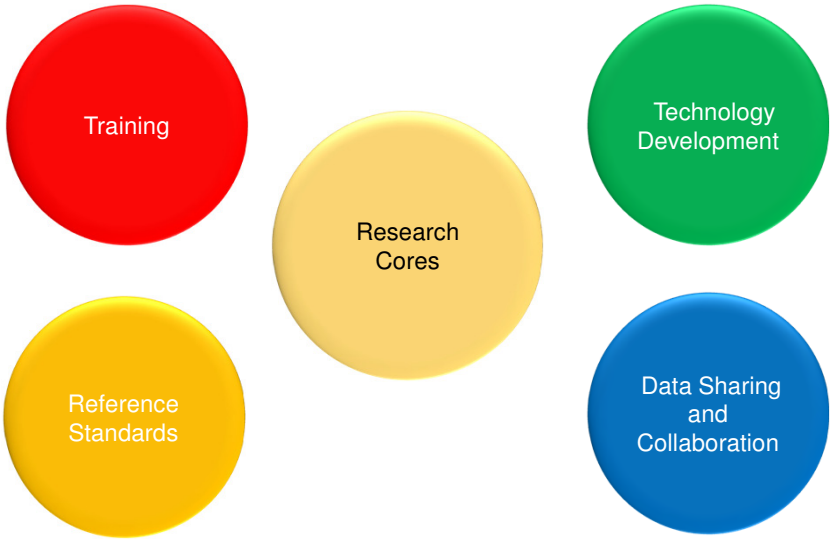
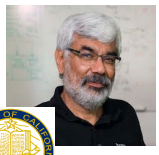
Metabolomics at the RTI RCMRC

Susan Sumner, PhD
Director, NIH Eastern Regional Metabolomics Resource Center
Director, Systems and Translational Sciences Center

NIH Common Fund Program – Building Metabolomics Capabilities



NIH Common Fund Metabolomics Centers

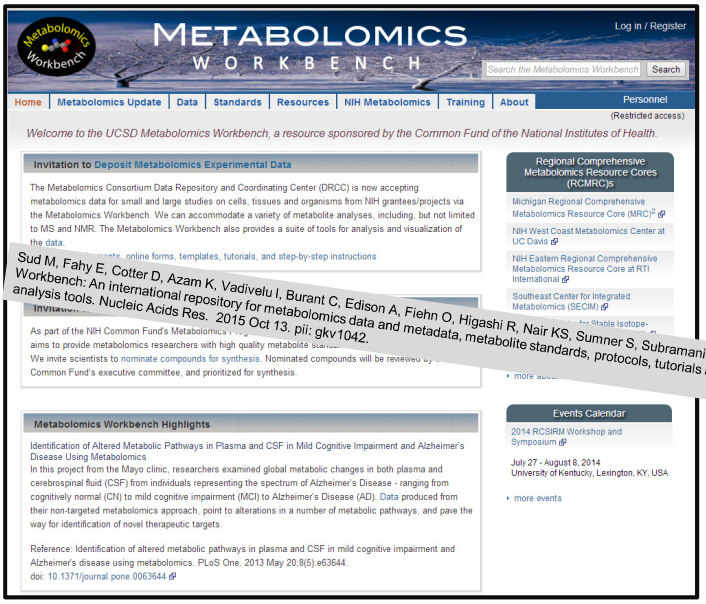






NIH Metabolomics Centers Ramp Up | November 4, 2013 Issue - Vol. 91 Issue 44 | Chemical & Engineering News. by Jyllian Kemsley

Metabolomics Workbench

http://www.metabolomicsworkbench.org/

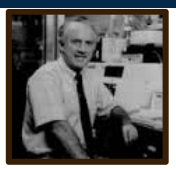


Sud M, Fahy E, Cotter D, Azam K, Vadivelu I, Burant C, Edison A, Fiehn O, Higashi R, Nair KS, Sumner S, Subramaniam S. Metabolomics Workbench: An international repository for metabolomics data and metadata, metabolite standards, protocols, tutorials and training, and analysis tools. Nucleic Acids Res. 2015 Oct 13. pii: gkv1042.

Shankar Subramaniam

Hands on Training

Stephen Barnes



Stephen Barnes, University of Alabama

- Week Long Course- June/July
- Experimental design, sample collection and storage, data capture, processing, statistical and multivariate analysis
- Mass Spectrometry and NMR Metabolomics
- http://www.uab.edu/proteomics/metabolomics/workshop/workshop

Web-based Metabolomics Learning

- Martin Kohlmeier, University of North Carolina at Chapel Hill

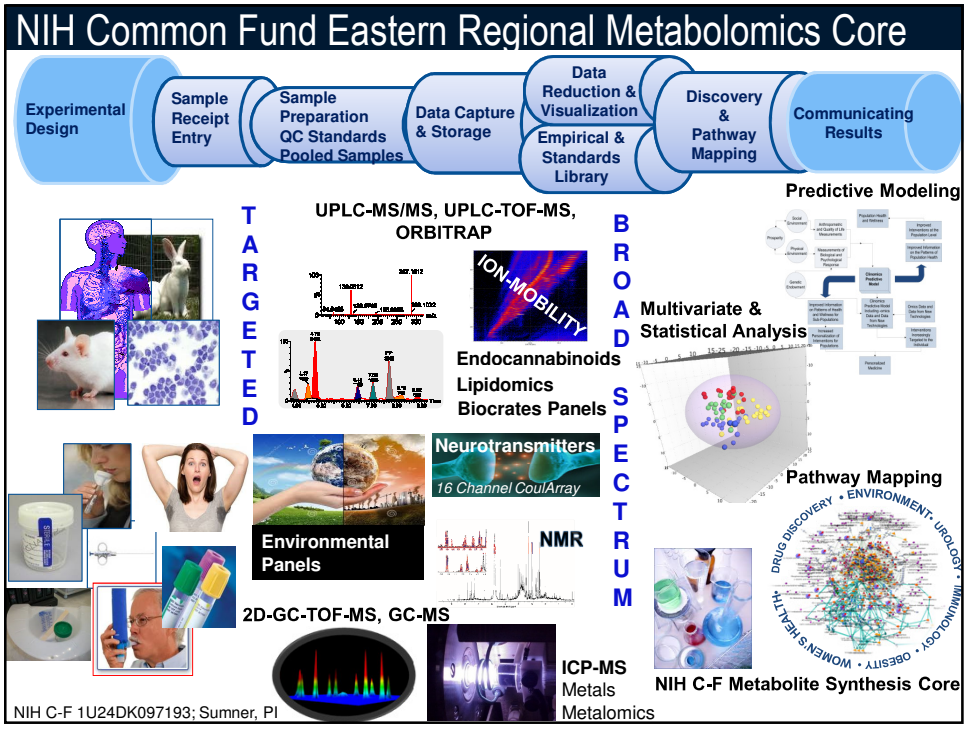
http://metabolomicsinmedicine.org/

Martin Kohlmeier

Metabotype

Johnson CH, et al. 2012. Annu. Rev. Pharmacol. Toxicol. 52:37–56

Studies have shown metabolomics signatures (the metabotype) to correlate with gender, race, age, ethnicity, drugs, chemicals, stress, weight status, mental health status, blood pressure, many disease states, behaviors, nutrition, gut microbiome....



Application Areas of the RTI RCMRC

Research Areas

- Treatment
- Intervention
- Foods
  - Fat, Soy, Casein, Rice
- Exposure
  - Metals, PAHs, Wood Smoke, PM2.5
- Mental Health
  - Schizophrenia, Bipolar Disorder, Anxiety
- Development
- Reproduction
- Cancer
- Climate Change
- Rare Disease
- Infection
- Opthamology
- Dentistry

Over 150 Research Collaborations

Organizations

Harvard
Columbia
UPenn
UDC
UCSD
Duke
UNC-CH
NCSU
U Louisville
U Montanna
Vanderbilt
Johns Hopkins
Nationwide Children's Hospital
NC Museum of Sciences
Howard University
Moffiat Cancer
ECU
WFU
NCA&T
LRRI
RTI
NYU
U Iowa
NCRC
UAB
Fort Bragg

Sample Types

- Serum
- Plasma
- Feces
- Urine
- Saliva
- Sweat
- Kidney
- Liver
- Brain
- Ovary
- Eye
- Lung
- Muscle
- Mussel
- Rice

Origin

Humans
- Elderly
- Adults
- Children
- Neonate
- Pregnant

Models
- Primates
- Rodents
- Aquatic

Insects
Cells

Targeted and Untargeted Analysis

Comparing States of Wellness and Sickness

Proteins

Pathways

Metabolit

Traditional Clinical Parameters

More sensitive and early markers for disease detection and staging

Markers to monitor

- efficacy
- adverse response
- relapse
- transplantation

Mechanistic insights from pathway analysis

Target Identification

RTI RCMRC

Early Serum Markers of 3rd Trimester Placental Abruption

- Placental abruption (PA) is an ischemic placental disorder that results from premature separation of the placenta before delivery and occurs in 1% of all pregnancies. It is associated with preterm delivery, fetal death, maternal hemorrhagic shock, and renal failure.
- Difficult to diagnose
  - Not a universally accepted definition
    - PA in the study samples was based on medical record review
  - Most common symptoms are vaginal bleeding and complaints of abdominal pain and uterine contractions.
- Goal of this study was to determine biomarkers from the 2nd trimester serum that predicts PA in the 3rd trimester

- Samples from the Abruption Study (Swedish Medical Center, WA)
  - Serum collected at the time of recruitment (approximately 16 weeks gestation)
  - Cases were identified that had at least two of the three clinical criteria:
    - *Vaginal bleeding* at ≥20 weeks in gestation accompanied and either non-reassuring fetal status or uterine tenderness/hypertonic uterus (without another identified cause)
    - At delivery, the placenta showed evidence of *tightly adherent clot and/or retroplacental bleeding*
    - *Sonographically diagnosed abruption*

Collaboration with Michelle Williams (Harvard University)

RTI RCMRC

Nine Metabolites were Significantly Associated with PA ($p<0.05$)

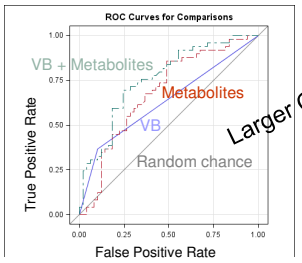
p180 Biocrates kit for the simultaneous quantification of 188 compounds

- free carnitine
- 40 acylcarnitines (Cx:y)
- 21 amino acids (19 proteinogenic amino acids, citrulline and ornithine)
- 21 biogenic amines
- hexose (sum of hexoses – about 90–95% glucose)
- 90 glycerophospholipids (14 lysophosphatidylcholines (lysoPC)
- 76 phosphatidylcholines (PC diacyl (aa) and acyl-alkyl (ae)
- 15 sphingolipids (SMx:y)

RTI RCMRC

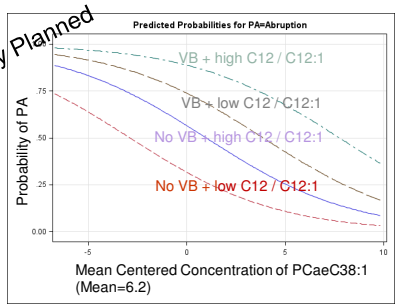
Logistic regression was used to model the probability of PA in the 3rd trimester based on serum biomarkers in 2nd trimester

	Area Under the ROC Curve (95% CI)	Model AUC Compared to VB Model AUC	Error Rate	Brier Score	R2	Bayes Information Criteria (BIC)
Model						
Vaginal Bleeding Only	0.63 (0.55, 0.71)	---	0.37	0.23	0.10	135.0
Metabolites Only	0.68 (0.58, 0.79)	p=0.48	0.37	0.22	0.10	139.3
Vaginal Bleeding + Metabolites	0.76 (0.66, 0.85)	p=0.003	0.29	0.20	0.19	133.1



ROC Curve (Area)
VB=0.63 Metabolites=0.68 VB + Metabolites=0.76

Larger Cohort Study Planned



RTI RCMRC

Pathways and Recommendations towards a Clinical Trial

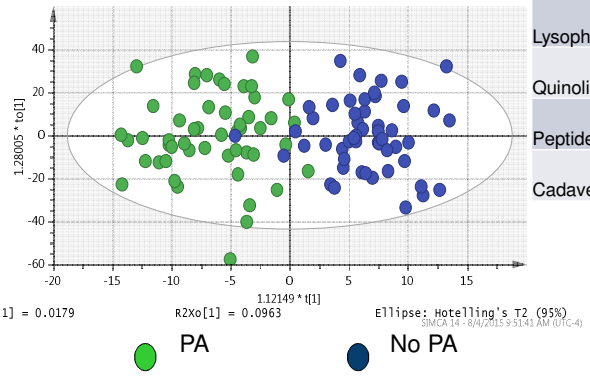
- **The probability of PA was increased with an increase in acylcarnitines and a decrease in phosphatidylcholine**.
  - These related pathways (acylcarnitine or phosphaticholine) branch … etabolite diacylglycerol. Diacylglycerol is transformed to the endocann… arachidononylglycerol (2-AG). 2-AG is converted to p… ers.
  - Vaswani demonstrated that the enzyme prost… 2 (PTGS-2), which converts 2-AG to pr… in the aging placenta, consisten… ulated … ance of PTGS-2 in preterm labor. Thes… may play a role in the pathogenesis of…
- Low-do… …nt women who are at high risk for a… …2 is a major target of nonsteroidal anti-inf… …cluding aspirin
- Res… …oline Plays Critical Role in Pregnancy and Women's Heal… …2015, 09:00 ET from The Choline Information Council
- Whole genome expression microarrays for healthy pregnant women taking choline at ~ AI level or ~ 2 x the AI level identified higher maternal choline lowers the concentrations of fms-like tyrosine kinase-1 (sFLT1) which is known to disable proteins that cause blood vessel growth, and indicate that choline may improve placental angiogenesis.

RTI RCMRC

Gelaye B, Sumner S, McRitchie S, Carlson JE, Ananth CV, Enquobahrie DA, Chunfang Q, Sorensen TK, Williams MA (2016). Maternal Early Pregnancy Serum Metabolomics Profile and Abnormal Vaginal Bleeding as Predictors of Placental Abruption: PlosOne 11(6)

Broad Spectrum Metabolomics

UPLC-TOF-MS of Serum



Library Matched Metabolites	Some VIPs
Beta-Cortol	3.1
5-Aminopentanal	3.1
Lysophosphatidylcholine	3.1
Quinoline alkaloid	2.6
Peptides	2.5, 2.6
Cadaverine	2.5

RTI RCMRC

Metabolomics and Autism

RTI RCMRC

Chinese Han population

3 - 6 years old

Discovery phase

- Autistic subject[illegible]59 M/14 F)
- Neurot[illegible]ubjects n=63 (5[illegible]

Validation phase

- Autistic[illegible]
- N[illegible]
- M[illegible]

Exclusion

- Aspe[illegible] syndr[illegible] develo[illegible]isorder not otherwis[illegible]pecified, Fragile-X syndrome

Fasting plasma analyzed with UPLC-MS

Diagnosis Panels Used

- Autism Beh[illegible]Checklist, Childhood Auti[illegible]les, [illegible]ient development

[illegible]s

[illegible]rgartens in [illegible]ically [illegible] no features of [illegible]al delay or autistic traits.

Discovery: 17 metabolites identified

Validation: 11 metabolites validated

sphingosine 1-phosphate

docosahexaenoic acid

Decanoylcarnitine, pregnanetriol uric acid, epoxyoctadecenoic acid, docosapentaenoic acid, adrenic acid, LPA(18:2(9Z,12Z)/0:0), LysoPE(0:0/16:0), LysoPE(18:0/0:0)

DHA is a supplement used in ASD

Sphingomyelin metabolism and fatty acid metabolism associated with ASD

Biological psychiatry 2004;55(3):323-6.

Autism : the international journal of research and practice 2012.

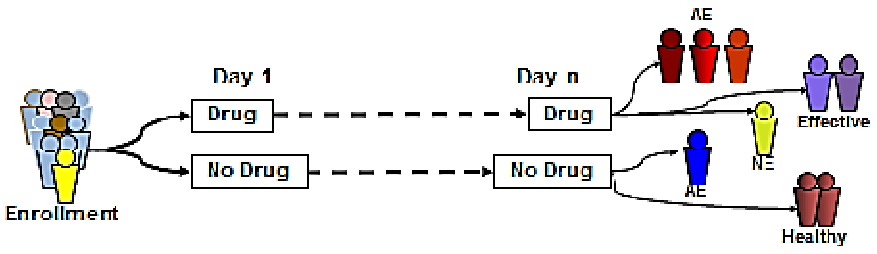
Prostaglandins, leukotrienes, and essential fatty acids 2009;80(4):221-7.

British Journal of Nutrition 2010;103(8):1160-7.

Wang et al (2016). Potential serum biomarkers from metabolomics study of autism potential serum biomarkers from metabolomics study of autism. *Journal of Psychiatry and Neuroscience*, *41*(1), 27–37.

Biomarkers in Preclinical and Clinical Research

Predicting Response to Treatment

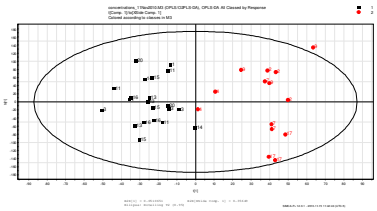


RTI RCMRC

Adolescent Obesity and Response to Intervention

RTI RCMRC

- Urine samples were obtained from adolescents participating in a 3 week healthy weight camp for overweight children.
- Children were provided a standardized meal plan, counseling, and fun physical activities.
- Some children had a clinically significant decrease in BMI, while others did not.
- Significant changes in the urinary metabolome occurred over the 3 week period.



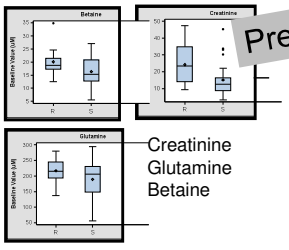
At baseline the branched-chain amino acids (BCAA) valine, leucine, and 2-oxoisocaproate were at lower levels in responders compared with non-responders to weight loss. Other investigators have found high levels of plasma BCAA (valine, isoleucine, phenylalanine, tyrosine & leucine) to be predictive of development of diabetes.

Pathmasiri et al., 2012, Integrating metabolomic signatures and psychosocial parameters in responsivity to an immersion treatment model for adolescent obesity. *Metabolomics*, *8*(6), 1037–1051.

Mechanisms of Childhood Glucocorticoid Resistance

The SSNS model has good quality fit statistics:
$R^2X(cum)=0.911$ $Q^2(cum)=0.595$.
The SSNR model did not have good quality fit statistics.

Baseline: SS vs SR



Creatinine
Glutamine
Betaine

Preliminary data, unpublished

- Blood was collected by the Midwest Pediatric Nephrology Consortium from 26 children with steroid sensitive (**SS**) and 14 children with steroid resistant (**SR**) nephrotic syndrome (NS)
- Collected prior to beginning treatment, and after ~7 weeks of daily oral glucocorticoids.
- Plasma was analyzed using broad spectrum metabolomics and quantitation.
- PCA of the pre- reatment SSNS groups biological variance between atment and non-treatment groups was greater than the individual variability.
- Compounds important for the differentiation of SSNS pre-and post-treatment included lipoproteins, and glucose.
- SSNS pre- and post-treatment plasma had $p \leq 0.05$ for 3-hydroxybutyrate, acetate, adipate, creatine, glucose, glycine, methylamine, pyruvate, tyrosine and valine.
- Alanine and o-phosphocholine levels had $p \leq 0.05$ for the pre and post treatment samples for SSNS and SRNS phenotypes.

Collaboration with
William Smoyer
Nationwide Children's Hospital

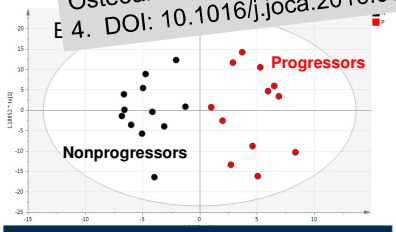
RTI RCMRC

Intensive Diet and Exercise for Arthritis (IDEA)

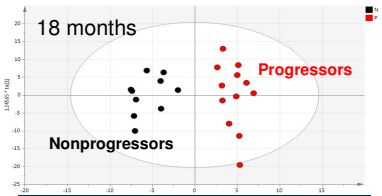
R Loeser
UNC-CH

- IDEA was a prospective, single-blind, randomized controlled trial that enrolled overweight osteoarthritis.
- Obese older adults with knee pain and radiographic evidence of OA were randomized into one of three18-month interventions (dietary weight loss-only; dietary weight loss-plus-exercise; or exercise-only).
- OA progression was determined by meas… space width from ba…
- N… di… pr… n
- Su… E… …ace.

Loeser RF, Pathmasiri W., Sumner S., McRitchie S., Beavers D., Saxena P., Nicklas B., Jordan J., Guermazi A, Hunter D., Messier S (2016). Association of urinary metabolites with radiographic progression of knee osteoarthritis in overweight and obese adults: An exploratory study. Osteoarthritis Cartilage S1063-4584(16)01072-4. DOI: 10.1016/j.joca.2016.03.011. PMID: 27012755



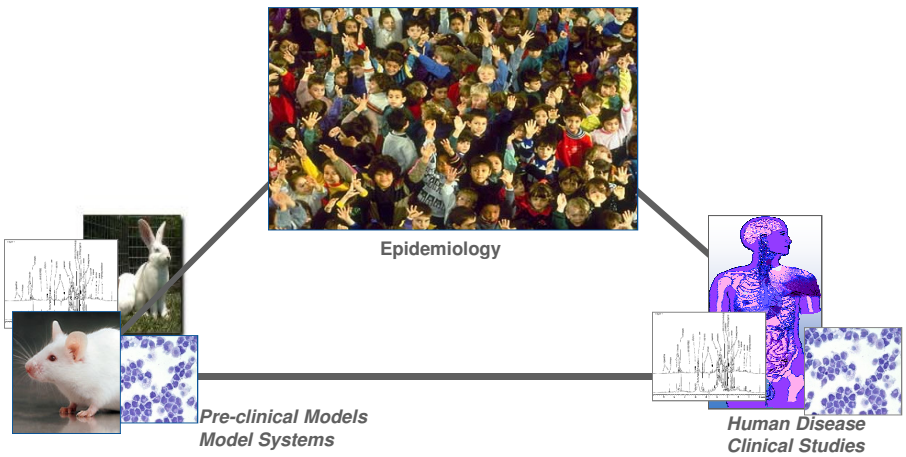
Glycolate, hippurate, and trigonelline



Alanine, N,N-dimethyglycine, glycolate, hippurate, histidine, and trigonelline

RTI RCMRC

Model Systems



RTI RCMRC

Western Diet and the Ovarian and Serum Metabolome

- Ovarian tissue and serum was collected from midlife cynomolgus monkeys (*Macaca fasicularis*) that had been fed either a Prudent or Western diet.
- Prudent diet monkeys were research naïve and had been exposed only to a commercial monkey chow diet: low in cholesterol (14 gram feed) and saturated fats, high in complex carbo
- Western diet monkeys were fed, for 2 years (116 mg/100 gram feed), saturated carbohydrates.
- UPLC-TOF-MS a ve targeted analysis revealed 13 amino , and 2 biogenic amines that were signif ent between the two diet groups for serum, and si were observed for the ovary extracts.
- This study demonstrated that dietary exposure had a significant impact on the serum and ovarian metabolome.
- Serum metabolites could be validated to serve as surrogates for ovarian quality.

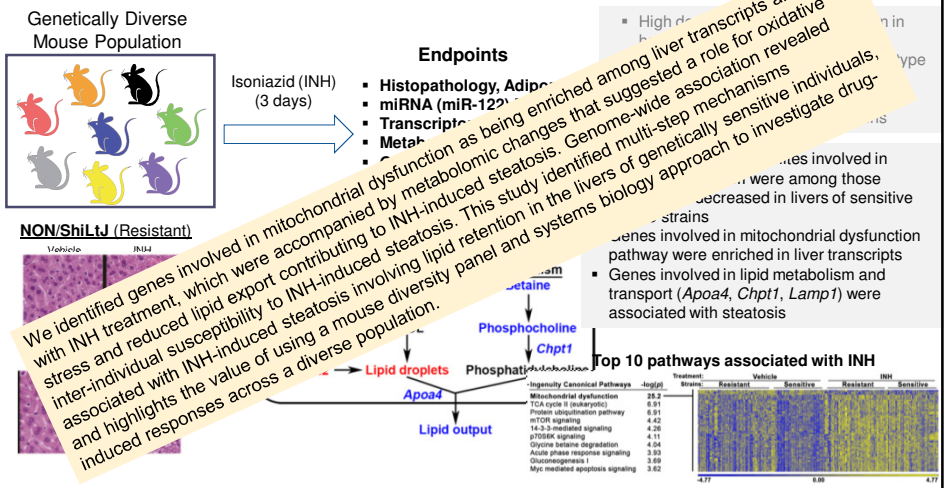
Susan E. Appt, Suraj Dhungana, James E. Carlson, Wimal Pathmasiri, Susan McRitchie, Matt Davis, Susan Sumner. Impact of Western Diet on the Ovarian and Serum Metabolome. Accepted. Maturitas

RTI RCMRC

Collaboration with Sue Apt, WFU

INH Drug Induced Liver Injury: Systems Biology

Sumner, et al.(2010). Metabolomics of urine for the assessment of microvesicular lipid accumulation in the liver following isoniazid exposure. *Metabolomics*, *6*(2), 238–249.



We identified genes involved in mitochondrial dysfunction as being enriched among liver transcripts altered with INH treatment, which were accompanied by metabolomic changes that suggested a role for oxidative stress and reduced lipid export contributing to INH-induced steatosis. Genome-wide association revealed inter-individual susceptibility to INH-induced steatosis. This study identified multi-step mechanisms associated with INH-induced steatosis involving lipid retention in the livers of genetically sensitive individuals, and highlights the value of using a mouse diversity panel and systems biology approach to investigate drug-induced responses across a diverse population.

- High d
- Genes involved in mitochondrial dysfunction pathway were enriched in liver transcripts
- Genes involved in lipid metabolism and transport (*Apoa4*, *Chpt1*, *Lamp1*) were associated with steatosis

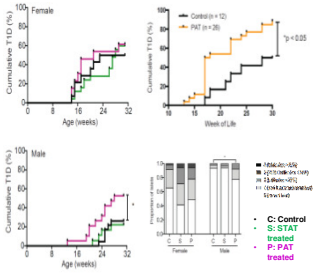
RTI RCMRC

Church et al, 2014. *A Systems Biology Approach Utilizing a Mouse Diversity Panel Identifies Genetic Differences Influencing Isoniazid-Induced Microvesicular Steatosis.* Tox. Sci. 140(2): 481-92.

Antibiotic Mediated Gut Microbiome Perturbation Accelerates Type 1 Diabetes

Martin Blaser
NYUMC

- Hypothesis: Early-life antibiotic use alters gut microbiota essential for immune development - promoting T1D development.
- Non-obese diabetic (NOD) mice were exposed to PAT or control
  - pulsed antibiotic treatment-macrolide tylosin
- By 31 weeks of age, control females had higher T1D incidence (50%) than males (26%).
- T1D incidence in males was significantly increased in PAT exposed- compared to controls.



These results provide evidence that early-life PAT exposure increase the development of T1D and accelerates the severity of insulitis

Alexandra Livanos

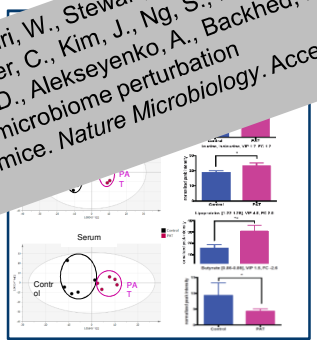
Antibiotic Mediated Gut Microbiome Perturbation in T1D

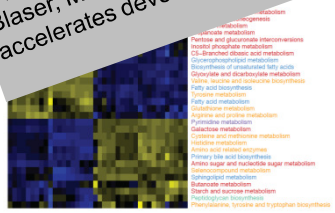
MICROBIOME

Microbiome analysis showed 32 genus-level taxa significantly enriched in controls and 7 enriched in PAT mice

Metagenome i
enrich

META





Livanos, A. E., Greiner, T. U., Vangay, P., Pathmasiri, W., Stewart, D., McRitchie, S., Li, H., Chung, J., Sohn, J., Kim, S., Gao, Z., Barber, C., Kim, J., Ng, S., Rogers, A. B., Sumner, S., Zhang, X., Cadwell, K., Knights, D., Alekseyenko, A., Backhed, F., & Blaser, M. J. (in press). Antibiotic-mediated gut microbiome perturbation accelerates development of type 1 diabetes in mice. *Nature Microbiology*. Accepted

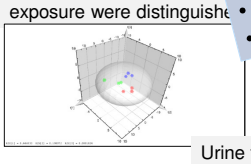
Metabolomics distinguished PAT exposed NOD from NOD control: including differences in amino acids, lipids and significant reduction in butyrate

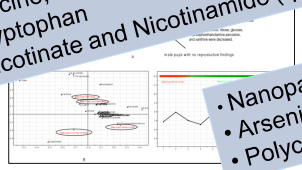
RTI RCMRC

in utero Exposure to Chemicals and Health Outcomes

- Phthalates, ubiquitous in the environment, have been characterized as endocrine disruptors.
- Pregnant rats were dosed with BBP for during gestation (gd 18-21): control, low dose (25 mg/kg), high dose (250 mg/kg).
- Urine was collected from dams gd 18 and pnd 21 [illegible] pups after weaning but before puberty (pnd 26), and [illegible] were collected at study termination.

Dams that received the h[illegible] dose, the low dose, or the vehicle at 21 days past exposure were distinguishe[illegible]

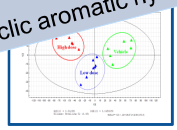


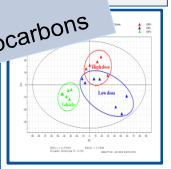


- Citrate Cycle
- Glycine, Serine, Threonine
- Tryptophan
- Nicotinate and Nicotinamide (quinolate)

(control vs BBP dose [illegible]e noted in levels of [illegible]etabolites in the brain (male and female pups, not d[illegible]s, and uterus (d[illegible]

- Nanoparticles
- Arsenic
- Polycyclic aromatic hyrocarbons





Urine from pups, on pnd 26, were distinguished by the dose the dam received during gestation.

Testes

Male pup brain

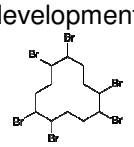
RTI RCMRC

Sumner et al., 2009. Metabolomics in the assessment of chemical-induced reproductive and developmental outcomes using non-invasive biological fluids: application to the study of butylbenzyl phthalate. *Journal of Applied Toxicology* and **Banerjee et al., 2012**. Metabolomics of brain and reproductive organs: characterizing the impact of gestational exposure to butylbenzyl phthalate on dams and resultant offspring *Metabolomics*

Neonatal Exposure to Brominated flame retardants

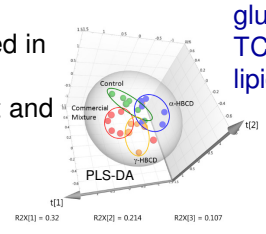
Hexabromocyclododecane (HBCD)

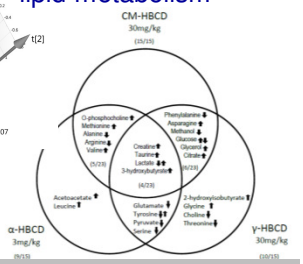
- High production volume flame retardant
  - Building insulation foams, electronics, and textiles
- Commercial mixture consists of 3 stereo isomers (α, β, γ)
  - α-HBCD (10%), β-HBCD (10%), γ-HBCD (80%)
- Shift from dominant γ to α detected in humans and wild life
- Implications in neurodevelopment and endocrine disruption



Mice exposed to α-, γ-, or CM-HBCD demonstrated differences in endogenous metabolites by treatment- and dose-groups. Metabolites involved in

amino acid metabolism
glycolysis
gluconeogenesis
TCA cycle
lipid metabolism



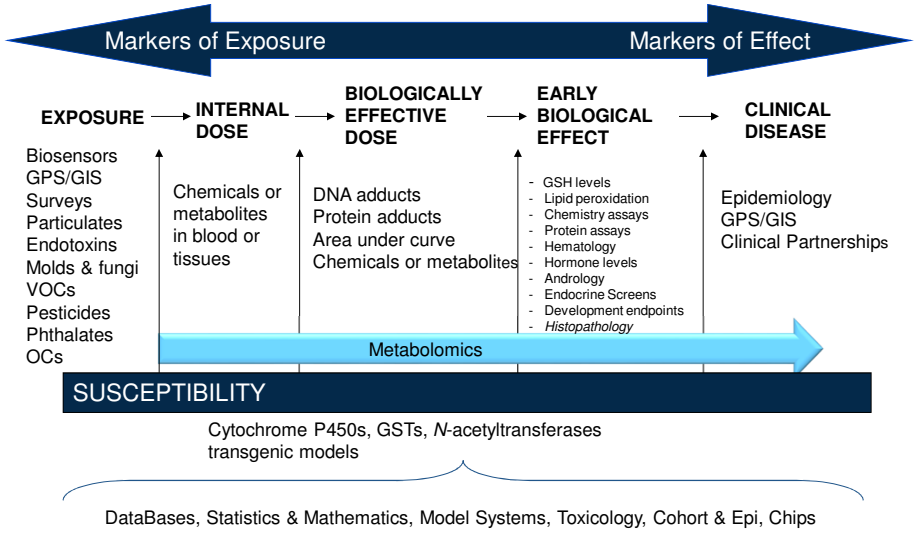


- PND 10 female C57BL/6 mice administered single dose of vehicle, HBCD α, γ, or commercial mixture (3, 10, or 30 mg/kg)
- Serum collected 4 days post-oral administering

RTI RCMRC

Szabo et al., 2016, Different serum metabolomics profiles in neonatal mice following oral brominated flame retardant exposures to hexabormocyclododecane (HBCD) alpha, gamma, and commercial mixture. Accepted, *EHP*

Biomarkers of Exposure, Dose, and Response



RTI RCMRC

Adapted from NRC 1989; Fennell 1996; Sumner, 2005

Birth and Early Childhood Outcomes

Collaboration with Frederica Perera (Columbia University)

- The Columbia Center for Children's Environmental Heath (CCCEH) is evaluating the effects of environmental exposures on women and newborns in inner-city communities in New York.
- Prospective birth cohort of nonsmoking African-American and Dominican women - Studies have found that environmental exposures during pregnancy adversely affect fetal development, childhood respiratory health, and the neurodevelopment of children.
- Polycyclic aromatic hydrocarbons (PAHs), are known to have endocrine disruption potential, and were the major pollutant studied.
- Metabolomics in exposure sciences has largely focused on comparing biochemical profiles of biological specimens in studies designed to reveal biomarkers that differentiate groups based on well-defined exposure characteristics.
- The use of metabolomics in health research has focused on comparing the profiles between and among study groups with well-defined health outcomes.
- We have used structural equation modeling (SEM) to conduct pathway analysis between environmental exposures, the metabotype of cord blood, and birth and early developmental health outcomes.

RTI RCMRC

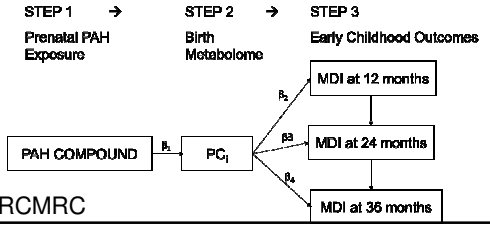
Exposure and Health Outcome Data

- Nine PAHs were determined from personal monitor worn over a 2 day period during the 3rd trimester of pregnancy.
- Levels of B[a]P-DNA adduct were measured in cord blood - as a proxy for total PAH-DNA adducts.
- Maternal urinary metabolic concentrations were measured for 10 PAH-derived metabolites.
- Birth weight and head circumference were recorded at the time of delivery.
- Mental Development Index (MDI) was calculated using the Bayley Scales of Infant Development at 12, 24, 36 months of age.

PAHs Measured in Personal Monitors
- Benz(a)anthracene
- Benzo(a)pyrene
- Benzo(b)fluroanthene
- Benzo(g,h,i)perylene
- Benzo(k)fluroanthene
- Chrysene
- Disbenz(a,h)anthracene
- Indeno(1,2,3-cd)pyrene
- Total PAH*
- Pyrene

DNA adducts in cord blood
- B[a]P-DNA adducts

PAHs in Maternal Urine
- 2-hydroxyfluorene
- 3-hydroxyfluorene
- 9-hydroxyfluorene
- 1-hydroxynaphthalene
- 2-hydroxynaphthalene
- 1-hydroxyphenanthrene
- 2-hydroxyphenanthrene
- 3-hydroxyphenanthrene
- 4-hydroxyphenanthrene
- 1-hydroxypyrene



βi is the standardized regression coefficient. The magnitude of the coefficient represents the strength of the effect, and the sign represents whether the effect is positive or negative.

RTI RCMRC

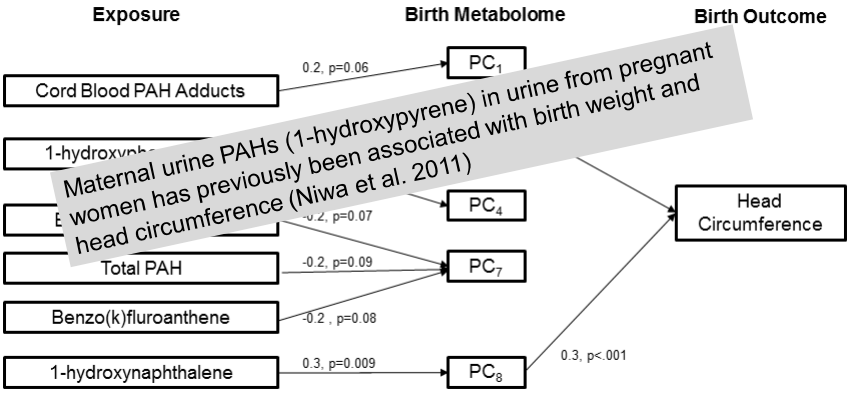
Nine Principal Components (PCs) Represent the Metabotypes (eigenvalue > 2)

- Nine principal components (PCs), with eigenvalue > 2, were selected to represent the metabotypes
  - Percentage of the variance explained = eigenvalue / total number of variables
- Each PC is a linear combination of the 141 transformed bins
  - The coefficient for each bin variable represents the weight of the bin variable in the PC, and the sum of the squares of the weights equals 1.

PCs	% Variance	Rank order of Library Matched Metabolites Listed by the Magnitude of the Coefficient
PC1	49.7%	*proline*, cholate, 3-hydroxybutyrate, lipoproteins, leucine, alanine, ….
PC2	9.0%	glucose, ribose, maltose, sucrose, mannose, unsaturated lipids, ….
PC3	6.3%	threonine, *proline*, ethanol, glucose, lipoproteins, 3-hydroxybutyrate…..
PC4	5.8%	3-hydroxybutyrate, unsaturated lipids, glucose, ribose, maltose….
PC5	4.6%	glucose, ethanol, glutamate, *proline*, pyruvate, lactate, ….
PC6	3.7%	aminoadipate, aminobutyrate, lysine, lactate, mannose, ….
PC7	2.2%	acetone, lipoproteins, unsaturated lipids, 3-hydroxybutyrate,….
PC8	2.1%	lipoproteins, propylene glycol, unsaturated lipids, choline, ….
PC9	1.6%	3-hydroxybutyrate, kynurenine, acetone, glutamate, …..

RTI RCMRC
Unpublished results

One Pathway Analysis Example- PAH Exposure to the Birth Metabolome and the Birth Outcome of Head Circumference



RTI RCMRC
Unpublished results

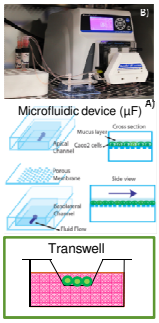
Exposure to Outcome

- Some exposure measures were associated with PCs which did not have an association with health outcomes, which could indicate that the environmental exposure impacts the metabotype with no health consequences, or just not those measured in this study.
- Some cord blood PCs were associated with adverse birth or childhood outcomes that developed at 12, 24, or 36 months that did not associate with exposures measured in this study.
  - These PCs could be explored as biomarkers to use for predicting outcomes in early childhood.
  - Bermúdez and coworkers have demonstrated correlations between levels of increased copper in cord blood and small for gestational age birth weight.
- Cord blood metabolites (e.g., choline, proline, glutamine, alanine, glucose, phenylalanine, and citrulline, and phospholipids) have been associate with low birth weight (Ivorra et al. 2012; Ciborowski et al. 2015; Horgan et al. 2011), growth restriction (Favrettoto et al. 2012), or fetal growth and neurodevelopment (Neu 2001; Poindexter et al. 2006).
- Metabolite perturbations associated with MDI include:
  - Taurine, lactate and N-acetyl aspartate metabolism, and glucose levels have been associated with later adverse neonatal/infant neurodevelopment (Wharton et al. 2004, Duvanel et al. 1999; Lucas et al. 1988, Barkovich et al. 1999).
  - Choline, phospholipids, and betaine are known metabolites involved in the brain development (Zeisel, 2006).

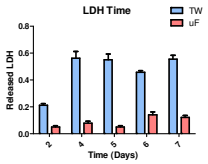
RTI RCMRC
Unpublished results

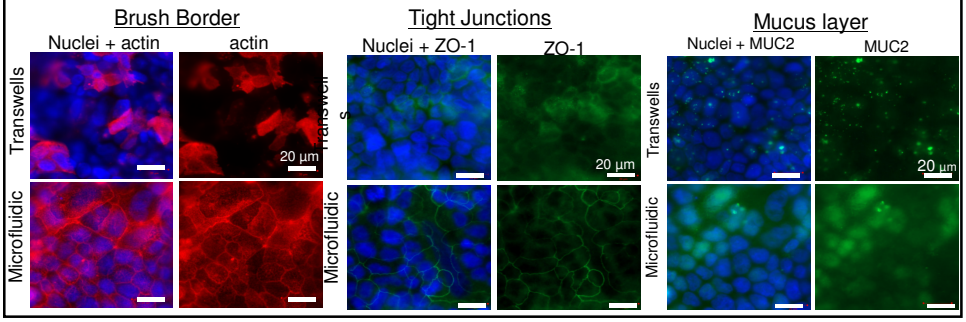
Biomedical Engineering

RTI RCMRC

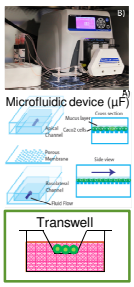


- Cell physiology was compared between transwells and microfluidic devices to understand the effect of fluid dynamics
- Released LDH in the cell media was higher in the media of transwells compared to μF.
- Caco-2 cells culture in μF have a higher production of mucin (MUC2), more defined tight junctions and a better developed brush boarder than transwells.

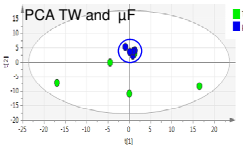




Transwell vs Microfluidic Caco-2 Cells

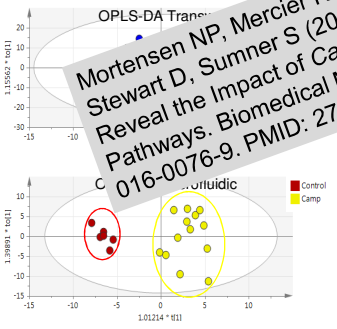


Fluid dynamics effect on cell metabolism



Differential pathways

Campylobacter jejuni 81-176 inf



Mortensen NP, Mercier KA, McRitchie S, Cavallo T, Pathmasiri W, Stewart D, Sumner S (2016). Microfluidics Meets Metabolomics to Reveal the Impact of *Campylobacter jejuni* Infection on Biochemical Pathways. Biomedical Microdevices 18(3):51. doi: 10.1007/s10544-016-0076-9. PMID: 27231016

pylobacter vs. Control

Proline
cetate
Glutamate
Pyroglutamate
ucose
Glutamate
3-Methyl-2-oxovalerate
Isoleucine
Leucine
Malonate
Methionine
N-Acetylaspartate
Phenylalanine
Pyruvate
t-Methylhistidine
Valine

RTI RCMRC

STS Center


Wimal Pathmasiri
NMR & GC-MS


Jim Carlson
LC- and GC-MS


Jessica Gooding
LC-MS


Kelly Mercier
NMR


Susan McRitchie
Data Analysis


Zach Acuff
Biostatistics

Bob Clark
Genetics


Jason Burgess
Program
Coordinator


Andrew Novokhatny
NMR and QC


Aurora Cabrera
LC-MS/MS


Jocelin Spruill
GC-MS
Neurotransmitter


Tammy Cavallo
Biology and QC


Delisha Stewart
Cell Biology


Ninell Mortensen
Microbiology


Maria Moreno
Biology


Keith Levine
Metallomics


Yuanyuan Li
LC-MS


Rod Snyder
LC-MS


Sherry Black
In vivo and in vitro
Metabolism

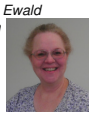

Scott Watson
Neurotranmitter
LC/MS


Skip Gaudette
Systems


Puvi Patel
In vitro metabolsm


Yan Lan Yueh
LC-MS

Rose Ewald
intern


Courtney
Whitaker
intern


Tim Fennell
Metabolism


Sue Clark
Administrative
Support

Melody Markley Model Systems

RTI RCMRC