CF – Metabolomics Annual Program Meeting
University of Michigan, Ann Arbor

Participants: Representatives from the laboratories and cores funded through the Program, NIH project scientists, Common Fund working group members, and EEC members.

Friday, September 27, 2013
Auditorium, Kellogg Eye Center
1000 Wall Street, Ann Arbor, MI 48105

12:00 – 1:00 Registration

Session: Introduction – overall Program vision and goals and specific meeting goals

1:00 – 1:15 Introduction/overview/general info (Phil Smith)

Session: Technology Development – report on goals and progress (David Balshaw, moderator)

1:15 – 3:15 Technology development R01s (15 min presentation + 5 min questions)

Evaluating the impact of metabolite extraction and separation on metabolomics data
Andrew Patterson, Pennsylvania State University – University Park
Slow Magic Angle Spinning NMR Metabolomics
Jian Zhi Hu, Battelle Pacific Northwest Laboratories
Mass Spectrometry/Ion Mobility Based Methods for High Throughput Lipidomics Analysis
Robert Barkley, Joseph Hankin, Robert Murphy, University of Colorado Denver
Multidimensional Metabolite Characterization with Ion Mobility Separations and Mass Spectrometry
Erin S. Baker, Battelle Pacific Northwest Laboratories
Chemoselective Platform for Large-Scale Metabolomics
Teresa W-M Fan, University of Louisville
Automating a Platform Towards High-Throughput Analysis of the Total Metabolome
Gary Joseph Patti, Washington University

3:15 – 4:15 Discussion of overall state of technology and needs in metabolomics

4:30 – 5:30 Keynote speaker (Michael Snyder)

5:30 Poster session/reception

Saturday, September 28, 2013
Auditorium, Kellogg Eye Center
1000 Wall Street, Ann Arbor, MI 48105

7:30 – 8:00 Registration

Session: RCMRCs (Padma Maruvada, moderator)

Current RCMRC Annual Reports (goals, infrastructure, activities, highlights)

8:00 – 8:40 University of Michigan (Charles Burant)
8:40 – 9:20 University of California, Davis (Oliver Fiehn)
9:20 – 10:00 Research Triangle Institute (Susan Sumner)

New RCMRCs (descriptions, planned activities and infrastructure)

10:00 – 10:20 University of Florida (Arthur Edison)
10:20 – 10:40 University of Kentucky (Rick Higashi)
10:40 – 11:00 Mayo Clinic (K. Sreekumar Nair)

11:00 – 12:00 Discussion of overall composition, outstanding issues

12:00 – 1:00 Lunch

Session: Metabolomics Training and Capacity-Building Related Activities (Barbara Spalholz, moderator)

1:00 – 1:20 Administrative supplement program report and discussion (Barbara Spalholz)
1:20 – 1:50 Overview of K award program (Dick Okita & K01s)
1:50 – 2:20 R25 courses and coordination with U24 and DRCC resources (Dick Okita & K25s)

Session: Data Repository and Coordinating Center Annual Report (Art Castle, moderator)

2:20 – 3:20 DRCC presentation (data sharing requirements, goals, metadata requirements, reports, infrastructure, program coordination and discussion)
3:20 – 3:35 Overview of Reference standards for metabolomics and introduction of Metabolite Synthesis contract (Pothur Srinivas)
3:35 – 3:55 Metabolite Synthesis (RTI)
3:55 – 4:15 Metabolite Synthesis (SRI)
4:15 – 5:00 P/F grants (Padma Maruvada)
5:00 – 5:15 Closing remarks (Charles Burant)

NIH Common Fund Metabolomics Program
Fiscal Year 2012 Awards

RFA-RM-11-020: Metabolomics Data Repository and Coordinating Center (DRCC) (U01)

Project Number: 1U01DK097430
Contact PI: Shankar Subramaniam
Institution: UNIVERSITY OF CALIFORNIA
Project Title: THE METABOLOMICS DATA CENTER AND WORKBENCH (MDCW)

RFA-RM-11-016: Regional Comprehensive Metabolomics Resource Cores (RCMRC) (U24)

Project Number: U24DK097153
Contact PI: CHARLES F BURANT
Institution: University of Michigan
Project Title: Michigan Regional Comprehensive Metabolomics Resource Core (MRC2)

Project Number: U24DK097154
Contact PI: OLIVER FIEHN
Institution: UNIVERSITY OF CALIFORNIA, DAVIS
Project Title: West Coast Central Comprehensive Metabolomics Resource Core (WC3MRC)

Project Number: U24DK097193
Contact PI: SUSAN J SUMNER
Institution: RESEARCH TRIANGLE INSTITUTE
Project Title: RTI's Regional Comprehensive Metabolomics Resource Center

FY2013 Awards:

Project Number: U24DK097209
Contact PI: ARTHUR S EDISON
Institution: UNIVERSITY OF FLORIDA
Project Title: Southeast Resource Center for Integrated Metabolomics (SECIM)

Project Number: U24DK097215
Contact PI: Richard M Higashi
Institution: UNIVERSITY OF KENTUCKY
Project Title: Resource Center for Stable Isotope-Resolved Metabolomics

Project Number: 1U24DK100469
Contact PI: SREEKUMARAN K Nair
Institution: MAYO CLINIC
Project Title: Mayo Clinic Metabolomics Resource Core

RFA-RM-11-019: Technology Development to Enable Large Scale Metabolomics Analyses (R01)

Project Number: R01 ES022190
Contact PI: Erin S Baker
Institution: BATTELLE PACIFIC NORTHWEST LABORATORIES
Project Title: Platform Providing Increased Throughput, Sensitivity and Specificity for Metabolomics

Project Number: R01 ES022191
Contact PI: TERESA W-M FAN
Institution: University of Louisville
Project Title: Integrated Chemoselective and Informatic Platform for Large-Scale Metabolomics

Project Number: R01ES022176
Contact PI: Jian Zhi Hu
Institution: BATTELLE PACIFIC NORTHWEST LABORATORIES
Project Title: Slow-MAS NMR Metabolomics

Project Number: R01ES022172
Contact PI: ROBERT C MURPHY
Institution: UNIVERSITY OF COLORADO DENVER
Project Title: High Throughput Lipidomics Analysis by MALDI/Ion Mobility Mass Spectrometry

Project Number: R01ES022186
Contact PI: Andrew Patterson
Institution: PENNSYLVANIA STATE UNIVERSITY-UNIV PARK
Project Title: Optimized Metabolite Extraction, Separation, and Identification for Metabolomics

Project Number: R01ES022181
Contact PI: Gary Joseph Patti
Institution: WASHINGTON UNIVERSITY
Project Title: Developing the Untargeted Metabolomic Workflow for High-Throughput Analyses

RFA-RM-11-017: Mentored Research Scientist Development Award in Metabolomics (K01)

Project Number: K01GM103821
Contact PI: Mary Cloud Bosworth Ammons
Institution: Montana State University
Project Title: Metabolomic Analysis as a Tool to Understanding the Use of Novel Therapeutics

Project Number: K01GM103817
Contact PI: Debby Ngo
Institution: Massachusetts General Hospital
Project Title: The Kynurenine Pathway in Pulmonary Arterial Hypertension

Project Number: K01GM103809
Contact PI: Vanessa V Phelan
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO
Project Title: The Interactive Metabolome of Polymicrobial Biofilms

Project Number: K01GM103806
Contact PI: JESSE ROWLEY
Institution: UNIVERSITY OF UTAH
Project Title: The Platelet Metabolome in Obesity

RFA-RM-11-018: Development of Courses or Workshops in Metabolomics (R25)

Project Number: R25GM103798
Contact PI: STEPHEN BARNES
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Project Title: UAB Metabolomics Workshop: from design to decision

Project Number: R25GM103802
Contact PI: MARTIN KOHLMEIER
Institution: UNIVERSITY OF NORTH CAROLINA CHAPEL HILL
Project Title: Online learning platform: introducing clinicians and researchers to metabolomics

NOT-RM-11-024: Administrative Supplements for Collaborative Activities to Promote Metabolomics Research

Project Number: R37DK58282
Contact PI: Domenico Accili
Institution: Columbia University Health Sciences
Project Title: Mouse models of insulin resistance

Project Number: R01DK047348
Contact PI: Hans-Rudolf Berthoud
Institution: Pennington Biomedical Research Center
Project Title: Neural mechanisms controlling food intake and body weight after bariatric surgery

Project Number: U01DK060990
Contact PI: Harold Feldman
Institution: University of Pennsylvania
Project Title: The Chronic Renal Insufficiency Cohort (CRIC) Study

Project Number:	R01ES019315
Contact PI:	Rebecca Fry
Institution:	University of North Carolina Chapel Hill
Project Title:	In Utero Exposure to Arsenic, Links to Epigenetic Alterations and Disease

Project Number:	R01HD065826
Contact PI:	Mari S Golub
Institution:	UNIVERSITY OF CALIFORN IA, DAVIS
Project Title:	Fluoxetine: Sensitive Ages and Genotypes for Adverse Effects in Juvenile Monkeys

Project Number:	R01GM086786
Contact PI:	Wendy Hanna-Rose
Institution:	Pennsylvania State University
Project Title:	Probing Function of NAM and NAD+ Salvage in Development and Aging in C. elegans

Project Number:	R01CA164492
Contact PI:	Stephen Kron
Institution:	University of Chicago
Project Title:	Radiation Response within the Tumor Microenvironment

Project Number:	R37DK043806
Contact PI:	Mitchell Lazar
Institution:	University of Pennsylvania
Project Title:	Thyroid Hormone Receptor: Regulation and Function

Project Number:	R01HL088533
Contact PI:	Ronglih Liao
Institution:	Brigham and Women's Hospital
Project Title:	Molecular Mechanisms and Treatment of Primary Amyloid (AL) Cardiomypathy

Project Number:	R01HL095479
Contact PI:	John Roback
Institution:	Emory University
Project Title:	Adverse Effect of RBC transfusions: A unifying hypothesis

Project Number:	R01CA152330
Contact PI:	Pankaj Seth
Institution:	Beth Israel Deaconess Medical Center
Project Title:	Pathway Specific Imaging in VHL Deficient Renal Cancer

Project Number: R01AR056973
Contact PI: Kristin Vandenborne
Institution: University of Florida
Project Title: Magnetic Resonance Imaging and Biomarkers for Muscular Dystrophy

Project Number: R01EY012224
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Abstract #1

2-aminoadipic acid is a novel biomarker of diabetes risk and modulates glucose homeostasis

Debby Ngo, M.D.
Massachusetts General Hospital

ABSTRACT

Improvements in metabolite profiling techniques are providing increased breadth of coverage of the human metabolome and may highlight novel biomarkers and pathways in common diseases such as diabetes. We recently developed a liquid chromatography-tandem mass spectrometry method capable of profiling 70 small molecules preferentially ionized using negative mode electrospray ionization, including intermediary organic acids, purines, pyrimidines and other compounds. We performed a nested case-control study of 188 individuals who developed diabetes and 188 propensity-matched controls from 2,422 normoglycemic participants followed for 12 years in the Framingham Heart Study. The metabolite most strongly associated with the risk of developing diabetes was 2-aminoadipic acid (2-AAA) ($p=0.0009$). Individuals with 2-AAA concentrations in the top quartile had >four-fold risk of developing diabetes (adjusted odds ratio, 4.5, 95% confidence interval, 1.9 to 10.9). These findings were replicated in the Malmö Diet and Cancer Study ($p=0.004$; pooled result, $p<0.0001$). Levels of 2-AAA were not well correlated with other metabolite biomarkers of diabetes, such as branched chain amino acids ($r=0.04$ to 0.24) and aromatic amino acids ($r=0.01$ to 0.13), suggesting they report on a distinct pathophysiological pathway. In experimental studies, administration of 2-AAA lowered fasting plasma glucose levels in mice fed both standard chow and high fat diets. Further, 2-AAA treatment enhanced insulin secretion from both a pancreatic beta cell line as well as murine and human islets. These data highlight a metabolite not previously associated with diabetes risk that is increased up to 12 years before the onset of overt disease. Our findings suggest that 2-AAA is a novel marker of diabetes risk and a potential modulator of glucose homeostasis in humans.

Abstract #2

Plasma metabolomics analysis after Roux-en-Y gastric bypass surgery in high-fat diet-induced obese rats

Peter Scherp, Ginger Ku, R Leigh Townsend, Laurel M Patterson, Michael B Mumphrey, Hans-Rudolf Berthoud

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Unlike drug and behavioral therapies, bariatric and metabolic surgeries particularly Roux-en-Y gastric bypass surgery (RYGB), efficiently reduce excess body weight and improves glucose homeostasis in a sustained fashion. These beneficial effects must ultimately be due to complex changes over time in the communication between the gut and other organs, including the brain, liver, muscle, and adipose tissue. A number of candidate mechanisms have been proposed, including changes in gut hormone secretion and signaling, the gut microbiome and its downstream signaling pathways, increased glucose disposal of the enlarged Roux- and common limbs, as well as crosstalk between the gut and the liver, muscle and adipose tissue. This latter possibility is suggested by observations of increased circulating levels of bile acids, known to signal the liver, brown adipose tissue and the brain. Therefore, the goal was to determine differences in the fasting levels bile acid profile in rats after Roux-en-Y gastric bypass surgery and in high-fat diet-induced sham-operated obese rats. In order to further understand the metabolic changes of RYGB surgery, we performed global metabolomics analysis on plasma samples from both RYGB and Sham rats seven months after surgery. Global metabolomics analysis was carried out using ultra-performance liquid chromatography – mass spectrometry (UPLC-MS^E) and allowed us to acquire low and high energy mass spectra from 3004 features in positive and 3529 features in negative acquisition mode of the mass spectrometer. Of these features, we identified a total of 39 compounds based on database searches and analysis of high-energy MS/MS spectra. The majority of these compounds were identified as bile acids, cholesterol metabolites and fatty acids. For more detailed analysis of changes of bile acids after RYGB surgery, we utilized a targeted mass spectrometry analysis approach to quantify a total of 13 bile acids from the same plasma samples as global metabolomics. Our results from global and targeted metabolomics show that cholesterol metabolites are reduced and that primary bile acids cholic and chenodeoxycholic acid increased by 6.4 fold and 20 fold, respectively. This increase of primary bile acids inversely correlates with the total fat weight and the weight of retroperitoneal and eipididymal fat pads. This suggests that RYGB surgery alters the turn-over of bile acids leading to an increased de novo synthesis of bile acids from cholesterol.

Supported by National Institutes of Health Grant DK047348 S1

Abstract #3

Vitamin D affects microbial metabolites in the urinary metabolome of mice.

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Background: Vitamin D_3 has known immunoregulatory roles and decreased levels of vitamin D_3 are linked to immune-mediated diseases, such as inflammatory bowel disease (IBD). Vitamin D_3 is hydroxylated to $25(OH)D_3$, and then again by the enzyme 1-α-hydroxylase (Cyp27B1) into $1,25(OH)_2D_3$. $1,25(OH)_2D_3$ is the biologically active form of vitamin D that binds to the vitamin D receptor (VDR) and regulates gene expression. Cyp27B1 knock-out (KO) mice cannot metabolize 25(OH)D3 into bio-active 1,25(OH)D3. These mice are more susceptible to mouse models of IBD than their WT littermates. Dysbiosis of the microbiome is also an important factor in susceptibility to diseases such as IBD, and the metabolome of IBD patients is altered with respect to bacterial metabolites. Furthermore, our preliminary data shows that vitamin D_3 status influences the communities of microbiota in the gut. However the mechanisms by which vitamin D_3 regulates the microbiota are unknown.

Objective: The objective was to explore novel roles for vitamin D_3 metabolites in regulating the host and the microbial metabolome.

Methods: Two different experimental designs were used to test the role of vitamin D in age- and sex-matched mice. To determine how $1,25(OH)_2D_3$ affects the metabolome, urine was collected from WT and Cyp27B1 KO mice. Extracts from Cyp27B1 WT and KO mice were profiled by ultra-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOFMS) and multivariate data analysis tools. To determine how vitamin D_3 levels affect the metabolome, urine was collected from WT mice before and after 14 days of 20,000 IU/d of vitamin D_3 supplementation. Urine extracts from mice before and after Vitamin D_3 dosing were compared using UPLC-QTOFMS and multivariate data analysis tools.

Results: Differences were found in the urinary metabolome of Cyp27B1 KO compared to WT controls. In addition, vitamin D_3 dosing of WT mice also resulted in changes in the urinary metabolome. Metabolites associated with the gut microbiota: xanthurenic acid, citric acid, betaine, xanthine, and cinnamoyl glycine were different in the two different experimental groups.

Conclusion: Alterations in the urine metabolome suggest a role for vitamin D_3 in regulating host and microbial metabolism. The data further suggest that the effects of vitamin D_3 or $1,25(OH)_2D_3$ on the gut microflora may not be the same. Metabolite analysis will aid our understanding of the mechanisms and pathways by which vitamin D_3 affects host health and the microbiome.

Abstract #4

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Metabolomics of fatty acid potentiation of glucose stimulated insulin secretion (GSIS)

Acute fatty acid exposure potentiates GSIS in β-cells though the signaling pathways that participate in the potentiation remain unclear. We tested the hypothesis that fatty acids may cause changes in glucose metabolism that lead to augmentation of insulin secretion. We assessed the alteration in the metabolome of INS-1 (832/3) cell following preincubation for 30 minutes with palmitic acid (PA, 500 μM) or BSA followed by stimulation with 16 mM unlabeled or U-[^{13}C] glucose for 5-60 minutes. Media was collected to assess insulin secretion and targeted metabolomic profiling was performed on cell extracts by LC-MS. Fatty acid exposure resulted in a 2-fold potentiation of GSIS at 60 minutes. Preincubation of INS-1 cells with palmitic acid resulted in a significant increase in plamitoyl-CoA, which fell by 50% within 5 minutes after addition of glucose. A significant, time-dependent rise in glycerol-3-phosphate (Go3P) found after glucose addition was attenuated by palmitic acid addition. 13C-glucose addition showed the rapid appearance of M+3 isotopomers of phosphatidic acid and diacylglycerol containing 32:0 fatty acids, indicating increased flux of glucose into the glycerolipids pathway via Go3P. Untargeted metabolomic profiling also detected the increases in potential signaling molecules including Palmitoyl taurine and palmitoyl glycine. The rapid esterification of lipids was accompanied by a decrease in static levels of glycolytic and pentose phosphate pathway intermediates metabolites and a 2-folds decrease in NADH/NAD+ ratio, presumably due to the consumption of NADH in the conversion of the glycolytic intermediate dihydroxy acetone phosphate to Go3P. The change in redox potential was accompanied by an increase in ^{13}C-isotopomer levels in TCA cycle intermediates and increased INS-1 cell oxygen consumption, indicating an increased flux of glucose through the TCA cycle. The increase in glucose utilization by palmitic acid was accompanied by decreases in malonyl-CoA levels and AMP-Kinase activation as evidenced by increased phosphorylation of acetyl-CoA carboxylase. Conclusion: These studies suggest that acute exposure to fatty acids may potentiate insulin secretion by augmenting the formation of signaling lipids as well as by increasing glycolytic flux and increasing glucose utilization in the TCA cycle where additional signaling molecules may be formed.

Abstract #5

Tools for Interpreting Metabolomics Data

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Recent progress in the field of metabolomics has created an opportunity to advance our understanding of physiological and pathological processes. It also posed a number of bioinformatics challenges associated with data analysis and interpretation. To date, there are only few tools that allow users to analyze metabolomics data and to link different types of omics data. Our recently developed tool Metscape is aimed at addressing these issues (http://metscape.ncibi.org). Metscape is a plugin for Cytoscape. It uses an internal Microsoft SQL Server database that integrates data from KEGG (http://www.genome.jp/kegg/) and the Edinburgh Human Metabolic Network (EHMN, http://www.ehmn.bioinformatics.ed.ac.uk/). It allows users to upload a list of metabolites with experimentally determined concentrations and map them to the reactions, genes and pathways. It also supports identification of enriched biological pathways from expression profiling data, building the networks of genes and metabolites involved in these pathways, and allow users to visualize the changes in the gene/metabolite data over time/experimental conditions.

While linking metabolites to metabolic pathways proved to be useful, only about half of experimentally detected metabolites can be mapped. Additional annotations are needed to enhance biological interpretation of metabolomics data. With this in mind, we recently developed a web-based tool Metab2MeSH that uses a statistical approach to annotate compounds with Medical Subject Headings (MeSH) (http://metab2mesh.ncibi.org). We will present our new tool MetDisease that uses the resulting data set to annotate compound networks with MeSH disease terms.

Abstract #6

Development of the UAB Metabolomics Workshop. S. Barnes[1], T. M. Beasley[2], H. Tiwari[2]. Department of Pharmacology & Toxicology[1] and Section on Statistical Genetics, Department of Biostatistics[2], University of Alabama at Birmingham.

As a test of the upcoming full (35-40 attendees) National Workshop on Metabolomics in 2014, a smaller workshop (20 attendees) was put on at UAB (July 22-25). This allowed us to develop (1) screening procedures for the attendees (online application, a rationale given by each applicant for their reason for attending, and support letters from two mentors); (2) infrastructure (hotels, meals, sites for the didactic and hands-on training); (3) faculty to provide didactic training and mechanisms for presenting didactic information; and (4) hands-on training (wet lab sample extraction, on instrument experiments and computational data analysis). The 20 attendees ranged from graduate students to full professors and there was a marked range of experience in metabolomics. Their opinions collected during and after the workshop have been analyzed statistically. Using a 1-5 scale with 5 being the best, the strength of the workshop was the quality of the faculty (Dr. Kathleen Stringer (U. Michigan – sample extraction), Dr. Natalie Serkova (U. Colorado Medical Center – NMR and NMR data processing), Dr. Olga Ilkayeva (Duke – targeted LC-MS), Dr. Xiuxia Du (UNC-Charlotte – MS data processing and statistical analysis) and Dr. Dean Jones (Emory – Pathway analysis) and the talks they presented. Drs. Stephen Barnes and Hemant Tiwari from UAB provided talks on an overview of metabolomics, experimental design, integrating metabolomics with other omics, and the future trends in –omics. In addition, three manufacturers gave talks (Brigitte Simons AB Sciex – SWATH analysis; Mark Bennett, NonLinear Dynamics – software analysis; and John Shockcor, Waters – a full clinical application based on LC-MS analysis). The hands-on aspects resulted in a wider range of opinions. The hands-on extraction session involved all 20 attendees using a yeast sample that was extracted using different protocols (initial washing with very cold methanol, ice-cold water, or ice-cold or room temperature physiological saline, each followed by extraction with cold methanol). These samples were carried forward to LC-MS analysis – setting up a MRM mass transition, product and precursor ion scanning for lipid classes, comprehensive (SWATH) lipid analysis, and a high mass accuracy and resolution analysis of lipid ions using FT-ICR MS. The attendees were broken into two groups of ten – one group did the LC-MS training modules, whereas the other went to the NMR hands-on session. These training sessions lasted 2-2.5 hr and for LC-MS, each segment lasted 30 min and involved 2-3 attendees. The NMR session was based on freeze-dried samples brought to UAB by Dr. Serkova. The hands-on computer session had the biggest variation in comments. This was a result of unfamiliarity of the attendees in using command line programming and a lack of student helpers providing support to the attendees. To meet the challenges of the larger 2014 workshop, we propose the following: the Workshop speakers will be augmented by experts in experimental design, untargeted metabolomics, trans-omics and pathway analysis. The workshop materials (lecture slides, hands-on protocols and datasets [raw and analyzed]) will be made available from the Cloud. The 3.5 day workshop should be extended to 4.5 days to increase time for hands-on sessions which will be pre-prepared. A decision also needs to be made about the experience level of the attendees, i.e., whether to restrict acceptance to beginners; otherwise, a multiple track Workshop should be created with a focus on data analysis for the more experienced investigators.

Abstract #7

High Resolution ^{1}H NMR Metabolic Profiling Using Slow Magic Angle Spinning

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NMR, a quantitative, non-destructive method that requires no or minimal sample preparation, is one of the leading analytical tools for metabonomic research. However, the requirement of sample volume of a few tens of μL (mg) or more for standard NMR metabolomics analysis limits its applications. This is especially a problem in research involving small laboratory animals such as mice, where animals need to be sacrificed to obtain adequate amount of tissue or blood for analysis. The sacrificing procedure makes it impossible to carry out a continuous study on a single animal over a long period of time. It is known that metabolomics data are sensitive to normal biological variation such as gender, age, and the health condition of individuals, etc. Often the changes of metabolite biomarkers due to normal biological variations are as big as the changes due to an insult, or stimulation that is intended to investigate. This makes metabolomics investigations expensive as a large number of animals need be used for establishing the biostatistics of a result. Thus, it is important to develop method that can perform metabolic profiling on small intact biological samples.

In this poster, we will introduce a non-destructive slow magic magic angle spinning (as slow as about 40 Hz) NMR technique that is capable of high resolution and high sensitivity metabolic profiling on biological samples, in particular tissue samples, with sample volume from as small as 200 nanoliters (nL) to as large as a milliliter or more using a single probe and using only a few minutes. The nanoliter capability may make it possible to follow the metabolic changes through a continued investigation on a single small laboratory animal over a long period of time using minimally invasive blood and tissue biopsy samples. While the milliliter capability would allow minimal destructive studies of intact biological object with size as large as a cm or more. This technique, after it is fully developed, has the potential to enable large scale metabolic profiling on intact biological tissues of various sizes that may have wide application in biomedical, clinical and translational researches.

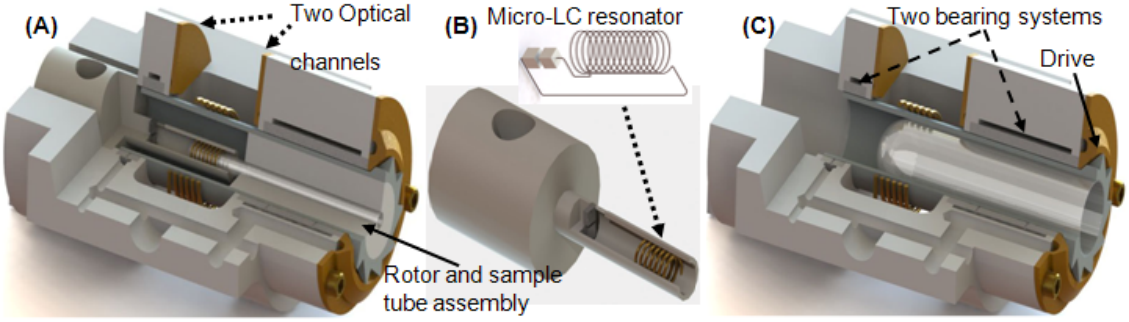

***Figure 1**. **The design of slow-MAS NMR metabolomics probe** that is capable of metabolic profiling on biological tissue and biofluids with volume as small as 200 nanoliters (0.2 mg) and to volume as large as 1 cm^3 or more (tissues only). (A) The design for small samples of variable sizes; (B) Highlighting the switchable plastic plug in (a) with the micro-LC(RF) resonator mounted inside the plug support; (C) The design for working with large biological objects of volume of 1 cm^3 or more by removing the switchable plug in (A).*

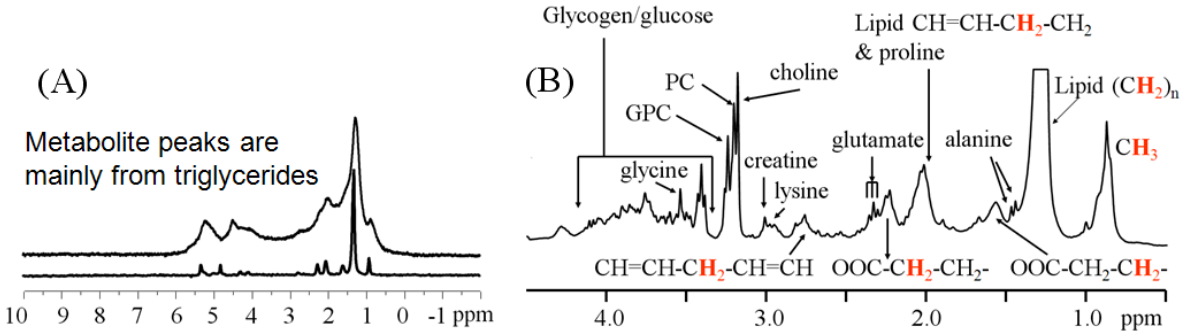

***Figure 2: Slow-MAS Preliminary Results. (A) Small volume biological tissue**: 2.6 minutes ^{1}H slow-MAS NMR spectra of 200 nanoliter (~0.2 mg) muscle from an obese mouse acquired at a sample spinning rate of 147 Hz (bottom trace) compared with spectrum acquired using standard method (top trace). Significant spectral resolution enhancement is obtained with slow-MAS. **(B) Large volume biological tissue:** ^{1}H slow-MAS NMR spectrum of a whole left lung lobe (~100 mg) from a 7-days post-silica exposed C57BL/6 mouse acquired at a sample spinning rate of 80 Hz. Many metabolites are observed.*

Abstract #8

Identifying Individual Differences of Fluoxetine Response in Juvenile Rhesus Monkeys by Metabolite Profiling

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Fluoxetine (Prozac®) is the only psychopharmacological agent approved by the FDA for children and is commonly used therapeutically in a variety of developmental disorders. Therapeutic response shows high individual variability, and severe side effects have been observed. A new collaboration using metabolomics to identify biomarkers of response to fluoxetine in a group of juvenile rhesus monkeys who are concurrently assessed for health, growth, sexual maturation, and behavior forms the base of this *NIH Common Fund Supplement Project*. It extends the developmental psychopharmacology goals of the parent grant to prediction of individual responses of children to this well-known psychopharmacological agent. Juvenile male rhesus monkeys currently being treated daily with fluoxetine (2.4 mg/kg-d) or vehicle (n=16/group) and evaluated for growth and behavioral functioning are sampled for blood, cerebrospinal fluid (CSF), and skin biopsies for fibroblast culture. The study group is also genotyped for MAOA and 5-HTTLPR polymorphisms. The samples are analyzed using a GC-TOF-MS platform in the *West Coast Metabolomics Center*. The metabolite signal intensity data are explored to determine markers which distinguish treated and untreated groups. Metabolite /behavior and metabolite/growth associations are evaluated using multivariate analysis of metabolite intensities versus apical variables of each behavioral/morphometric assay. Our preliminary analyses have identified plasma and CSF metabolites that distinguish fluoxetine- from vehicle-treated animals. Blood, CSF and fibroblasts will be further compared to optimize detection strategies. Finally, pathway analysis will be applied to the metabolites selected as biomarkers to elucidate the underlying biological targets.

Abstract #9

Metabolomics Data Analysis Framework – database and web portal for curation and analysis of mass-spectrometry based metabolomics data.

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Novel Aspect

An open source web portal and back-end database structure for quality control and exploratory analysis of mass-spectrometry based metabolomics data.

Introduction

Due to significant advances in analytical instrumentation mass-spectrometry based metabolomics experiments become more complex and produce large amounts of data. The tasks of quality control and consistent identification and quantitation of compounds across multiple data sets is a recognized bottleneck in the analysis workflow. In order to increase efficiency and quality of our service as a core metabolomics facility we augmented our existing LIM System with a package of web-based tools for in-depth quality control and data mining.

Overview

Metabolomics Data Analysis Framework (MDAF) has two major components – database and web-based analysis and visualization tool set. The database currently implemented in Oracle is shared with LIMS and provides access to all the logistics behind the core operation (user and sample information, methods, protocols, inventory), compound libraries and pre-processed experimental results. Web-based application (PHP/Apache/R) contains a number of modules that allow creating and editing data sets from available experimental results, evaluating quality of individual samples and data sets, search, browsing and exporting the data, performing exploratory data analysis, visualizing and exporting the results. Separate module is dedicated to managing compound libraries. There is also a set of generic tools for mass spectra interpretation (predict formula by mass/isotopic composition, calculate masses of adducts, predict isotopic pattern).

The system supports different levels of access to ensure proper data protection and sharing.

Conclusions

Presented software package extends the standard LIMS functionality to allow rigorous quality control of metabolomics data and exploratory data analysis. Its flexible architecture is open for further development and improvement.

Abstract #10

Metabolomics of Stored Red Blood Cells Prior to Transfusion: Reproducible, Donor-Specific Alterations in Numerous Metabolites

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1.

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Background: Population based studies suggest that red blood cells (RBCs) are therapeutically effective when collected, processed and stored for up to 42 days under validated conditions prior to transfusion. However, studies of RBC persistence in the circulation after transfusion have suggested that considerable donor-to-donor variability exists, and may affect transfusion efficacy. To investigate the underlying basis of donor variability, we collected and stored RBC units from several donors and quantified the kinetic changes in hundreds of chemical metabolites over 42 days.

Study Design and Methods: Leukoreduced AS-1 RBC units prepared from a total of 13 research donors were serially sampled for global metabolomic profiling over 42 days of refrigerated storage.

Results: Over several experiments, 185-264 defined metabolites were identified and quantified in stored RBC samples. These analyses confirmed well-recognized changes in several metabolites including 2,3-DPG, pyruvate and lactate; these metabolites were representative of the vast majority (83%) of identified RBC biochemicals in that their kinetic profiles were consistent between donors and on subsequent donations. In contrast, a second group of metabolites was identified (constituting 10% of the total) for which kinetic patterns differed significantly and reproducibly between donors. Metabolites in this category included caffeine, aspartate and catechol sulfate. The third group (7%) of biochemicals showed donor variability that was not reproducible. To begin to identify RBC biochemicals for which donor-specific variability correlated with transfusion efficacy, we repeated metabolomics on RBC units from research donors previously shown to have above- or below-average RBC survival following 42 day storage and autologous transfusion.

Conclusion: Using global metabolomic profiling, we identified several metabolites with donor-specific changes during storage. By examining metabolites in research donors with know RBC survival characteristics, we were able to identify a subset that correlate with RBC survival after transfusion and thus perhaps transfusion efficacy.

Abstract #11

Abstract

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An estimated $58 billion in medical costs are associated with complications of bacteria-contaminated chronic wounds, resulting in significant quality-of-life limitations for the over 18 million diabetics in the United States. Contributing to therapeutic resistance, contamination of wound beds by skin-resident, opportunistic pathogens are often characterized by the presence of the biofilm mode-of-growth. Bacterial biofilms are distinguished by phenotypic heterogeneity of a surface-attached community and are characterized by an altered sensitivity to antibiotics and immune defenses. Both methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* (MRSA and MSSA, respectively) are commonly found in the chronic wound bed and, in the biofilm mode-of-growth, contribute to chronicity of the wound. Transcriptomic and proteomic analysis of *S. aureus* biofilms have suggested that *S. aureus* biofilms exhibit an altered metabolic state relative to *S. aureus* grown in the planktonic mode-of-growth. Comparison of secreted and intracellular small molecule metabolites from MRSA and MSSA biofilm and planktonic cultures collected over multiple growth phase time courses demonstrates that biofilms utilize distinct metabolic mechanisms adapted to the biofilm mode of growth. ^{1}H nuclear magnetic resonance (NMR) metabolomics profiles identified through spectral feature analysis using the Chenomx™ metabolite database were used to classify and distinguish between growth phenotype and *S. aureus* strains. Pattern recognition assessment through principal component analysis (PCA) demonstrated that *S. aureus* biofilm mode-of-growth can be distinguished from its planktonic mode-of-growth based on distinct patterns of metabolite utilization which can serve as characteristic metabolic markers. Loading plots identified metabolites that significantly contributed to statistical clustering of the biofilm phenotype and provided insight into metabolic investments that characterized the biofilm phenotype.

Abstract #12

Title: **Large-scale metabolite determination using chemoselective approaches**

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ABSTRACT
Recent advances in metabolomics technologies, especially those that combine the complementarity of mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR) enables rapid analysis of thousands of peaks representing hundreds of metabolites. However, key barriers remain for large-scale metabolomics applications, most notably (i) higher throughput with robust metabolite assignment and quantification (ii) identification of unknown metabolites (iii) analysis of low abundance/labile metabolites (iv) reconstruction of metabolic networks and their regulation, (v) multicompartment metabolic flux analysis. We have adopted an integrated approach that uses chemoselective (CS) tagging of key metabolite functional groups to boost the speed and accuracy of metabolite identification while enhancing detection. Such tagged metabolites are readily amenable for multiplexed analysis, reliable quantification and pathway analysis via stable isotope encoding.

We have demonstrated these advantages with CS tagging of carbonyl-containing metabolites using an aminooxy-based probe (QDA, quaternary ammonium with C12 alkyl), followed by analysis of the derivatives from crude A549 cancer cell extracts using direct infusion nanoelectrospray, ultra high-resolution Fourier transform-ion cyclotron resonance-MS (FT-ICR-MS) at orders of magnitude higher sensitivity than the native metabolites. More than 500 C=O metabolite adducts were observed, for which the parent metabolites were not detectable without CS tagging. Quantification of metabolites (e.g. pyruvate and α–ketoglutarate or αKG) in crude cell extracts was achieved by establishing standard addition curves with $^{13}CD_3$-QDA adducts of authentic standards. Fractional enrichment analysis of ^{13}C-labeled pyruvate and αKG isotopologues in A549 cells given $^{13}C_6$-glucose and subjected to anti-cancer selenite treatment revealed compromised ^{13}C incorporation into different ^{13}C isotopologues of αKG and $^{13}C_3$-pyruvate. These results suggest that selenite acts at least in part by inhibiting the Krebs cycle and glycolysis.

To combine the power of FT-ICR-MS and NMR for structural elucidation, we introduced ^{15}N to QDA such that the 2- or 3-bond coupling between ^{15}N and metabolite ^{1}H in the adducts (R_1-O-^{15}N=C(R_2,R_3) where R_2 and R_3 derive from the target metabolite) can be analyzed by 2D ^{15}N-filtered heteronuclear NMR techniques such as long-range ^{1}H{^{15}N} HSQC and HSQC-TOCSY. This not only dramatically enriches C=O metabolite detection by NMR but also enables their identification using chemical shift data, the nature of the functional groups, and accurate mass from MS. Aldehydes were characterized by the aldehydic proton resonating at ca. 7.6 ppm, with variable ^{15}N shifts near 370 ppm whereas ketone compounds had ^{15}N shifts 20-30 ppm upfield of the aldehyde compounds.

Tailored chemoinformatic development is ongoing including methods for identifying functional groups (FG) from a MOL file representation of given metabolites in HMDB and KEGG databases, use of FG-encoded molecular formulae database for enhancing CS adduct identification based on accurate mass, mapping isomeric formulae in metabolite databases, and efficient algorithm for robust assignment of metabolites and labeled isotopologues.

Together, the combined approach promises to accelerate analytical throughput while widening the classes of analytes, including metabolically enriched isotopologues, far beyond current limits.

Abstract #13

Pathway Enrichment Analysis Based on Estimating the Underlying Interaction Network

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Pathway enrichment analysis has become a key tool for biomedical researchers to gain insight in the underlying biology of differentially expressed genes, proteins and metabolites. It reduces complexity and provides a systems-level view of changes in cellular activity in response to treatments and/or progression of disease states. Methods that use pathway topology information has been shown to outperform simpler methods based on over-representation analysis. However, despite significant progress on this task as evidenced by information available in knowledge data bases such as KEGG, Reactome, BioCarta, etc., the latter is not cell and/or condition under study specific. We propose to combine a network estimation procedure based on cell and condition specific Omics data with interaction information gleaned from biological data bases. The resulting pathway topology information is subsequently used for pathway enrichment analysis. The performance of the proposed methodology is illustrated using in-silico experiments and illustrated on a prostate cancer study involving metabolomics data.

Abstract #14

Metabolic Differences Between Light- and Dark-Adapted Mouse Retinas

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Purpose: In normal retinas, the production of large amounts of ATP, as well as exposure to UV light and high levels of oxygen from the choroidal circulation all give rise to reactive oxygen species (ROS) that can potentially damage proteins, lipids and DNA in photoreceptors. Additionally, these cells have high levels of photosensitive retinoid derivatives that are easily oxidized, becoming toxic to the cell. However photoreceptors in normal retinas are thought to protect themselves with a system of intracellular antioxidant enzymes and reducing agents. The goal of the present work is to define metabolic changes that occur in wild-type mouse retinas under cyclic light and dark-adapted conditions for future comparisons with animal models of retinal degeneration where light is often an exacerbating factor, as is also the case in humans.

Methods: C57BL/6J mice were raised under a normal light/dark cycle at ambient light levels of approximately 80 lux. At the age of approximately 1 month, mice were exposed to 250 lux for 3 h or adapted overnight in complete darkness. Light-adapted animals were euthanized, eyes were enucleated and the retinas dissected in PBS, followed by rapid freezing in liquid nitrogen. For dark-adapted mice, identical procedures were carried out under dim red lights. Samples were stored at -80 ˚C until processed for metabolic analysis. Four retinas from 2 mice (~15-20 mg total) were placed in a single tube and treated as a single sample. Each sample was homogenized in buffer containing 50:50 acetonitrile:water at a ratio of 1mg:25 µL tissue:buffer. A 320 µl aliquot of homogenate (12.8 mg tissue equivalent) was transferred to a tube and centrifuged. The supernatant was dried and re-suspended in 100 µl 95:5 H_2O:methanol for UPLC-TOF-MS analysis. All data were acquired on a G-2 SYNAPT-QTOF mass spectrometer equipped with the Acquity UPLC system (Waters Corporation, MA). A 10 µl injection was used for sample analysis and the metabolites were eluted from a BEH HSST3 column using a gradient separation. All UPLC-MS data were analyzed using TransOmics (Waters Corporation, MA) to determine the group differentiating markers of dark- and light-adapted retinas.

Results: Approximately 2,000 compound ions were detected. Differences in the metabolic profiles of retinas from dark- and light-adapted animals were observed using Orthogonal Projections to Latent Structure-Discriminant Analysis (OPLS-DA). OPLS-DA analysis identified group differentiating markers with a VIP (Variable influence on projection) value ≥ 2.0. Analysis of the group differentiating markers using the Human Metabolome Data Base revealed members of the retinol metabolism pathway (11-cis-Retinol, 11-cis-Retinal, All-trans-Retinoate, and 9-cis-Retinol). Several classes of lipids, amino acid derivatives and nucleotides were also distinguished between the dark- and light-adapted retina samples.

Conclusions: We evaluated the metabolic profile of wild type mice to determine the normal metabolic changes that occur in the retina under dark- and light-adapted conditions. Since light is an exacerbating factor in a number of retinal degenerative diseases, our results serve as a foundation for future studies to evaluate and understand changes in metabolism that occur in animal models for retinal degeneration. We anticipate that these studies will reveal novel pathways that lead to improved therapeutic strategies for retinal degeneration.

Abstract #15

Abstract

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In the context of metabolomics, reverse-phase chromatographic separations are undoubtedly the most prevalent. However despite their frequent use in metabolomics, the suitability of C18 column chemistries for separating the highly polar metabolites commonly found in biofluids, or tissue extracts remains questionable, with their impact on the study of the metabolome unknown. Considering a substantial number of the metabolites currently under investigation from biological samples are extremely polar, and subsequently poorly retained by reverse-phase column chemistries, alternative chromatographic strategies should be considered, and their effect on data sets characterized. Therefore, we are exploring the use of a variety of column chemistries including amide, amino, and silica, along with alternative reversed phase materials such as phenyl, phenyl-hexyl, and graphite. Mixed-mode column technologies containing both reversed phase and ion exchange functionalities will also be examined. Of particular interest, is the use of hydrophilic interaction liquid chromatography (HILIC) to improve the retention and separation of polar or charged metabolites. HILIC column chemistries are typically composed of a zwitterionic stationary phase that promotes retention and separation through enhanced partitioning on the adsorbed water layer, and through weak electrostatic interactions of the stationary phase. Optimizing the separation of hydrophilic metabolites will improve the quality of metabolomic studies, since poor retention can cause signal suppression resulting in compromised quantification, and reduced overall coverage of the metabolome. Using a chemically defined artificial liver metabolome comprised of known metabolites at defined concentrations, optimal chromatographic conditions and column chemistries for the separation of various classes of compounds including amino acids, bile acids, carnitines, acyl-coA's, nucleosides mono-, di-, and triphosphates, fatty acids, ceramides, eicosanoids, glycerolipids, phospholipids, and sterols will be determined. Chromatographic methodologies will be further developed based on mass spectrometry platform with specific adjustments being made for either targeted metabolomics profiling studies via multiple reaction monitoring, or high resolution full scan MS analysis of complex mixtures for non-targeted open profiling studies. The developed methods will then be used to analyze extracts from cultured cells and liver tissue homogenate from both healthy and diseased mice to evaluate how perturbing the system via dietary changes or acetaminophen-induced liver toxicity affects metabolite identification.

Abstract #16

Platelet Specific Knockout of Mitofusin 2 Leads to Impaired Mitochondrial Function and Altered Platelet Platelet Reactivity.

K01 Mentored research in Metabolomics update.

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It is well known that platelets from obese individuals, who are at risk for platelet mediated cardiovascular events, are hyper-reactive. The mechanism for this is unclear. Metabolomics analysis of platelets from obese individuals compared to healthy controls suggested mitochondrial dysfunction may be present. In addition, transcript expression levels for mitochondrial related transcripts, including Mitofusin 2 (*Mfn2*), are altered in platelets from obese individuals. To specifically test the role of Mfn2 in altered platelet reactivity, we have generated platelet specific Mfn2 deficient mice (Mfn2 KO). Seahorse analysis of MFN2 KO mouse platelets demonstrated both lower baseline and lower maximal oxygen consumption rates (OCR) compared to wild type (WT) littermate controls. Mfn2 KO mice displayed heightened reactivity compared to controls in response to Convulxin and Par4 agonists. Platelets from mice fed a high fat diet (HFD) for 10 weeks, also demonstrated heightened reactivity in response to Convulxin, but in contrast to Mfn2 KO mice, displayed increased baseline and maximal OCR compared to mice on a low fat diet (LFD). Additionally, changes in the metabolome of platelets from HFD versus LFD fed mice were observed. The significance of these changes, and their relationship to Mfn2 expression remain to be determined.

Abstract #17

Title: Developing a High-Throughput Platform for Structural Characterization of Metabolites during Untargeted Profiling

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The objective of untargeted metabolomics is to measure as many metabolites as possible from a biological specimen without bias. Over the last decade, advances in mass spectrometry and bioinformatic software has enabled the detection of thousands of peaks in most research samples. To date, however, the ability to structurally characterize large numbers of these peaks has been limited and represents a major barrier in the growth of the field. Here we describe a metabolomic platform to facilitate structural identification of compounds in untargeted analyses. Our workflow relies on the acquisition of MS1 and MS2 data simultaneously in addition to data processing by the software packages XCMS Online and decoMS2. XCMS Online uses MS1 data for quantitation while decoMS2 deconvolves MS2 data such that purified spectra can be matched to the METLIN metabolite MS2 library for qualitiative identification. We demonstrate the power of this platform by applying it to astrocyte cell cultures challenged with lipopolysaccharide, an endotoxin known to activate an immune response and cytokine production in astrocytes.

Abstract #18

NIH Regional Comprehensive Metabolomics Resource Core at RTI International (RTI RCMRC)

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Abstract:
NIH Regional Comprehensive Metabolomics Resource Core at RTI International (RTI RCMRC) has a wide range of instrumentation to facilitate broad spectrum and targeted metabolomics analysis of polar or nonpolar components, as well as methods for targeted analysis of metabolites and minerals. Current analytical platforms available to collaborators include: Nuclear magnetic resonance (NMR) spectroscopy, Liquid chromatography-mass spectrometry (LC-MS/MS, UPLC-Q-TOF-MS), Gas chromatography-mass spectrometry (GC-MS, 2D-GCTOFMS), Inductively-coupled-plasma mass spectrometry (ICP-MS) and MALDI imaging. The RTI RCMRC has experience with analysis of cells, organ tissue (e.g., liver, uterus, testes, brain, muscle, lung, breast tissue, retina), biological fluids (e.g., urine, serum, plasma, amniotic fluid), fecal and cecal matter. More recently, RTI RCMRC has worked to examine exhaled breath from human subjects or animal models. Following signal detection, RTI scientists apply statistical and mathematical tools (e.g., Umetrics, Spotfire, SAS) and use their expertise to identify data trends that show the correlation of specific signals with the phenotypic response under investigation. Identified signals are mapped to biochemical pathways through the use of specialized software, such as GeneGo, and expert biochemist interpretation to derive biomarkers and mechanistic insights. RTI RCMRC has several ongoing collaborative projects spanning diverse research areas. Highlights of metabolomics analysis in the areas of drug-induced liver injury (DILI), nanotoxicity, osteoarthritis, immunology and food metabolism will be summarized.

Abstract #19

Metabolomics and Fluxomics in Mammalian Tissue: Method Optimization and Insights into Metabolite Flux during Exercise

Charles R. Evans, Katherine A. Overmyer, Nathan Qi and Charles F. Burant

Mammalian tissue such as skeletal muscle, liver, and adipose are widely-studied targets of metabolomics experiments, since alteration in function of major organs is a key feature of diseases including cancer and diabetes. Metabolomics of organ tissue introduces particular challenges regarding tissue collection and data normalization which have not been as thoroughly studied as many other aspects of sample preparation. We present experimental results from targeted and untargeted LC-MS analysis of rodent tissue collected under various modes of commonly-used anesthesia. Different modes of anesthesia resulted in significant alterations in central carbon metabolism including changes in hexose phosphates, lactate, TCA cycle intermediates, and nucleotide phosphates. Based on these findings we have developed refined methods for tissue collection and extraction for metabolomics studies. We have applied these methods to the study of metabolite concentrations and metabolic flux during exercise. In these experiments, carbon-13 enriched stable-isotope tracers were administered to rodents immediately prior to treadmill exercise. Mass isotopomer analysis allowed detection of tracer incorporation into downstream metabolites and enabled assessment of relative flux under different exercise conditions. These experiments demonstrate the feasibility of these methods for studies of animal models of disease and their potential for studies involving human subjects.

Abstract #20

Vanessa Phelan
University of California, San Diego

Microbial biofilms are ubiquitous throughout nature. These surface-associated heterogeneous mixtures of microbes are found on surfaces ranging from rocks, soil and surgical equipment to the oral cavity, intestine and skin of humans. When at equilibrium, the microorganisms of these biofilms coexist in stable communities. However, when these communities are perturbed, the effect can be unregulated growth of opportunistic pathogens such as *Pseudomonas aeruginosa*. Biofilms are typically investigated as single species, but in nature are diverse microbial communities. The members of these polymicrobial biofilms work together leading to increased resistance to antibiotic therapy, immune responses and disinfectants. Many of the interactions involved in microbial collaboration within polymicrobial biofilms are dependent upon secreted molecules. Yet, remarkably, no studies address the convergence of multiples signals produced by multiple microorganisms in biofilms. In cystic fibrosis patients, a genetic defect in chloride transport prevents effective mucociliary removal of microbes. Despite aggressive antibiotic therapy, polymicrobial biofilms formed within the lungs persist.

By co-culturing the most common co-isolates from cystic fibrosis lung infections as polymicrobial biofilms, we investigate the role of metabolic exchange between microbes in biofilm maturation. We hypothesize that microbial isolates of biofilms interact at a chemical level and that a convergence of these multiple chemical signals determine polymicrobial biofilm maturation. Recent advances in mass spectrometry based imaging (IMS) methods allow for the characterization of the spatial and chemical distribution of extracellular metabolites in colony biofilm interactions. Additionally, the recently developed nanospray desorption electrospray ionization (nanoDESI) allows for sampling and microextraction of the biofilm colony's surface. Using these two advanced technologies, we will identify the chemical basis of microbial competition within biofilms. To investigate these metabolic interactions, we co-culture and monitor the metabolic changes when five organisms commonly associated with cystic fibrosis are co-cultured as a single biofilm. This provides direct insight into the multiplexed molecular signals controlling mixed species biofilm formation and is the first investigation to define a complex molecular network between organisms in a system-wide fashion.

Abstract #21

Targeting cancer metabolic reprogramming to inhibit DNA repair and block cell immortality: Metabolomics and mechanisms

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Along with increased interest in cancer metabolism over the past decade has come the understanding that cancer metabolic reprogramming that extends far beyond the Warburg effect. Important concepts such as glutamine addiction and the emerging links to epigenetics have helped push metabolism to the forefront of emerging cancer targets. In turn, along with discovery of new pathways, new small molecules have been discovered targeting metabolic pathways or serving as oncometabolites. The current paradigm holds that metabolic reprogramming arises in response to increased demands for building blocks to support cancer cell growth. However, along with deregulated proliferation, cancer cells also display genomic instability and cell immortality. Our studies are directed at testing how metabolic reprogramming might impinge on cellular responses to DNA double strand breaks including DNA repair, DNA damage checkpoints, apoptosis and cellular senescence.

Our work, along with other recent studies, has revealed that disrupting glucose or glutamine metabolism can sensitize cancer cells to genotoxic stress. To dissect mechanisms, we are using global metabolomic profiling as a tool to identify key metabolites associated with rapid DNA repair and resistance to senescence in cancer cells. Using MCF7 breast cancer as a model, we have screened nearly 30 drugs and oncometabolites targeting different key pathways including glycolysis, TCA cycle, glutaminolysis, serine synthesis, PPP, and HBP for effects on response to ionizing radiation. As controls, we cultured cells in media with altered levels of glucose and/or glutamine. Metabolites extracted with cold methanol were analyzed by LC-MS(/MS) with isotopic standards. Ions were identified and data visualized with MAVEN software. The resulting patterns of response, reflecting changes in up to 100 metabolites, were also examined by both unsupervised and pathway-driven analysis. This work is *en route* to populating a map of perturbations which may offer insights regarding crosstalk among pathways and the origins of complexity in the metabolic network. However, our analysis to date has yet to reveal a specific metabolite as the critical mediator.

Cell-based studies show that both glucose and glutamine metabolism contribute to cancer cell resistance to senescence, likely reflecting a dual contribution to pathways including the TCA cycle and HBP. Ongoing work is revealing how the balance of glucose and glutamine metabolites determines DNA damage response. Current work is focused on dissecting metabolic regulation of methylation states of histones at sites of double strand breaks, a well studied determinant of DNA repair and damage signaling.

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Abstract #22

Metabolomic Measurements in Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder that is due to a mutation in the dystrophin gene. DMD has a prevalence of 1 in 3,600-6,000 male births and is characterized by progressive muscle deterioration, loss of functional abilities, and reduced life expectancy. Treatments have shown limited effectiveness [e.g., corticosteroids] and currently there is no cure for DMD. However, there are a number of interventions that have shown promise in preclinical and early clinical trials, including adeno-associated virus delivery of microdystrophin genes, exon skipping, and small molecule therapies, such as stop codon suppression drugs. As a result, there is a pressing need for sensitive and reliable biomarkers capable of tracking disease progression and efficacy of intervention strategies in clinical trials.

This supplemental project has the unique opportunity to advance the development of analytical tools linking the metabolome to the genomic and physiological status. The parent project follows 100 boys with DMD (ages 5-14 years) and has developed a comprehensive centralized bioinformatics infrastructure that permits the mining of all the functional, genetic, and magnetic resonance imaging and spectroscopy parameters acquired in this well characterized subject cohort.

Despite promising results looking at serum proteins (MMP-9[1], osteopontin[2]), SNPs[3], and microRNAs[4], currently there is no accepted serum or urine biomarker for the detection of disease progression in DMD. The serum/urine proteome/metabolome of boys with DMD is expected to be complex, representing intersecting pathways between mitochondrial dysfunction, inflammation, muscle damage and remodeling in both skeletal and cardiac muscle. To investigate the possible complications, preliminary studies are conducted using a murine DMD model, the *mdx* mouse. Serum samples from mdx mice and controls were run using a new high temperature superconducting NMR probe allowing for both ^{1}H and ^{13}C metabolomics. Correlations between ^{13}C and ^{1}H spectra showed a unique perspective of metabolites in DMD versus control samples and allowed identification of metabolites that could not be made using proton spectroscopy alone. Similar methods were used to investigate the metabolomes of the *mdx* mice tissue, the direct site of disease. This revealed a metabolic profile similar to that found in the serum. The *mdx* model was used as a proof of principle that the metabolic profile of the non-invasive serum was correlated to the metabolome at the tissue level.

From these preliminary results, we feel confident in translating this technique to human samples. We currently have an approved IRB addendum on the parent grant, ImagingDMD, to collect blood and urine samples from boys in the age range of 5-14. To date, a total of 24 participants have consented to provide urine samples (92%) and 75% have consented to both blood and urine collection. The parent grant provides unprecedented access to information on the primary genetic defect, status of disease progression/severity as assessed by loss in muscle function as well as MRI, demographics, medication/supplements, corticosteroids use, quality of life questionnaires, and daily activity. We will use the metabolomics data to add to this database and begin making multivariate correlations between multiple data variables in search of a sensitive biomarker.

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Abstract #23

Metabolomic Characterization of Histone Deacetylase 3 Function in Liver and Muscle

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Abstract

Histone deacetylase 3 (HDAC3) is an ubiquitously-expressed epigenomic modifier involved in gene expression repression by various nuclear hormone receptors. We have previously shown that genomic occupancy of HDAC3 in mouse liver is rhythmic and controlled by the circadian clock through nuclear receptor Rev-erbα &β. Depletion of HDAC3 in liver enhances de novo lipogenesis and results in severe hepatic steatosis, but paradoxically improves insulin sensitivity and reduces glucose production. Different lipid species have been proposed to cause or protect against insulin resistance. Here we perform lipidomic profiling of HDAC3 depleted livers to provide new insights into lipid-induced insulin resistance. In contrast to liver, muscle-specific depletion of HDAC3 induces insulin resistance and glucose intolerance. Here we perform both static metabolomic profiling and dynamic flux tracing of glucose utilization using 13C-glucose in HDAC3-depleted muscles. We find that there is decreased glycolytic flux and a likely reciprocal increase in anaplerotic processes coupled with amino acid catabolism in the absence of HDAC3 in muscles. These findings, in combination with epigenomic and transcriptomic analyses, will shed light on how the epigenome integrates various hormonal signals in regulation of intermediary metabolism in liver and muscle.

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Abstract #24

Probing functions of NAM and NAD^+ salvage in development and cell survival in *C. elegans*.

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Dietary deficiency of nicotinamide and nicotinic acid (alternate forms of vitamin B3) causes severe dysfunction in multiple mammalian organs. These metabolites are substrates for biosynthesis of nicotinamide adenine dinucleotide (NAD^+), but how this relates to the molecular mechanisms causing organ pathologies is unclear. The parent grant is focused on understanding how the metabolism of NAD^+, nicotinamide and nicotinic acid is coordinated amongst tissues on an organism-wide level and on deciphering the mechanisms by which changes in the availability of NAD^+ and its biosynthetic precursors affect physiology and development using the *C. elegans* model. Our metabolomics supplement allows us to expand our aims from targeted analysis of a few metabolites specifically in NAD^+ biosynthetic pathways to widespread characterization of global changes that occur upon perturbation of vitamin B3 availability and processing.

Our studies make use of a strain with a mutation in the first enzyme of the salvage pathway for biosynthesis of NAD^+, PNC-1 nicotinamidase. PNC-1 converts nicotinamide (NAM) to nicotinic acid (NA). Mutation of *pnc-1* results in distinct developmental and functional defects in the reproductive system and other physiological abnormalities, some of which arise due to decreased NAD^+ biosynthesis, some due to accumulation of nicotinamide and some by a combination of both effects. We will present our analysis of specific phenotypes with a focus on our use of metabolomics approaches to shed light on the specific biology.

neuroendocrine uv1 cell necrotic death: uv1 neuroendocrine cells necrose in *pnc-1* mutants because of NAM accumulation. Global metabolomics analysis demonstrated that glutamate is elevated in *pnc-1* mutants and in wild-type animals supplemented with excess NAM. Moreover, this elevation in glutamate levels, like the cell death phenotype, was not corrected by supplementation of mutants with nicotinic acid. Genetic analysis confirms that glutamate signaling contributes to NAM toxicity.

Lipid homeostasis: NAM-induced uv1 cell death can be completely prevented by activation of EGF signaling. Using genetic approaches we have revealed a role for phosphocholine synthesis downstream of EGF signaling in promoting cell survival. Because *pnc-1* mutants also have a fat storage defect, we are using metabolic approaches to probe the impact of EGF and PNC-1 on lipid metabolism.

Abstract #25

Targeted Metabolomic Analysis of Nucleosides in biological fluids by Isotope Dilution Liquid Chromatography electrospray ionization tandem Mass Spectrometry

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Altered nucleoside metabolism plays a critical role in many proliferative disorders such as diabetic complications, heart failure, uremia and cancer. Key components of this metabolic pathway include Uracil, Pseudouridine and Dimethyl Guanosine which are part of the ribonucleic acid (RNA) structure and physiology and Allantoin which is related Purine degradation and oxidative stress. Several analytical methods are used to measure extremely polar nucleotide metabolites in biological samples but they have inherent issues that include lack of specificity, sensitivity, and difficulty in sample preparation. We have developed a facile assay to reproducibly measure nucleosides in biological fluids. Using liquid chromatography electrospray ionization mass spectrometry (LC/ESI/MS), we optimized chromatographic separation techniques to achieve a highly sensitive assay.

Samples were protein precipitated with v/v acetyl nitrile after stable isotope dilution. The nucleosides were separated on Luna C_{18} column. The mobile phase was 0.1% formic acid and acetonitrile with 0.1% formic acid. The gradient was raised to 10% acetonitrile in 3 minutes, and then a linear gradient of 10% to 100% acetonitrile, for 3 to 10 minutes followed by isocratic 100% acetonitrile, for 10 to11 minutes with a flow rate of 0.2 ml/min. Flow injection analysis (FIA) was used to optimize the fragmentor voltage, collision energy and cell acceleration voltage for nucleotide metabolites. Multiple reaction monitoring (MRM) experiments in ESI positive ion mode was developed to quantify the level of nucleoside metabolites from various biological samples.

We developed a method for quantifying these metabolites using Luna C_{18} column with MRM tandem mass spectrometry. Different LC columns including Hydrophilic interaction chromatography (HILIC) and various solvent and ion pairing systems were attempted with search for optimal peak shape, sensitivity and retention time stability as goal. To optimize the MRM parameters 50 pmol of each nucleoside metabolite was first analyzed by FIA method at different fragmentor voltages, cell acceration voltage and collision energy. Several solvent systems were evaluated to obtain good peak resolution in LC and maximal ion intensity in the MS detector.

The transitions of the *m/z* 113 to *m/z* 70 and *m/z* 116 to *m/z* 71 were monitored in MRM mode for Uracil and $^{13}C_1{}^{15}N_2$ Uracil, respectively. The transitions monitored were *m/z* 245 to *m/z* 209 for Pseudouridine; *m/z* 312 to *m/z* 180 for N^2,N^2-dimethylguanosine, and *m/z* 159 to *m/z* 116 for allantoin and *m/z* 161 to *m/z* 118 for ^{13}C Allantoin respectively. Calibration curves were created with the concentration of the nucleoside spiked in the biological matrix. Extraction efficiency and quality control were monitored with the peak area of the labeled stable isotopes in control plasma samples spaced throughout the run to monitor intra and inter run efficiency. The coefficients of variation for human plasma with in each run were 9.2%, 7.5%, 5.2%, and 13.8 % for Uracil, Allantoin, Pseudouridine, and Dimethyl Guanosine respectively. The inter run coefficient of variation was around 20% between different run days. The method was validated with mice plasma, human plasma and human urine.