted MS-MS fragments for the molecular species observed in the positive or negative ion mode. For positive ions, the unfiltered data gave 600-800 sum composition species and 900-1500 MS<sup>2</sup> spectra representing possible isomeric species.

The data for each run were aligned within a chromatographic time window and positive and negative ion annotations were merged into the results table. This approach provides lipid annotation that reflects the appropriate level of MS<sup>2</sup> fragment ions from the complete dataset giving higher confidence in lipid identifications. The results were then filtered by main adduct ion, ID quality, signal-to-noise, peak area and relative standard deviation; manual integration was performed if necessary prior to estimating concentration relative to an internal standard for each lipid class. These results demonstrate that in a 60 min LC-MS run that it is possible to separate and identify over a thousand isomeric lipid species from human plasma using a C30 UHPLC column.

## 31. Bridging a gap between cytochrome *bc*<sub>1</sub> complex structure and function

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Cytochrome (cyt) *bc*<sub>1</sub> is an energy-conserving complex that functions in the inner mitochondrial membrane and in the plasma membrane of bacteria as part of the electron transfer chain. The complex contains two active sites, Q<sub>o</sub>- and Q<sub>i</sub>-sites, where the substrate quinol/quinone is either oxidized or reduced, respectively. Although the electron transfer steps of cyt *bc*<sub>1</sub> operation have been studied extensively, the exact proton transfers, especially at the Q<sub>o</sub> site, are unknown. In fact, there is no experimentally acquired structural data regarding the binding mode of the substrate at the Q<sub>o</sub>-site. Here, the cyt *bc*<sub>1</sub> complex of bacterium *Rhodobacter capsulatus* was studied using extensive atomistic molecular dynamics (MD) simulations. Ligand-binding modes are presented for both unoxidized and oxidized forms of the substrate at the Q<sub>o</sub>-site based on the MD simulations. Furthermore, the computations suggest that specific water molecules are needed for the quinol oxidation and to prevent short-circuit suppression at the Q<sub>o</sub>-site. With the Q<sub>i</sub>-site, the MD simulations underline the possible role of negatively charged cardiolipins for quinone reduction.

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## 32. Unsaturated phosphatidylinositols promote cotton fiber cell growth

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Cotton fibers are differentiated from the ovule epidermal cells and undergo rapid elongation, which may activate membrane lipid biosynthesis. In this work, cotton lipids were extracted and separated from fast-elongating wild-type upland cotton fibers, wild-type ovules with fibers removed and ovules from the *fuzzless-lintless (fl)* mutant harvested at 10 day post-anthesis (dpa). By applying targeted lipidomics analyses, we showed that fiber cells contained significantly higher amount of phosphatidylinositol (PI) than that extracted from ovule samples of both genotypes, with 34:3 (16:0, 18:3) being the most predominant molecular species. Exogenously applied PI containing C18:3 or C18:3 per se in ovule culture medium significantly promoted fiber cell growth. Quantitative analysis of total fatty acids showed that linolenic acid (C18:3) is the most abundant unsaturated fatty acids in fiber cells. The genes encoding fatty acid desaturases ( $\Delta^{15}$ GhFAD), PI synthase (PIS) and PI kinase (PIK) were expressed in a fiber-preferential manner. Further analysis of phosphatidylinositol monophosphate (PIP) indicated that elongating fibers contained four- to five- fold higher amounts of PIP 34:3 than those from the ovules. Exogenously applied C18:3, Soybean L- $\alpha$ -PI and PIP 34:3 promoted significant fiber growth, whereas a liver PI lacking C18:3 moiety, linoleic acid and PIP 36:2 were completely ineffective. Furthermore, cotton plants expressing virus-induced gene-silencing constructs that specifically suppressed *Gh* $\Delta^{15}$ FAD, *Gh*PIS or *Gh*PIK expression, resulted in significantly short-fibered phenotypes. Our data provide the basis for in-depth studies on the roles of PI and PIP in mediating cotton fiber growth.

Qin, Y.M., and Zhu, Y.X. (2011). How cotton fibers elongate: a tale of linear cell-growth mode. *Curr. Opin. Plant Biol.* 14, 106-111.

Shi, Y.H., Zhu, S.W., Mao, X.Z., Feng, J.X., Qin, Y.M., Zhang, L., Cheng, J., Wei, L.P., Wang, Z.Y., and Zhu, Y.X. (2006). Transcriptome profiling, molecular biological, and physiological studies reveal a major role for ethylene in cotton fiber cell elongation. *Plant Cell.* 18, 651-664.

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

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

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### 34. Programming macrophage inflammation-resolution: The role of $\omega$ -3 polyunsaturated fatty acids

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**Objective:** Macrophages are important to study in the context of inflammation given that their physiological locality situates these cells to respond to the initial inflammatory insult. Dietary supplementation with omega-3 polyunsaturated fatty acids ( $\omega$ -3 PUFAs) have been shown to decrease levels of inflammatory mediators produced by macrophages, yet little is known with regard to their therapeutic mechanism of action(s). This study is set to determine if the introduction of  $\omega$ -3 PUFAs promote inflammation-resolution as a result of modifying the functional phenotype and/or molecular pathway profile of activated macrophages during an inflammatory cascade. **Methods:** As a model for macrophage immune cell function, RAW264.7 cells, a murine-derived macrophage cell line, was exposed to inflammatory stimuli following pre-treatment with n-3 PUFAs. Cells were treated with different doses/combinations of alpha linolenic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) prior to macrophage activation to assess the combinatory impact of dietary n-3-PUFAs. To model an inflammatory event, RAW264.7 were stimulated with bacterial lipopolysaccharide (LPS). Cell culture supernatants and cell lysates were collected and analyzed for different biomarkers of inflammation. The tests performed included Griess reaction (to measure the production of nitrite) and western blots (to measure levels of inflammatory proteins). **Results:** A decrease in the inflammatory markers cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were detected by western blot analyses with various concentrations and DHA and EPA, compared to control samples. The most significant decreases in nitrite levels were observed in cultures treated alone or in combination with DHA. Current studies are underway to evaluate changes in the functional phenotype of macrophages (i.e. phagocytic ability, TNF-alpha expression) following  $\omega$ -3 PUFA treatment and LPS activation. **Clinical Implications:** Understanding the biological role of n-3 PUFAs in inflammation will advance the knowledge-base into the therapeutic role it can play in reducing inflammation and developing novel therapies to treat chronic inflammatory diseases.

## 35. Apolipoprotein A-I Binding Protein regulates macrophage polarization in the tumor microenvironment

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Apolipoprotein A-I Binding Protein (AIBP) is a highly conserved intracellular and secreted protein. Our laboratory recently demonstrated in zebrafish that extracellular AIBP mediates cholesterol efflux from endothelial cells, which in turn disrupts lipid rafts and limits angiogenic signaling. While several *in vitro* studies on AIBP have been published, little is known about its *in vivo* functions. To demonstrate the conserved function of AIBP and investigate its other roles, our laboratory has generated *Aibp*<sup>-/-</sup> mice, which are viable and fertile. In preliminary studies we found that *Aibp*<sup>-/-</sup> mice fed a high fat diet have an increased content of M1-polarized macrophages in the white adipose tissue in comparison to wild type mice, and that *Aibp*<sup>-/-</sup> mice exhibit increased levels of inflammatory cytokines. We hypothesized that AIBP plays a role in macrophage polarization and inflammation, especially under pathological conditions. Indeed, when mice were implanted with subcutaneous B16 melanoma, the tumors in *Aibp*<sup>-/-</sup> mice had significantly greater presence of M1 macrophages. Immunohistochemistry showed that AIBP is present in macrophages in inflamed tissues, such as atherosclerotic lesions and the lungs from pneumonia patients. However, thioglycollate-elicited peritoneal macrophages lacking AIBP expression do not exhibit any impairment in their ability to polarize to M1 or M2 states, instead suggesting that deficiency in secreted extracellular AIBP may be responsible for the M1 phenotype observed in *Aibp*<sup>-/-</sup> mice. Indeed, pretreating macrophages with recombinant AIBP protein prior to polarization stimuli resulted in suppression of M1 polarization. A better understanding of AIBP's regulation of inflammation and macrophage polarization will provide new mechanistic insights and targeted therapies tailored to the inflammatory milieu.

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## 36. Oxidized phospholipids regulate macrophage bioenergetics through a TLR2-dependent ceramide accumulation.

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Mitochondrial dysfunction plays a key role in diabetes and obesity. Recent studies identify a link between mitochondrial function and inflammatory signaling of macrophages. During the development of obesity macrophages accumulate in chronically inflamed adipose tissue, an environment of great oxidative stress, characterized by the formation of bioactive oxidized phospholipids (OxPL). Our lab has previously shown that OxPL induce TLR2-dependent inflammatory gene expression in macrophages. To test the effects of OxPL on macrophage bioenergetics, we treated bone marrow-derived macrophages (BMDMs) from C57BL/6J (WT) and TLR2-KO mice with 10µg/mL of 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine oxidation products (OxPAPC). Using MitoTracker Red staining, we observe that mitochondrial membrane potential, an indicator of mitochondrial function, is decreased in OxPL-treated macrophages. Using an extracellular flux analyzer (Seahorse) to assess mitochondrial function through the measurement of oxygen consumption rate (OCR), we find that OxPL inhibit macrophage OCR in a TLR2-dependent manner. Since ceramides have been shown to inhibit mitochondrial function, we used liquid chromatography-mass spectrometry to show that OxPL induce ceramide accumulation in macrophages, which was also dependent on TLR2. Using an inhibitor of *de novo* ceramide biosynthesis, Myriocin, we confirmed that ceramides are at least partially responsible for inhibition OCR by OxPL. Furthermore, OxPL also inhibit succinate dehydrogenase (electron transport chain complex II) mRNA expression and increase the glycolytic capacity of macrophages. Finally, we identify 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine (PGPC) as a component of OxPAPC responsible for inhibition of OCR and increase in glycolysis. Taken together, our results show that OxPL induce a bioenergetics shift in macrophages by inhibiting mitochondrial function through a TLR2-ceramide dependent manner and increasing glycolytic capacity.

### 37. Lipidomics profiles reveal altered sphingolipids in Fanconi Anemia cells

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Fanconi anemia (FA) is a chromosome instability syndrome whose clinical manifestations typically present during childhood. FA patients are extremely susceptible to cancers including head and neck and other squamous cell carcinomas (SCCs). Clinical management of this tumor type in FA remains dismal with 2 year survival below 50%. Furthermore, molecular and chemical mechanisms underlying FA SCC pathogenesis are poorly understood thus limiting effective treatment development. By using an unbiased metabolomics approach in FA knockdown systems, we found that sphingolipid metabolism is altered in the FA deficient SCC cells and in a non-tumorigenic human keratinocyte cell line, i.e. human near diploid immortalized keratinocytes that form skin (NIKS). Glycosphingolipids were identified among the differentially regulated metabolites in FA deficient SCC and NIKS cells along with sphingomyelin and phosphatidylinositol. Targeted analysis by ultra-performance liquid chromatography coupled with triple quadrupole mass spectrometry (UPLC-MS/MS) confirmed that the identities and quantities of these glycosphingolipids are elevated in the FA deficient cells. Functional studies revealed their important activities in the induction of advanced SCC tumor phenotypes. Taken together, our studies suggest that altered sphingolipid metabolism is an important factor in FA susceptibility to SCC progression.

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### 38. Regulation of the inflammatory and monocyte recruitment pathways in HAECs by PEIPC and EI

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Oxidation products of 1-palmitoyl-2-arachidonoyl-*sn*-glycerol-2-phosphatidylcholine (PAPC), referred to as OxPAPC, and an active component, 1-palmitoyl-2-(5,6-epoxyisoprostanoyl E<sub>2</sub>)-*sn*-glycero-3-phosphatidylcholine (PEIPC), accumulate in atherosclerotic lesions and other sites of chronic inflammation and regulate over 1000 genes in human aortic endothelial cells (HAEC), including upregulation of monocyte binding. Using microarray analysis, we also determined that the degradation product of PEIPC, the epoxyisoprostanone EI, also regulates several of these same key genes. Surprisingly, we also demonstrated that EI strongly downregulates several key genes involved in monocyte binding, including MCP-1. The focus in our work is now to elucidate the pathways by which EI affects monocyte binding in HAECs and what effects EI pretreatment has on key inflammatory mediators, PEIPC, IL1-beta, and TNF-alpha in HAECs. We previously demonstrated that PEIPC covalently binds to a number of proteins in HAEC via binding to specific cysteines, and the main goals that this work focuses on are to determine key proteins that are bound by OxPAPC, PEIPC, and EI and to determine the overlap in bound proteins. Subsequently we will test the effects that silencing of these proteins has on the inflammatory and monocyte binding response to these lipids in HAECs. These studies are currently focused on testing the binding of these lipids to candidate proteins VEGFR2, EP2, and GRP78 with biotinylated OxPAPC. The next steps will be to test binding using biotinylated EI to determine EI binding to these proteins and to perform silencing studies to determine regulation of monocyte recruitment pathway by this binding.



## 39. Alterations in lipid metabolism due to gravitational effects

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One of the biggest challenges facing human space exploration are the adverse health outcomes astronauts acquire during spaceflight. Microgravity can be achieved during spaceflight or other modeled conditions and significantly alters numerous biological processes. Medical data from International Space Station astronauts reveal they suffer from skeletal/cardiac muscle atrophy and loss of bone density.

There is evidence to suggest that alterations in lipid homeostasis are involved in the impairment observed in the musculoskeletal system when organisms are subjected to microgravity conditions. Post-spaceflight muscle biopsies of astronauts, heart muscles of rats subjected to microgravity on Spacelab 3, human mesenchymal stem cells grown in modeled microgravity (MMG), and the proximal tibial and humeral metaphyses of suspended rats, all show an increase in intracellular stored lipids.

To understand this phenomenon, we analyzed microarray data of yeast cells grown in MMG. Data was obtained from the Gene Expression Omnibus (NIH). We chose *S. cerevisiae* as a model organism due to the tremendous amount of biological information available and the significant similarities in lipid metabolism between yeast and mammals. We found that 33% of the 150 yeast genes associated with lipid homeostasis maintenance under the Gene Ontology (GO) term “lipid metabolic process” in the Saccharomyces Genome Database showed more than a 1.5 fold change in expression compared to cells grown in normal gravity.

Through secondary analysis of existing expression data, promoter data, and pathway reconstruction tools, we have inferred a transcriptional regulatory network that describes the changes in lipid metabolism that take place during microgravity conditions. We are currently examining how MMG affects free fatty acid-induced lipotoxicity in *S. cerevisiae*. Identifying the mechanisms by which changes in gravity produce perturbations in lipid homeostasis will be greatly useful for comprehending and managing lipid-related disorders in a variety of habitats, whether in Earth or Space.

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## 40. Nano-LC-MS/MS for the quantitation of prostanoids in immune cells

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A sensitive quantitation method for prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), PGD<sub>2</sub>, 6-keto PGF<sub>1α</sub>, PGF<sub>2α</sub> and thromboxane B<sub>2</sub> (TXB<sub>2</sub>) produced by immune cells has been developed and validated. The challenge in developing this method was the limited number of immune cells that could be isolated from murine tissue (40,000 mast cells) or blood (5,000 T cells). Current methods for prostanoid quantitation in cells require cell counts in the magnitude of 200,000 to 2,000,000. Thus, our aim was to develop a more sensitive method to measure prostanoid concentrations from a limited number of cells.

Mast cells were isolated from paws of mice after inducing an inflammation by local injection of zymosan. In contrast, prostanoids generated by T lymphocytes were isolated from blood of two different groups of mice: one group had been sensitized with oxazolone and the other group was non-sensitized. Both groups were challenged with the allergen before taking the blood.

An Eksigent nano-LC 2D Ultra system equipped with a C8 column was used for chromatographic separation. It was connected to a hybrid triple quadrupole – ion trap mass spectrometer 5500 QTRAP (AB Sciex) via a nanospray ion source.

A validation according to FDA guidelines was done successfully in terms of linearity, precision, accuracy, recovery, stability and lower limit of quantitation (LLOQ). The LLOQ were 75 fg on column for PGE<sub>2</sub> and PGD<sub>2</sub> and 112.5 fg on column for 6-keto PGF<sub>1α</sub>, PGF<sub>2α</sub> and TXB<sub>2</sub>, respectively. In the mast cells, we found that the production of PGE<sub>2</sub> due to an inflammatory stimulus increases significantly stronger than the synthesis of the other prostanoids. In contrast, the two groups of T cells did not show any difference in prostanoid production.

The sensitivity, selectivity and reliability of the developed method make it suitable for the study of the roles of prostanoids in various physiological and pathophysiological processes.

## 41. Lipid profiling in rheumatoid arthritis patients indicates response to rituximab treatment

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Background: Rheumatoid arthritis (RA) is a common autoimmune disease, with no known cure, that often results in disability, reduced life expectancy, and increased risk of comorbidities [1]. Many patients do not respond well to first-line therapy, anti-TNF treatment, and subsequently are treated with anti-B (rituximab), anti-T (abatacept), or anti-IL6 therapies, until an acceptable level of disease management is achieved [2]. We have previously shown that modulation of phospholipid biosynthesis influences inflammatory arthritis and may be an effective treatment strategy [3]. Thus, we hypothesized that characterization of patients' lipid profiles, utilizing a high-performance benchtop mass spectrometer could represent a useful clinical tool for understanding and improving patient outcomes. Methods: 23 active seropositive RA patients on concomitant methotrexate were treated with rituximab. Clinical outcome was assessed 6 months after treatment. Patients were classified as responders and non-responders according to the American College of Rheumatology (ACR) improvement criteria, at a 20% level (ACR20). A Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer was used to analyze lipids isolated from unfiltered peripheral blood specimens collected at baseline and 6 months after rituximab therapy [4]. Data processing and statistical analysis were performed in the MATLAB programming environment and pathway analysis was performed using VANTED software. Significant lipids were identified and pathway analysis was used to correlate clinical outcome and lipid profiling. Results: Prior to treatment, most observed classes of glycerophospholipids were downregulated in rituximab responders, including phosphatidylethanolamines, phosphatidylserines, and phosphatidylglycerols. However, the opposite trend was observed in phosphatidylinositols. Following treatment with rituximab, several of these trends were reverse or eliminated, suggesting that response to rituximab is related to shifts in phospholipid composition in responders. Conclusion: The relationship between blood lipid profiles and patient response to rituximab therapy suggests that the application of lipid profiling is a promising clinical tool for RA.

<sup>1</sup>Edwards JCW, et al. *New England Journal of Medicine* 2004;350:2572.

<sup>2</sup>Cuchacovich M, et al. *Clinical Rheumatology* 2014;33:1707.

<sup>3</sup>Guma M, et al. *Annals of the Rheumatic Diseases* 2014.

<sup>4</sup>Tiziani S, et al. *Analytical Biochemistry* 2008;377:16-23.

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## 42. Lipidomic “deep profiling” reveals uncommon phosphorylated sphingolipids in different organisms

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We recently introduced a strategy to capture phospho-monoester lipids and improve the detection of long-chain base phosphates (LCB-Ps, e.g., sphingosine-1-phosphate) by liquid chromatography-mass spectrometry. Novel LCB-Ps were discovered and characterized in tissues from human and mouse, as well in *D. melanogaster* and yeast. These findings have immediate relevance for our understanding of sphingosine-1-phosphate biosynthesis, signaling, and degradation in different organisms.

### **43. Identification of small subunit of serine palmitoyltransferase a as a lysophosphatidylinositol acyltransferase 1-interacting protein**

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Lysophosphatidylinositol acyltransferase 1 (LPIAT1), also known as MBOAT7, is a phospholipid acyltransferase that selectively incorporates arachidonic acid (AA) into sn-2 position of phosphatidylinositol (PI). We previously demonstrated that LPIAT1 regulates AA content in PI and plays a crucial role in brain development in mice. However, how LPIAT1 is regulated and which proteins function cooperatively with LPIAT1 are unknown. In this study, using a split-ubiquitin membrane yeast two-hybrid system, we identified the small subunit of serine palmitoyltransferase a (ssSPTa) as an LPIAT1-interacting protein. Knockdown of ssSPTa decreased the LPIAT1-dependent incorporation of exogenous AA into PI but did not affect the in vitro enzyme activity of LPIAT1 in the microsomal fraction. Interestingly, knockdown of ssSPTa decreased the protein level of LPIAT1 in the crude mitochondrial fraction but not in total homogenate or the microsomal fraction. LPIAT1 was localized to the mitochondria-associated membrane (MAM), where AA-selective acyl-CoA synthetase is enriched. These results suggest that ssSPTa plays a role in fatty acid remodeling of PI, probably by facilitating the MAM localization of LPIAT1.

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### **44. Aldehyde dehydrogenases as a source of fatty acids in cancer stem cells \***

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Aldehyde dehydrogenases are a class of rate limiting enzymes traditionally thought to be responsible for ridding cells of toxic waste. Cancer cells, with their increased need for biomolecules to generate daughter cells, have been known to co-opt biochemical pathways to serve this need.

Through a metabolism focused shRNA screen performed exclusively in primary murine leukemic stem cells and their normal hematopoietic counterparts in the presence of supporting bone marrow stroma, our laboratory has identified a particular aldehyde dehydrogenase, Aldehyde Dehydrogenase 3a2, the depletion of which results in death of leukemic stem cells while sparing normal hematopoietic cells. Depletion of this enzyme prolonged survival in mice transplanted with Acute Myeloid Leukemia (AML) and decreased growth of human AML samples in culture. From clinical data, patients with normal cytogenetic AML and low levels of this enzyme at diagnosis have longer overall survival.

Our mechanistic studies suggest that the effect observed in leukemia stem cells after depletion of Aldh3a2 may be to a lack of the availability of fatty acids, which are the biochemical products of this enzyme's activity and are needed for the generation of new cancer cells. Cancer stem cells have not previously been known to co-opt what is thought to be a primarily recycling pathway for the generation of biomolecules to enable survival and propagation.

Our future work will explore the possibility of hitherto unidentified or underappreciated roles in cancer stem cells of recycling enzymes such as Aldh3a2. These enzymes promise to be unique metabolic vulnerabilities for these cell populations. This work is likely to lead the way for the search of previously unsuspected therapeutic targets in the treatment of AML.