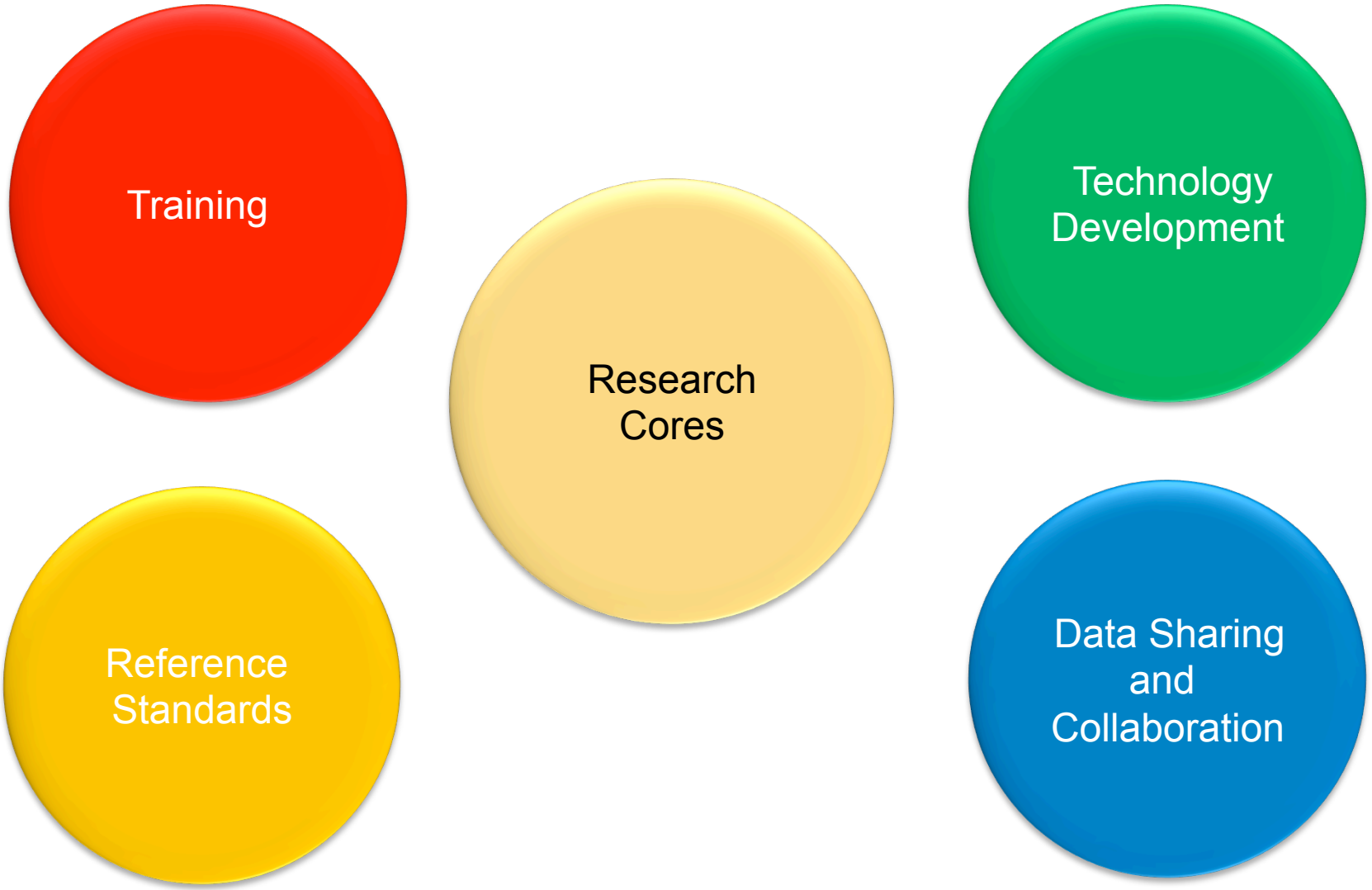
Metabolomics in Nutrition Research, and Implications in Blood Type Research

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

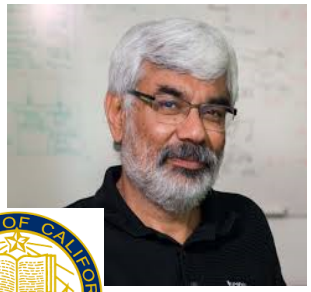
Learning Objectives

- Where to learn more: NIH Common Fund Metabolomics Program
- What is Metabolomics and when is it useful?
- Study Design Considerations for Clinical Trials
- Metabolomics Experimental Workflow and Data Interpretation
- This presentation will cover several applications of metabolomics
  - Responsivity to healthy life-style weight loss
  - Impact of sub-therapeutic doses of antibiotics
  - Weight status and the response to vaccination
  - Diet and ovarian health
  - Pregnancy complications and target identification
  - Autism and nutritional supplementation
  - Individual Variability

NIH Common Fund Program – Building Metabolomics Capabilities



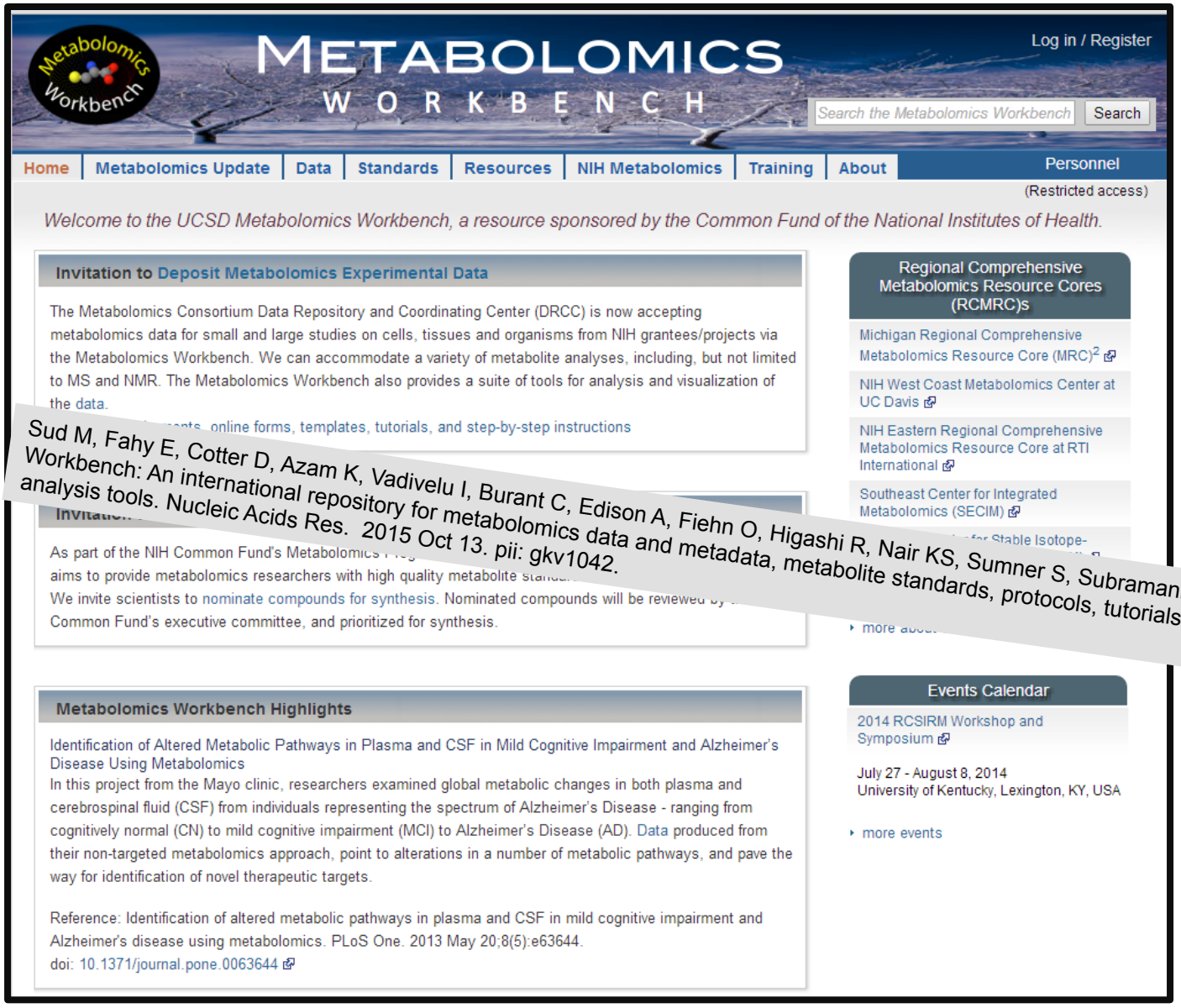
NIH Common Fund Metabolomics Centers



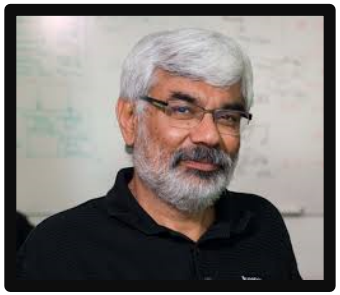
NIH Metabolomics Centers Ramp Up | November 4, 2013 Issue - Vol. 91 Issue 44 | Chemical & Engineering News. by Jyllian Kemsley

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

Metabolomics

- Metabolomics involves the analysis of the low molecular weight complement of cells, tissues, or biological fluids.

- Metabolomics makes it feasible to uniquely profile the biochemistry of an individual or system.
  - Metabonomics is used to determine the pattern of changes (and related metabolites) arising from disease, dysfunction, disorder, or from the therapeutic or adverse effects of xenobiotics

- This leading-edge method has come to the fore to reveal biomarkers for the early detection and diagnosis of disease, to monitor therapeutic treatments, and to provide insights into biological mechanisms.

Why Metabolomics?

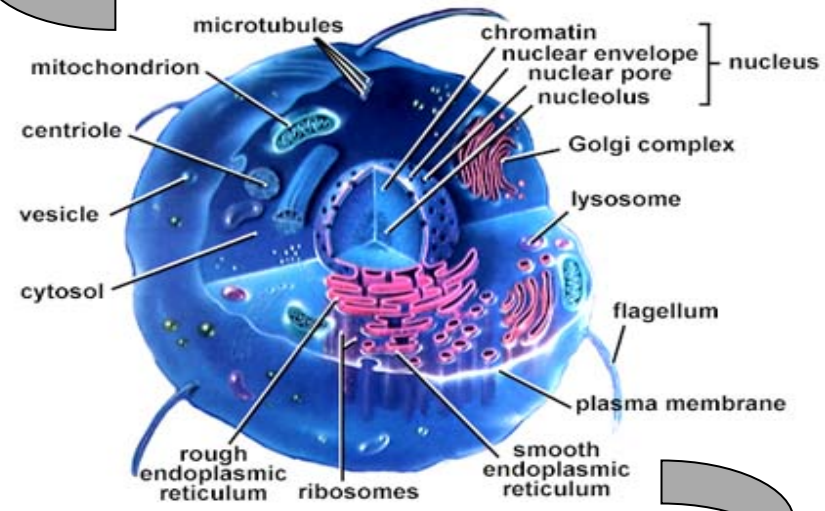
- Specific genes can be identified that define individuals' at risk for a disease, dysfunction, or disorder, or response to treatments.

- Diseases or responses can occur at the level of the proteome; and proteins and metabolites can inform us about the state of a disease, dysfunction or disorder at any given time.

Metabolomics of Cells, Tissues, and Biological Fluids

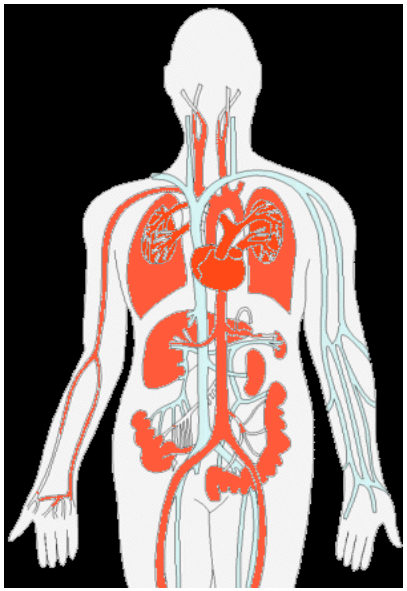
Cells / Organ

Released metabolites



Cytosolic metabolites

System

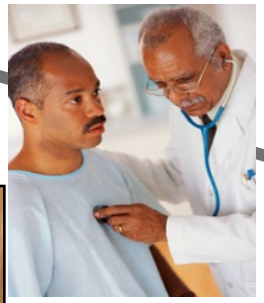


Serum
Urine
Saliva
Breath
Plasma
CSF
Feces

Signatures or Profiles

Discrete peaks ⇨ *Diagnostics*

Comparing States of Wellness and Sickness

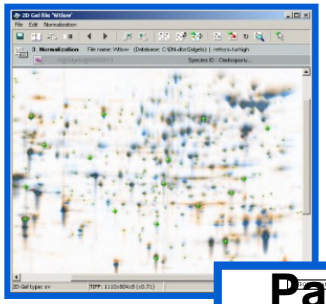





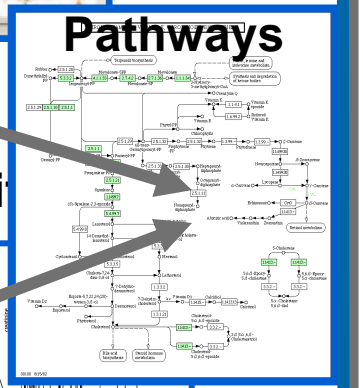




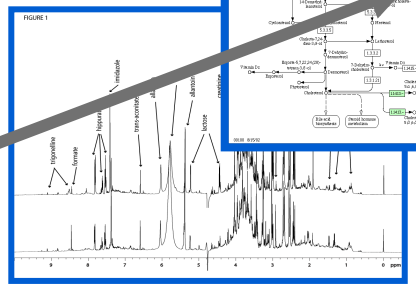
Proteins



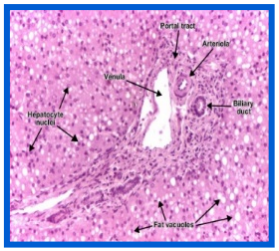
Pathways



Metaboli



Traditional Clinical Parameters



More sensitive and early markers for disease detection and staging

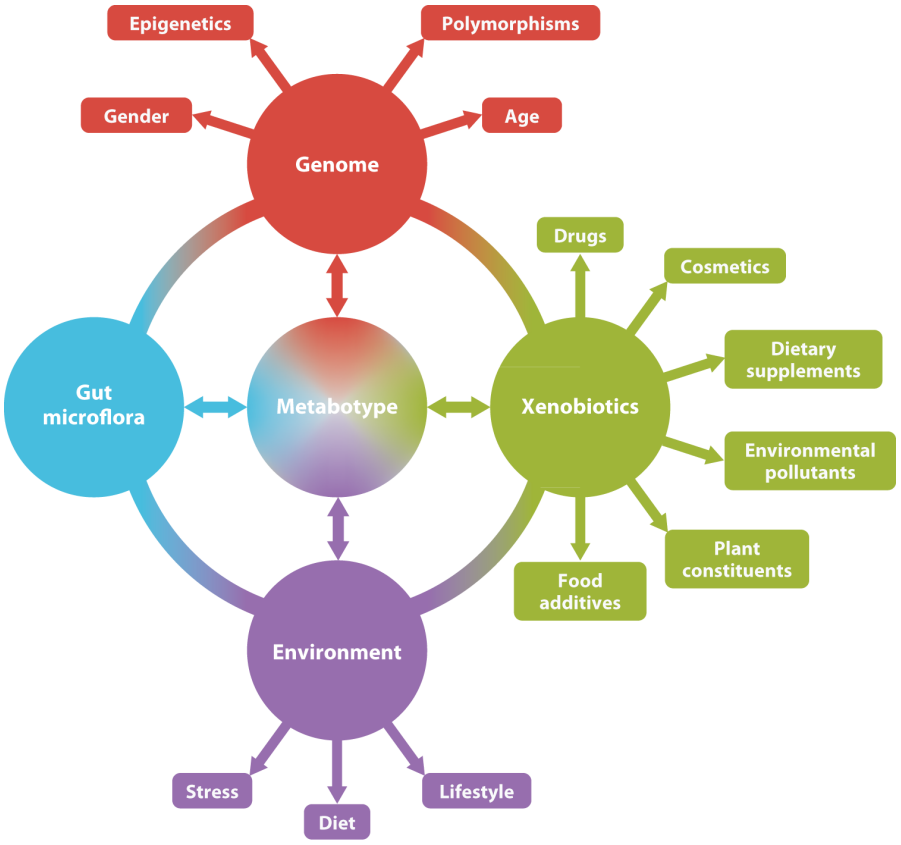
Markers to monitor

- efficacy
- adverse response
- relapse
- transplantation

Mechanistic insights from pathway analysis

Target Identification

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....

Study Design Considerations

- Study Design
  - Gender, race, ethnicity, age, exposures (drugs, chemicals, stress, city, etc..) all contribute to the metabotype

- Sample Collection and Storage
  - Consistency in collection and processing
    - blood to serum (over ice?), or blood to plasma (anticoagulant?)
  - Storage consistency (vials, temperature, freeze thaws, etc.)
  - Selection of chemicals for extraction of samples

NIH Common Fund Eastern Regional Metabolomics Core

Experimental Design

Sample Receipt Entry

Sample Preparation QC Standards Pooled Samples

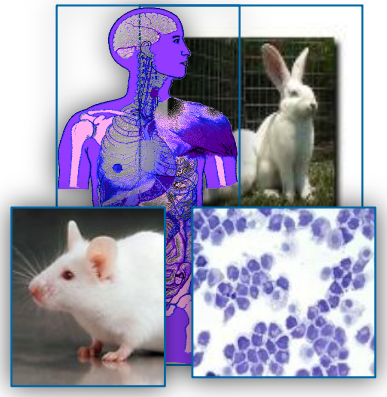
Data Capture & Storage

Data Reduction & Visualization

Empirical & Standards Library

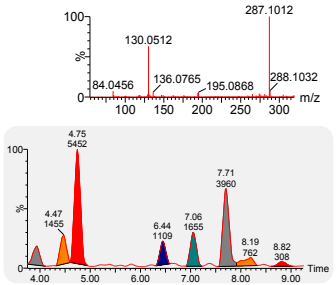
Discovery & Pathway Mapping

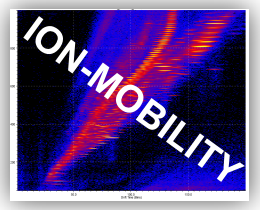
Communicating Results



TARGETED

UPLC-MS/MS, UPLC-TOF-MS, ORBITRAP





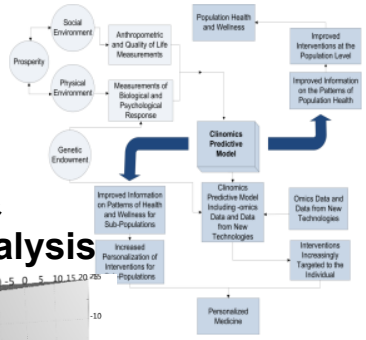
Endocannabinoids

Lipidomics

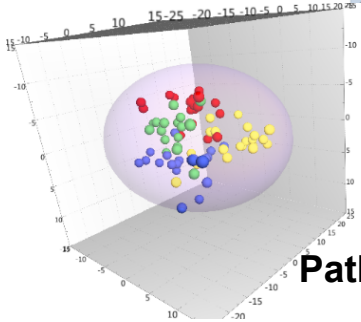
Biocrates Panels

BROAD SPECTRUM

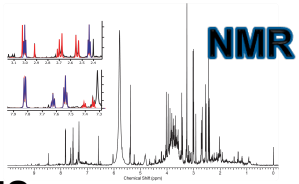
Predictive Modeling



Multivariate & Statistical Analysis

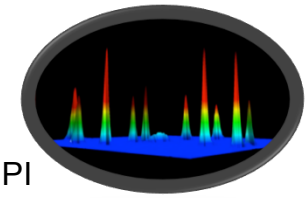


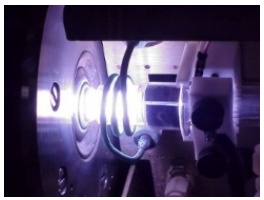
Environmental Panels



Pathway Mapping

2D-GC-TOF-MS, GC-MS





ICP-MS

Metals

Metalomics

NIH C-F Metabolite Synthesis Core

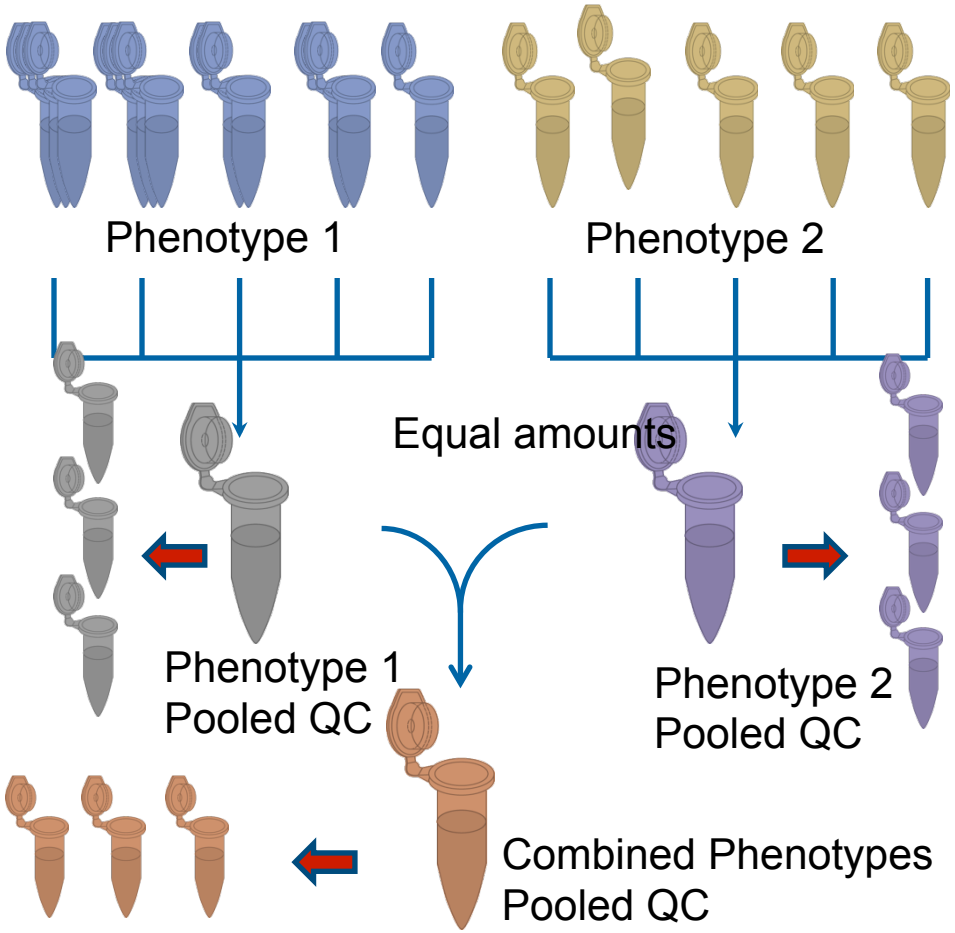
NIH C-F 1U24DK097193; Sumner, PI

Analysis Considerations

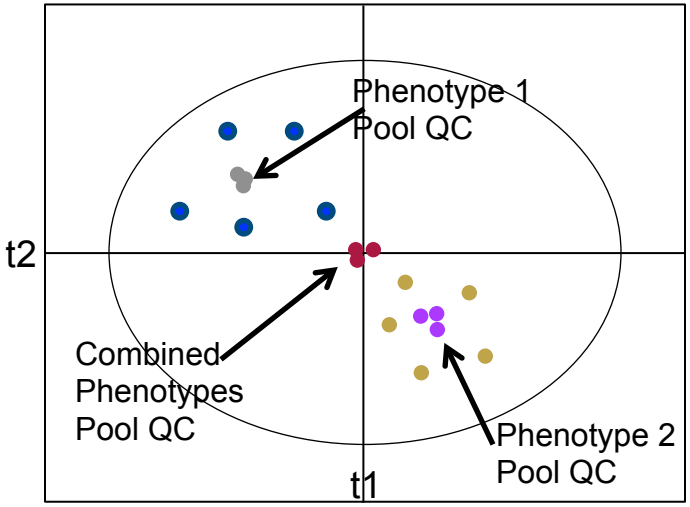
- Instrument Response and Drift
  - Consistency in parameters for each sample run
  - Create phenotypic pools as well pre- and post- run standards

- Data Analysis
  - Data quality, spectral alignment, formatting, etc.
  - Check the standards and the pooled samples!!

Quality Control Pool Samples



- Aliquots from each sample in the study phenotype are pooled (phenotypic pool)
- Equal amount of each phenotypic pools are pooled (Combined phenotypic pool)
- Replicates of pools are processed and randomized with the study samples



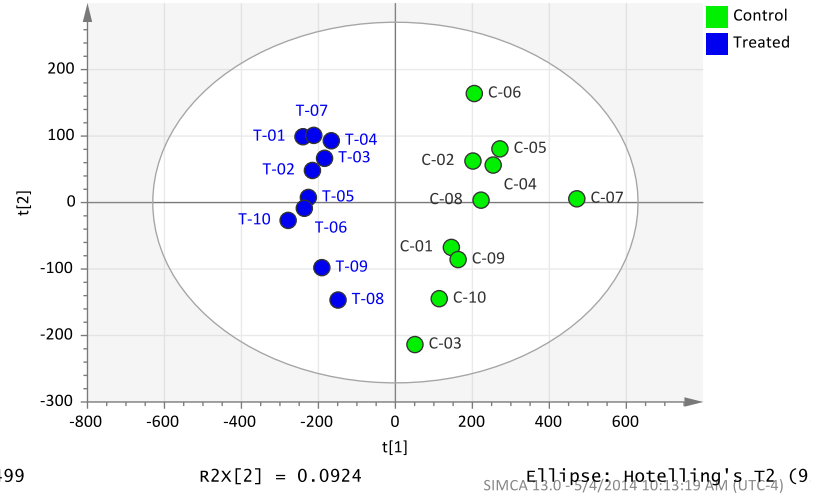
Data Analysis Methods

Data analysis methods can include:

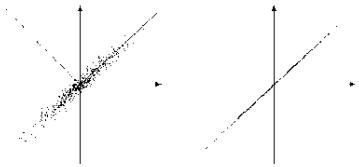
- Descriptive Statistics
- Hypothesis Testing
- Multivariate Analyses
- Linear Regression
- Logistic Regression
- Structural Equation Modeling
- Integration of Data (e.g., genomics, microbiome)
- Pathway Analyses

Multivariate Analysis

Principal component analysis (**PCA**) is a statistical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called **principal components**



Principle component analysis (PCA)

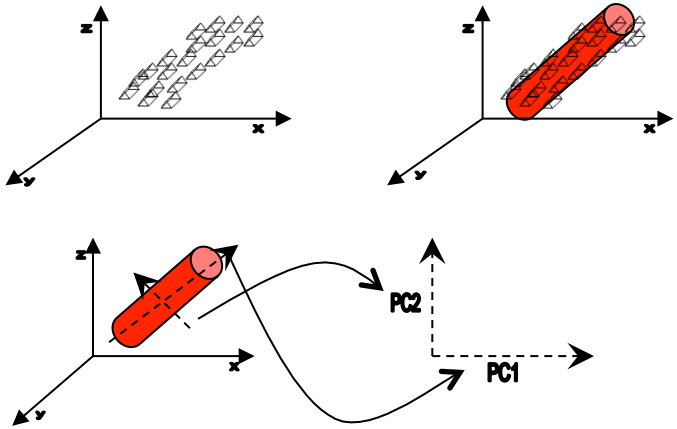


(a) A data set and its principal axes. (b) Reduction of the data to one dimension.

PCA - reduce the dimensionality of data

goal - to minimize the error by doing some transformation to another basis

The eigenspaces that retain the most significant amount of information are those that correspond to the largest eigenvalues



Application Areas

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

Nutrition Research Applications

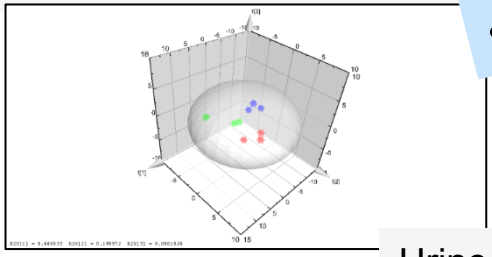
- In nutrition research, metabolomics holds promise for determining biomarkers for the early diagnosis of disease, for understanding how weight and diet influence health outcomes and the responsivity to treatment, and for determining perturbations in biochemical pathways related to exposure, or disease, for informing the development of intervention strategies
  - Responsivity to healthy life-style weight loss program
  - Impact of sub-therapeutic doses of antibiotics
  - Weight status and the response to vaccination
  - Diet and ovarian health
  - Pregnancy complications and target identification
  - Autism and nutritional supplementation.

- While we are aware that the biochemistry of blood groups differ, research on the metabotype of blood type is at its infancy.
  - provide compelling evidence that the metabolic profiles of individuals differs by blood type, and that these biochemical difference may be associated with known increased risks for disease, and provide a means for intervention strategy.

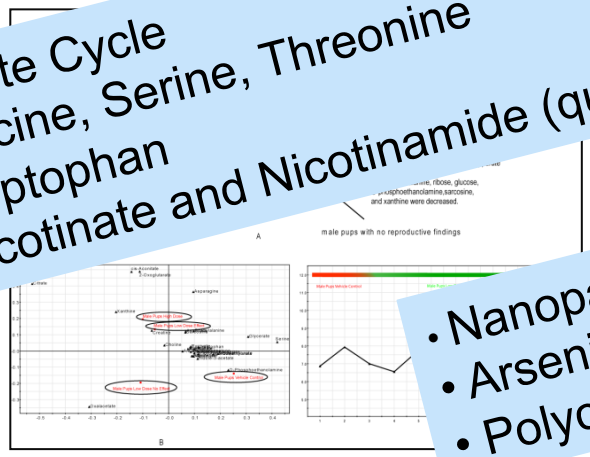
in utero Exposure to Chemicals and Health Outcomes

- 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]om pups after weaning but before puberty (pnd 26), and [illegible]vere collected at study termination.

Dams that received the h
dose, the low dose, or the
vehicle at 21 days past
exposure were distinguish



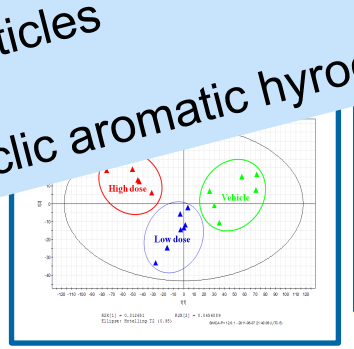
- Citrate Cycle
- Glycine, Serine, Threonine
- Tryptophan
- Nicotinate and Nicotinamide (quinolate)



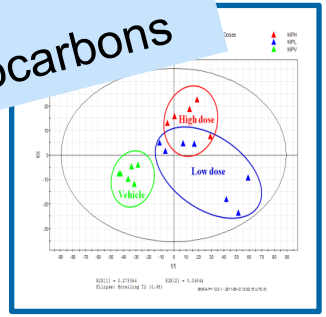
Urine from pups, on pnd 26, were distinguished by the dose the dam received during gestation.

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

- Nanoparticles
- Arsenic
- Polycyclic aromatic hyrocarbons



Testes



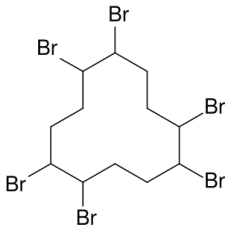
Male pup brain

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

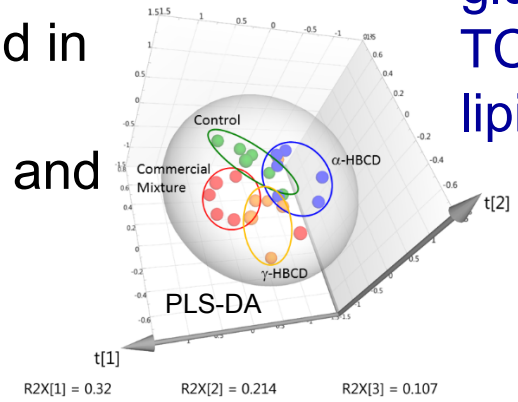
Hexabromocyclododecane (HBCD)

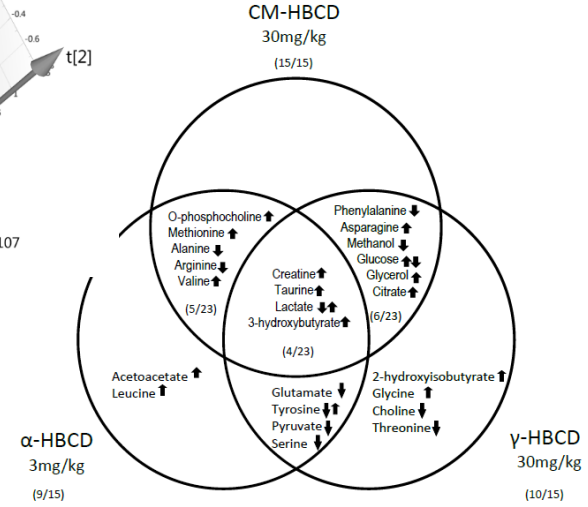
- High production volume flame retardant
  - Building insulation foams, electronics, and textiles
- Commercial mixture consists of 3 stereo isomers (α, β, γ)
  - α-HBCD (10%), β-HBCD (10%), γ-HBCD (80%)
- Shift from dominant γ to α detected in humans and wild life
- Implications in neurodevelopment and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



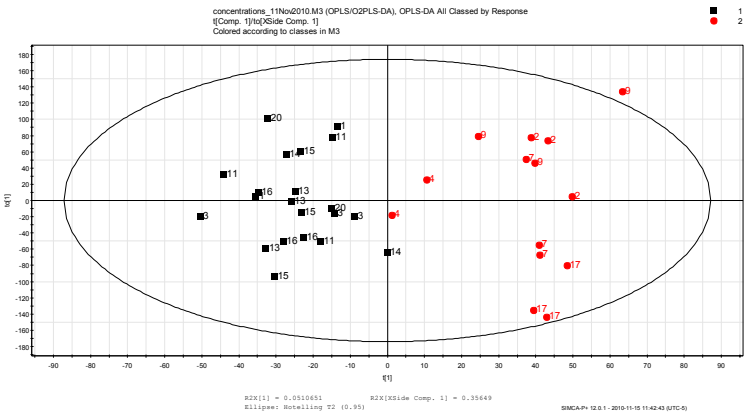


- PND 10 female C57BL/6 mice administered single dose of vehicle, HBCD α, γ, or commercial mixture (3, 10, or 30 mg/kg)
- Serum collected 4 days post-oral administering

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*

Adolescent Obesity and Response to Intervention

- 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.

Metabolomics in Diet

- ***Diet and Ovarian Health***. Non-human primates fed prudent or western diets. Folate synthesis, oxygen signaling, fatty acid oxidation, oxidative damage, reactive oxygen species. – with Sue Appt, DVM, WFU.

- ***Influenza and Obesity***. Flu in a diet-induced obesity model, and in mice lacking leptin receptor signaling: High-fat diet-induced and genetic-induced obese mice exhibited greater pH1N1 mortality, lung inflammatory responses, and excess lung damage despite similar levels of viral burden compared with lean control mice. Metabolites were perturbed by obesity both prior to and during infection- fatty acid, phospholipid, and nucleotide metabolism. –M Beck, PhD, UNC-CH.
  - Milner et al., 2015, Obesity increases mortality and modulates the lung metabolome during pandemic H1N1 influenza virus infection in mice. *Journal of Immunology*, *194*(10), 4846–4859.

Metabolomics and Autism

Chinese Han population

3 - 6 years old

Discovery phase

- Autistic subject
- Neurot

Validation phase

- Autistic
- M

Exclusion

- Aspe
  syndr
  disorde
  Fragile-

Fasting plasma analyzed with UPLC-MS

Diagnosis Panels Used

- Autism Be Checklist, Childhood Auti les,

Sphingomyelin metabolism and fatty acid metabolism associated with ASD

DHA is a supplement used in 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 .

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)

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.

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)

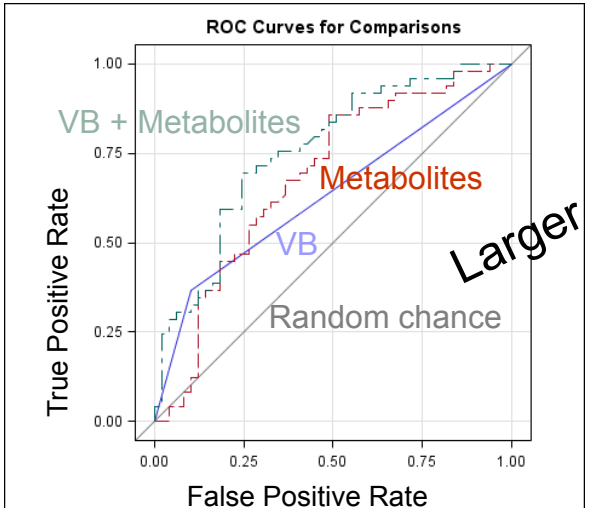
Nine Metabolites were Significantly Associated with PA ($p<0.05$)

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)

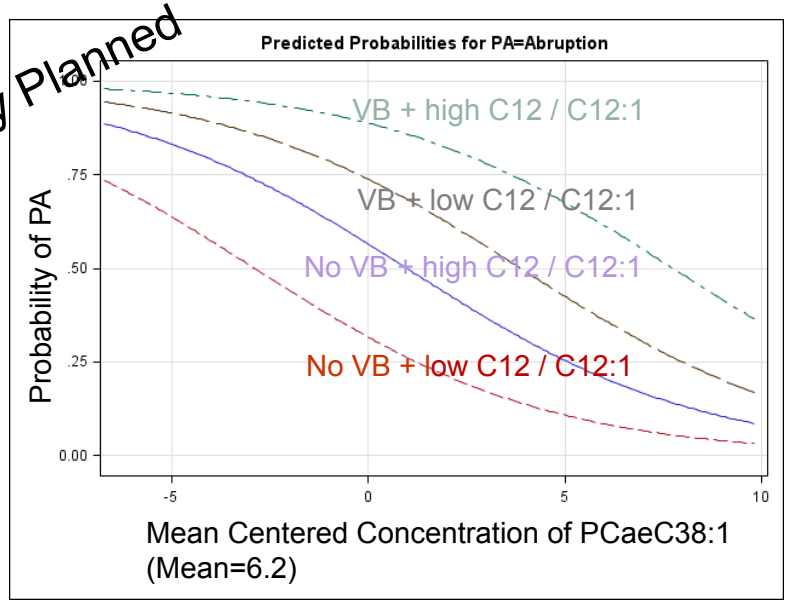
Logistic regression was used to model the probability of PA in the 3rd trimester based on serum biomarkers in 2nd trimester

	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



Pathways and PA

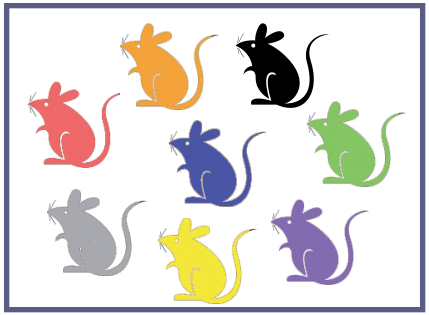
The probability of PA was increased with an increase in acylcarnitines and a decrease in phosphatidylcholine.

- These related pathways (acylcarnitine or phosphaticholine) branch at the metabolite diacylglycerol
  - Diacylglycerol is transformed to the endocannabinoid 2-arachidononylglycerol (2-AG), and 2-AG is converted to prostogladin glycerol esters.
- Vaswani et al. recently demonstrated that the enzyme prostaglandin-endoperoxide synthase-2 (PTGS-2), which converts 2-AG to prostaglandin glycerol esters, is down-regulated in the aging placenta
  - consistent with studies investigating the importance of PTGS-2 in preterm labor, and suggest that PTSGS-2 may play a role in the pathogenesis of abruption
- Aspirin, a COX-2 inhibitor, is used in extremely high risk pregnancies
- Choline/phosphatiylcholine are important in pregnancy and fetal development.
- Acylcarnitines are markers for mitochondrial function and reflect metabolic processes involved in long-chain fatty acid metabolism
  - they are synthesized by the enzyme carnitine palmitoyltransferase 1 (CPT 1) that is known to be responsible for the transport of fatty acids into the mitochondrial matrix
- Incomplete fatty acid oxidation results in elevated acylcarnitine concentrations, which is used in newborn screening to detect metabolic disorders.
- Alterations in concentrations of acylcarnitines have measured in women with gestational diabetes mellitus, or hypertensive disorders of pregnancy

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.

Genetically Diverse Mouse Population



Isoniazid (INH) (3 days)

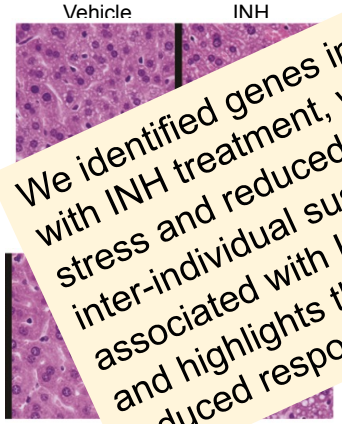
Endpoints

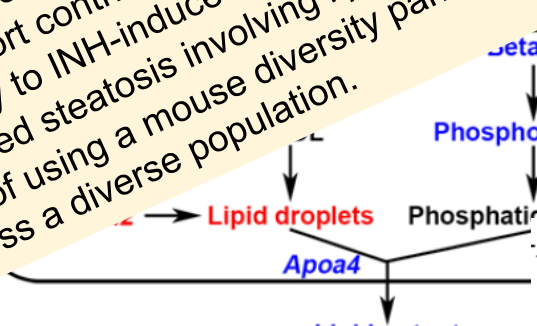
- **Histopathology, Adipo**
- **miRNA (miR-122)**
- **Transcripto**
- **Metab**

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… in h…
- …ype
- …ns
- …ites involved in … were among those … decreased in livers of sensitive … strains
- 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

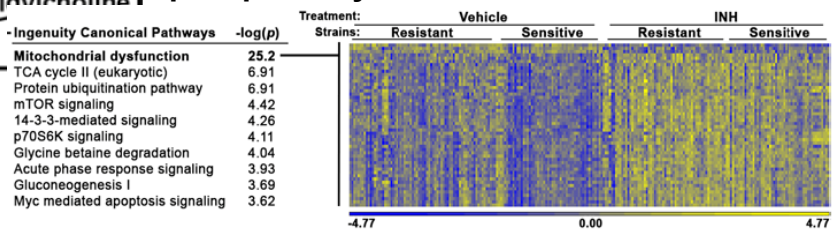
NON/ShiLtJ (Resistant)





Top 10 pathways associated with INH

Ingenuity Canonical Pathways	-log(p)
Mitochondrial dysfunction	25.2
TCA cycle II (eukaryotic)	6.91
Protein ubiquitination pathway	6.91
mTOR signaling	4.42
14-3-3-mediated signaling	4.26
p70S6K signaling	4.11
Glycine betaine degradation	4.04
Acute phase response signaling	3.93
Gluconeogenesis I	3.69
Myc mediated apoptosis signaling	3.62

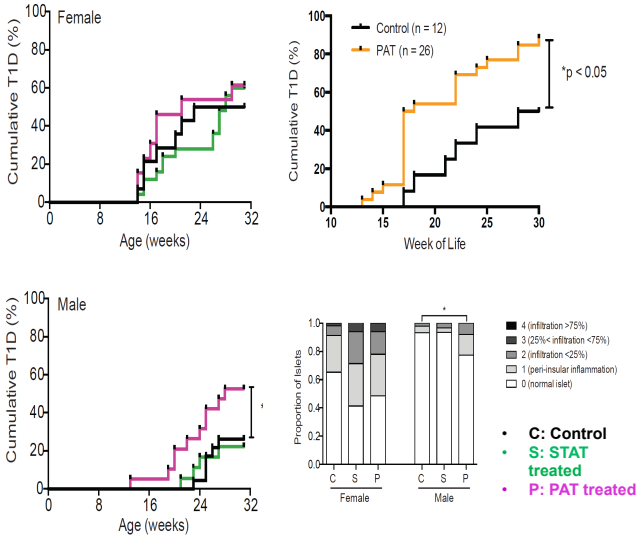


Church et al, 2014. *A Systems Biology Approach Utilizing a Mouse Diversity Panel Identifies Genetic Differences Influencing Isoniazid-Induced Microvesicular Steatosis.* Tox. Sci. 140(2): 481-92.

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

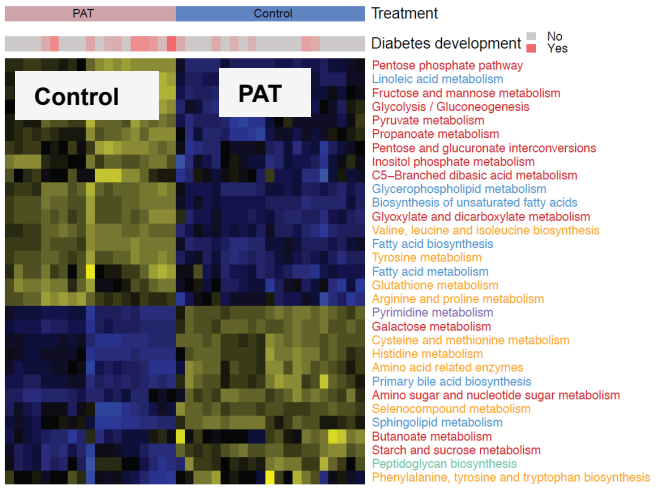
Alexandra Livanos

Antibiotic Mediated Gut Microbiome Perturbation in T1D

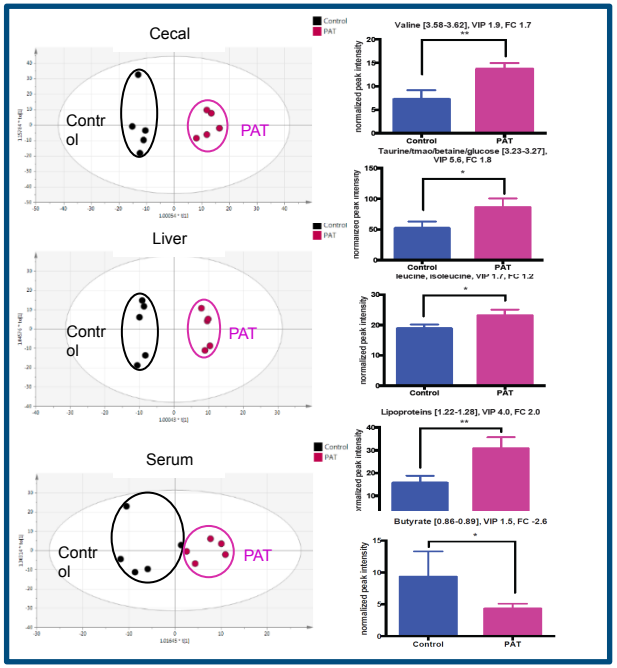
MICROBIOME

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

Metagenome in PAT mice were enriched in lipid, AA metabolism and reduced for butyrate

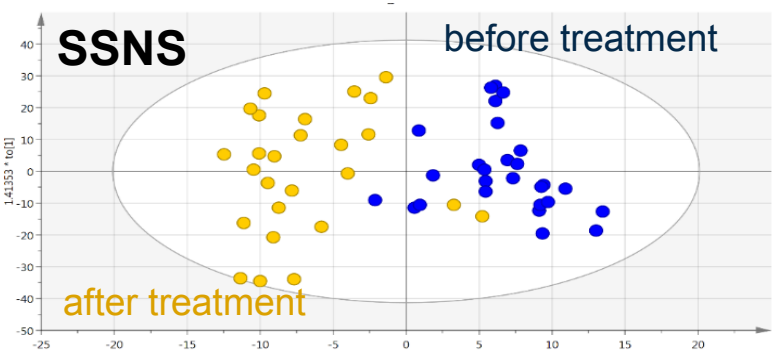


METABOLOMICS



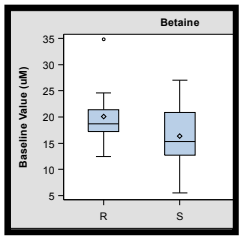
Metabolomics distinguished PAT exposed NOD from NOD control: including differences in amino acids, lipids and significant reduction in butyrate

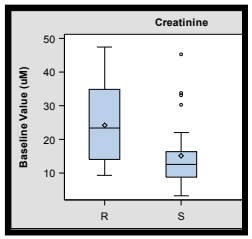
Mechanisms of Childhood Glucocorticoid Resistance

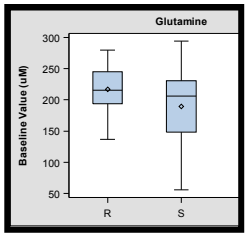


The SSNS model has good quality fit statistics:
R^{2X}(cum)=0.911 Q^2(cum)=0.595.
The SSNR model did not have good quality fit statistics.

Baseline: SS vs SR







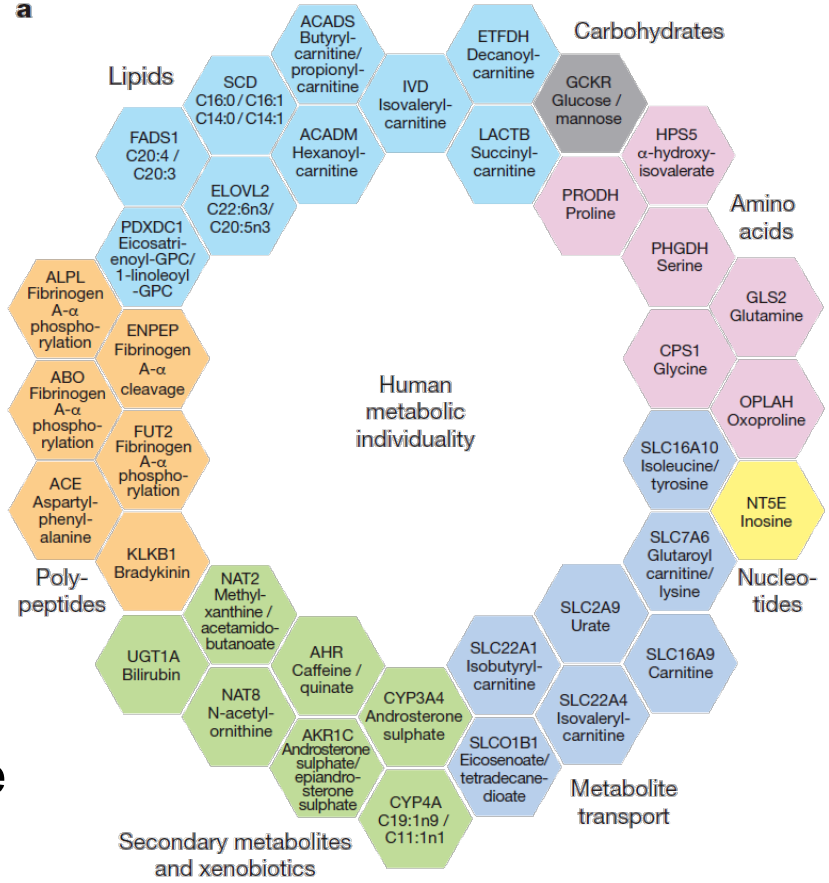
Creatinine
Glutamine
Betaine

Collaboration with
William Smoyer
Nationwide Children's Hospital

- 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- and post-treatment SSNS groups demonstrated that the biological variance between the treatment 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.

Human Metabolic Individuality

- Analysis of genotype dependent metabolic phenotypes using GWAS with non-targeted metabolomics via UPLC-MS and GC-MS
- Metabolic profiling on fasting serum from the Cooperative Health Research in the Region of Augsburg (KORA) F4 study (n=1,768) and British TwinsUK study (n=1,052)
- Assessed the association of approximately 600,000 genotyped SNPs with more than 37,000 metabolite signals by fitting linear models in each cohort to the log-transform of metabolite signals (adjusted for age gender and family structure)



Genetic basis of human metabolic individuality
37 Loci Correlated with Specific Disease

*Suhre et al. Human metabolic individuality in biomedical and pharmaceutical research, Nature 2011, 477, 54-62

Human Metabolic Individuality

Example Disease Related Associations

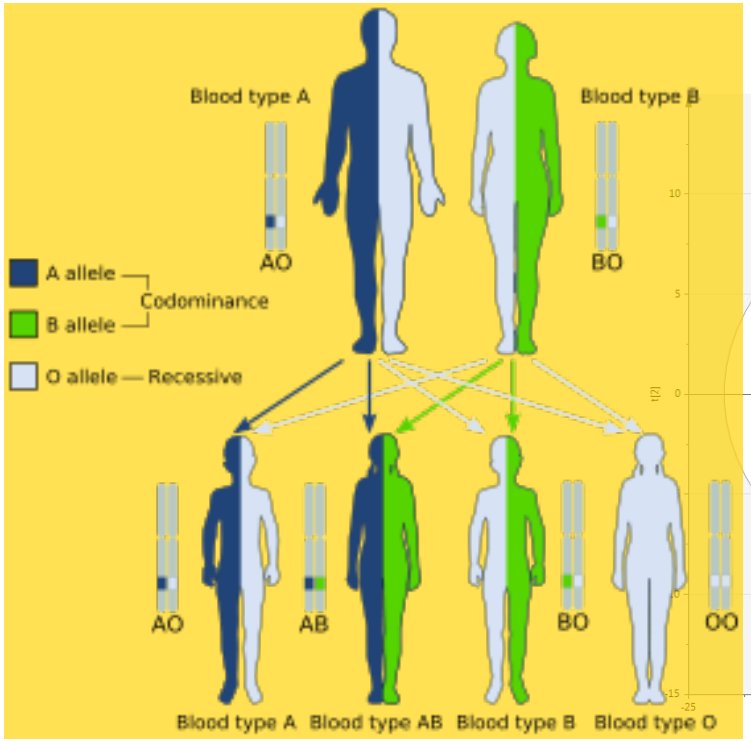
- Kidney disorders
  - Established a link between N-acetyltransferase 8 locus, N-acetylornithine, and eGFR, and CKD.
- Type 2 diabetes
  - Glucokinase regulator (GCKR) is a associated with diabetes and cardiometabolic related traits. This locus has a highly significant association with mannose:glucose ratios.
- Gout
  - SLC2A9 (GLUT9) which transports uric acid is highly associated with Urate metabolite levels

*Suhre et al..: Human metabolic individuality in biomedical and pharmaceutical research, Nature 2011, 477, 54-62

What's blood type got to do with metabolomics?

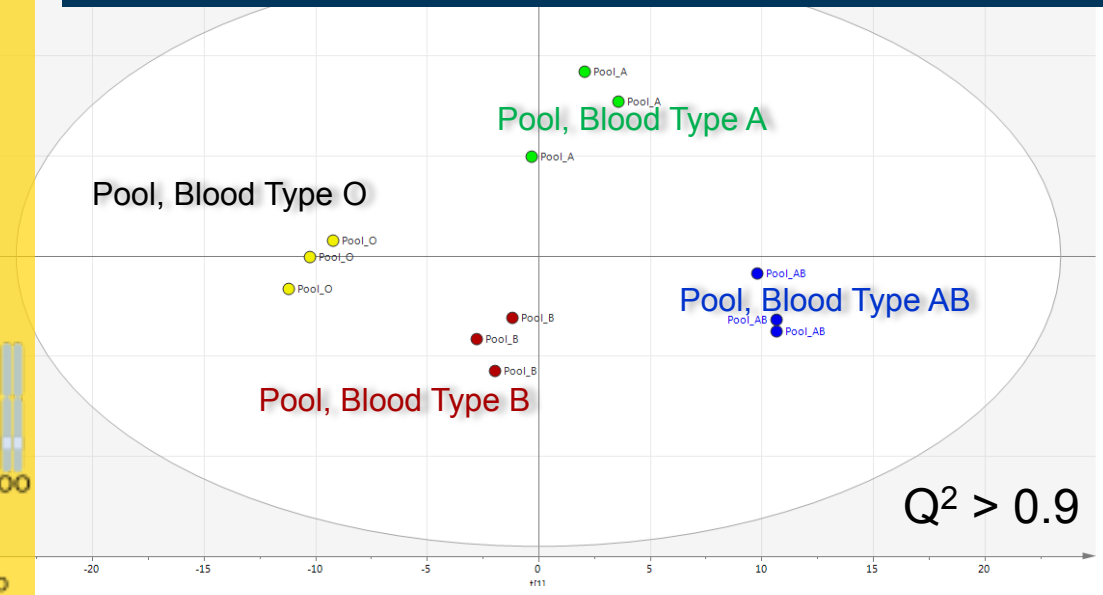
ABO blood type is located on chromosome 9 (9q34.1) - ABO glycosyltransferase.

The three main allelic forms are A, B, and O - each responsible for the production of its glycoprotein.



- Serum from 40 white healthy males AB, O, A, and B blood types
- Each of the 40 samples were prepared and run individually, and triplicates of each blood group were pooled and analyzed.

PCA of Pooled Samples



Metabotype of each Blood Type

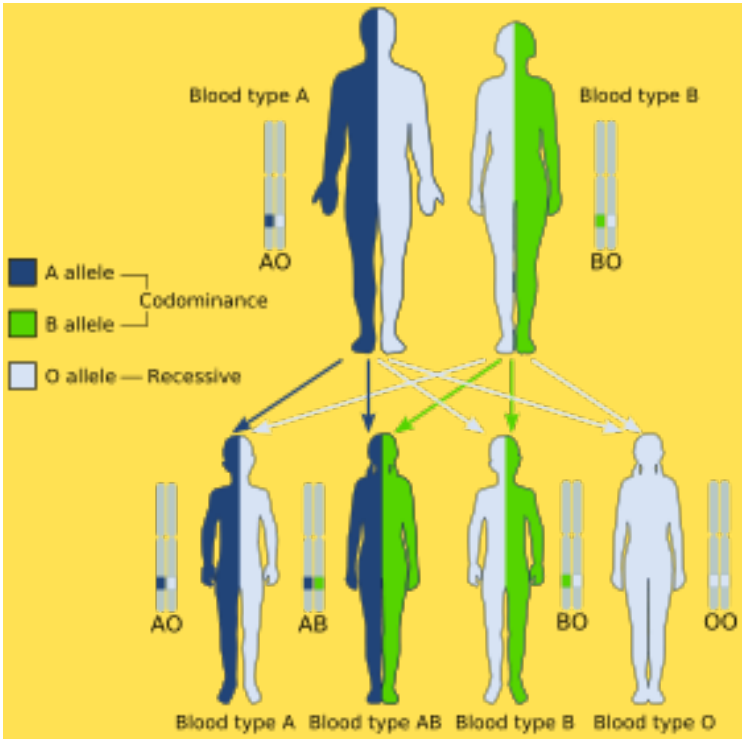
Blood Type X vs All Others	Metabolites Important to Defining Blood Type X
O	2-Hydroxy-3-methylvalerate, 2-Hydroxybutyrate,2-Hydroxyvalerate, 2-Oxocaproate, 2-Oxovalerate, 3-Hydroxybutyrate, 5-hydroxylysine, Agmatine, Alanine, Betaine, Butyrate, Carnitine, Choline, Dimethylamine, Fatty Acids, Fructose, Galactarate, Glucose, Glutamate, Glutamine, Glutathione, Glycerol, Isoleucine, Lactate, Leucine, Lipoproteins, Lysine, N-acetyl aminoacids, O-Acetylcarnitine, O-Acetyocholine, O-Phosphocholine, Phenylacetate, Proline, Pyroglutamate, sn-Glycerophosphocholine, Succinate, Taurine, TMAO, Galactonate, Unsaturated lipids, Valine
A	2-Hydroxy-3-methylvalerate, 2-Hydroxyvalerate, 2-oxocaproate, 3-Hydroxybutyrate, 3-Methyl-2-oxovalerate, Agmatine, Betaine, Butyrate, Carnitine, Choline, Creatine, Creatinine, Fatty Acids, Fructose, Glucose, Glutamate, Glutamine, Isoleucine, Lactate, Leucine, Lipoproteins, Lysine, N-acetyl aminoacids,O-Acetyocholine, O-Phosphocholine, Phenylacetate, Phenylalanine, Proline, sn-Glycerophosphocholine, TMAO, Tryptophan, Unsaturated lipids, Valine
B	2-Hydroxy-3-methylvalerate, 2-hydroxybutyrate, 2-hydroxyisocaproate, 2-Oxocaproate,3-Hydroxybutyrate, 3-Methyl-2-oxovalerate, 3-Phenyllactate, 4-Aminobutyrate, Acetate, Acetoacetate, Asparagine, Betaine, Butyrate, Carnitine, Choline, Creatine, Glucose, Glutamate, Glutamine, Glutarate, Glutathione, Glycerol, Lactate, Leucine, Lipids, Lipoproteins, Lysine, N,N-dimethylglycine, N-Acetyl aminoacids, N-Acetylglutamine, N-Methylhydantoin, O-Phosphocholine, Phenylacetate, Phenylalanine, Proline, Pyroglutamate, sn-Glycerophosphocholine, Taurine, Trimethylamine N-oxide, Tryptophan, Valine
AB	N-Acetyltyrosine, O-Acetylcarnitine, O-Acetyocholine, p-Cresol, Phenylacetate, TMAO, 2-Hydroxybutyrate, 2-Hydroxyisocaproate, 2-Oxocaproate, 4-Aminobutyrate, 5-Hydroxylysine, Acetate, Alanine, Carnitine, Citrate, Fructose, Galactarate, Glutamate, Glutathione, Glycerol, Lipids, Phenylalanine, Pyroglutamate, Sarcosine, sn-Glycerophosphocholine, Succinate, Taurine, Galactonate, Unsaturated lipids, 2-Hydroxy-3-methylvalerate, 2-Hydroxyvalerate, 3-Hydroxybutyrate, Betaine, Choline,

Blood Type and Disease

- The Suhre et al. “Human metabolic individuality in biomedical and pharmaceutical research” GWAS study demonstrated links between SNPs, specific metabolites, and disease.
- There are known risks of specific blood types and diseases.
- Can we determine links between the metabotype of the blood type and disease?
- Can we determine links between secretor status, the metabotype of the blood type, and disease?

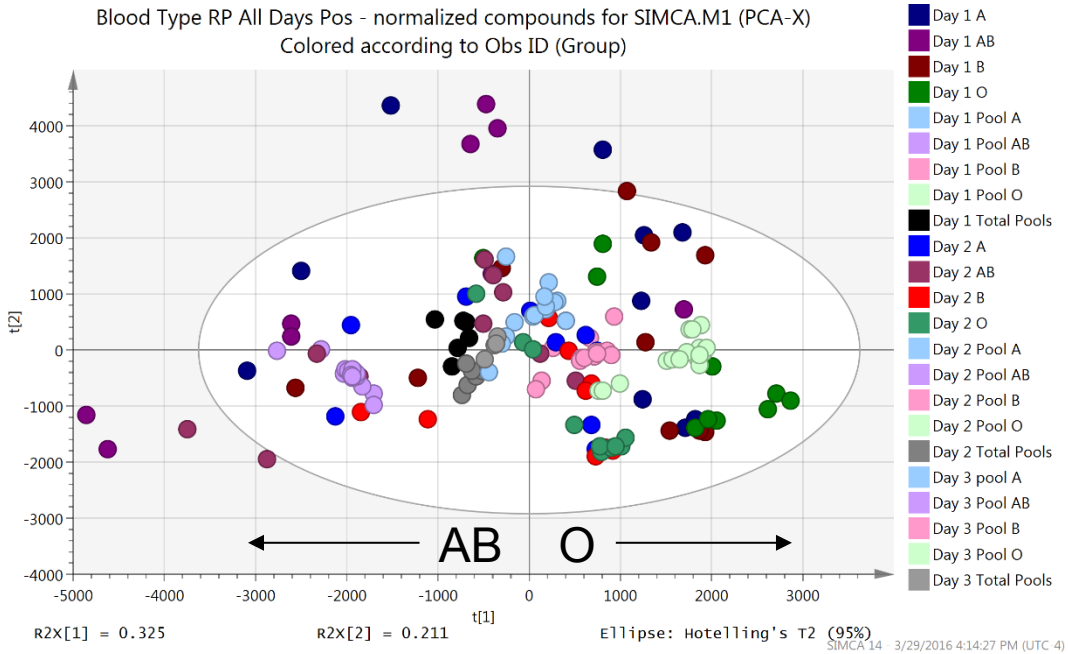
Disease	Disease Metabolites Important to Projection	Blood Type and Risk for Disease	Blood Type Metabolites Important to Projection
Diabetes Type 2 Obesity	**Glucose**, **Lipoproteins**, **Plasma phospholipids**, **Glycerol**, **Leucine**, Isoleucine, **Valine**, **Amino acids** and metabolites, Fatty acids- unsaturated and saturated, Uric acid, TCA-cycle metabolites, **Tyrosine**, **Phenylalanine**, Esterified fatty acids (EFAs) and non-esterified fatty acids (NEFAs), Acylcarnitines, β-hydroxybutyrate, **Sulfur amino acids**, Bile acids	Non-O blood Groups (B as an example)	2-Hydroxy-3-methylvalerate, 2-hydroxybutyrate,2-hydroxyisocaproate, 2-Oxocaproate,3-Hydroxybutyrate, 3-Methyl-2-oxovalerate, 3-Phenyllactate, 4-Aminobutyrate, Acetate, Acetoacetate, **Asparagine**, Betaine, Butyrate, Carnitine, Choline, Creatine, **Glucose**, **Glutamate**, **Glutamine**, Glutarate, Glutathione, **Glycerol**, Lactate, **Leucine**, **Lipids**, **Lipoproteins**, Lysine, N,N-dimethylglycine, N-Acetyl aminoacids, N-Acetylglutamine, N-Methylhydantoin, O-Phosphocholine, **sugars,** Phenylacetate, **Phenylalanine**, **Proline**, Pyroglutamate, sn-Glycerophosphocholine, **Taurine**, Trimethylamine N-oxide, **Tryptophan**, **Valine**

AB vs O Metabotypes: UPLC-TOF-MS



- Serum from 40 white healthy males AB, A, B, and O
- UPLC-TOF-MS

PCA of All Samples



Links Between Metabolites, Disease, and Blood Type

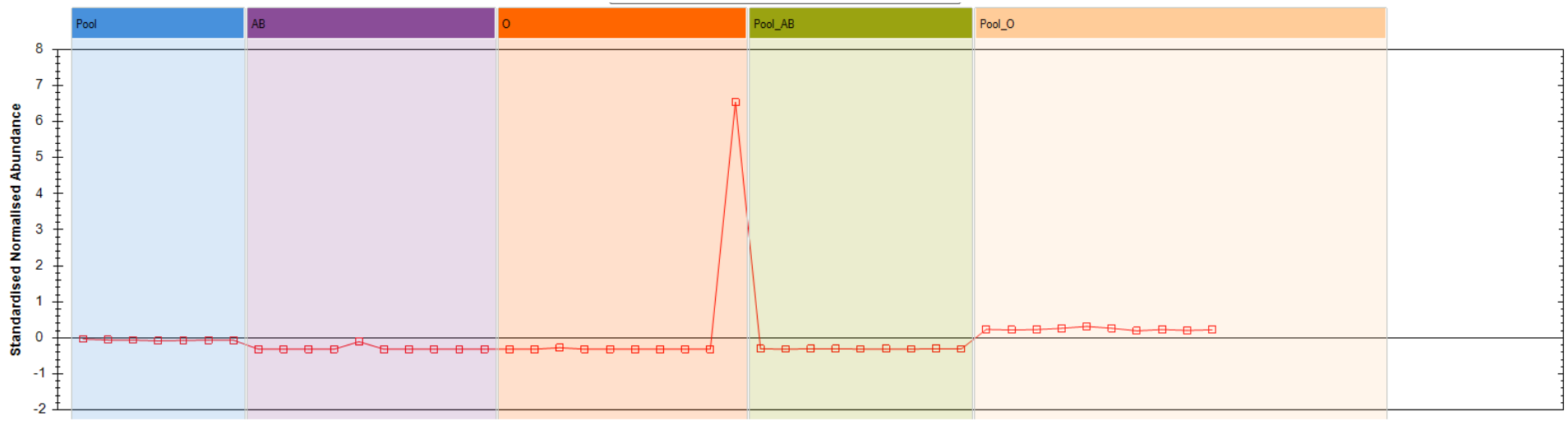
- **3-Decaprenyl-4-hydroxybenzoic acid** (DHB) is 2.7 fold higher in AB vs O blood groups ($p < 0.001$).
  - DHB in humans is involved in the biosynthesis of coenzyme Q10.
  - Reduced CoQ10 levels is a typical feature of PD patients, and associated with mitochondrial energy production deficit.
  - Franchini and Liumbruno: Blood Transfus. 2016 Mar; 14(2): 158–159.
    - The AB blood type and increased coagulation factor VIII levels were associated with a higher incidence of cognitive decline.
- **Opiorphin** is 3.6 fold higher in AB than O ($p= 0.01$).
  - Opiorphin is an endogenous compound first isolated from human saliva, and is a natural antinociceptive modulator of opioid-dependent pathways.
  - The odds ratio for AB blood group in opioid addicts is 3.98 compared to non-addicts ($p < .001$)
  - Afltoonian et al., 2011. Possible association between human blood types and opioid addiction Am J Addict. Nov-Dec;20(6):581-4
  - Non-O blood groups are associated with the ADRA2C 322-325 deletion variant which has been associated with higher pain perception and cognitive responses.
    - Kohil et al., 2010. Eur J Pain. Effects of variation in the human alpha2A- and alpha2C-adrenoceptor genes on cognitive tasks and pain perception Feb;14(2):154-9.

Links Between Metabolites, Disease, and Blood Type

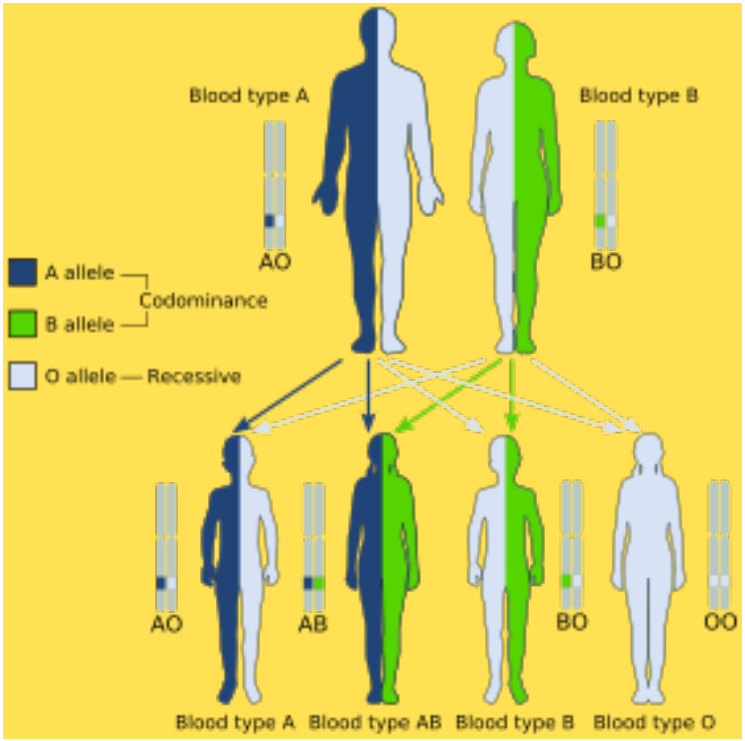
- **Uric Acid** is 1.6-fold lower in AB vs O ($p < 0.001$).
  - High blood concentrations of uric acid can lead to gout and are associated with other medical conditions including diabetes and the formation of ammonium acid urate kidney stones.
    - Anecdotal - O is more likely to have gout
    - Acheson, 1970. Epidemiology of serum uric acid and gout: an example of the complexities of multifactorial causation. Proc R Soc Med. 1970 Feb; 63(2): 193–197
    - Guo et al., Intestinal Microbiota Distinguish Gout Patients from Healthy Humans. Sci Rep 2016 Feb 8;6:20602. doi: 10.1038/srep20602.
    - Mkivuokko et al., 2012. J. Association between the ABO blood group and the human intestinal microbiota composition. *BMC Microbiology*

Lipid Profiling of AB vs O Blood Groups

- Esomeprazole and 5-hydroxyesomeprazole
- Used to treat Peptic Ulcers and Gastro-esophageal reflus (GERD)
- Type O is more likely to develop GERD and peptic ulcers
  - Garratty (2000) Blood groups and disease: A historical perspective. *Transfusion Medicine Reviews*,14:291-301

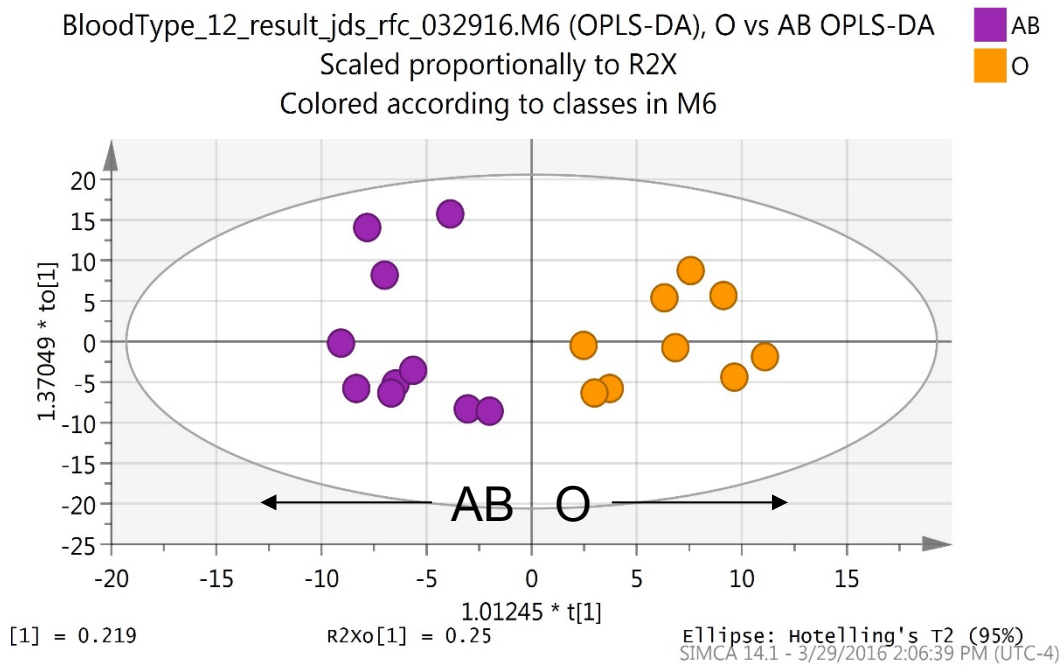


AB vs O Metabotypes: GC-TOF-MS



- Serum from 40 white healthy females AB and O
- GC-TOF-MS: Fiehn Method

OPLSDA



GC-TOF-MS: AB vs O

Metabolite	p-value
alanine	0.033
arachidic acid	0.009
behenic acid	0.021
glucose	<.001
glutamine	0.01
glycerol	0.034
glycine	0.05
histidine	0.019
isoleucine	0.012
leucine	0.007
lysine	0.039
myristic acid	0.053
octadecanol	0.033
palmitoleic acid	0.041
phenylalanine	0.038
proline	0.031
serine	0.028
stearic acid	0.051
tyrosine	0.027
Valine	0.009

- **Branched Chain Amino Acids** are higher in AB
  - BCAAs been associated with increased risk for diabetes
    - Diabetes is higher in non-O blood groups
- **Tyrosine** is involved in the production of the stress neurotransmitters epinephrine and norepinephrine. It is taken as a supplement to fight off depression and boost dopamine levels
  - Tyrosine is higher in the AB blood type
    - Type O blood type have more depression and intense anxiety
      - Anderson and Stern, 2015, Blood Type matters for Brain Health. Scientific American
      - Singg and Lewis, 2001, Depression and Blood Types. Psycol Rep.
- **Palmitoleic acid is an unsaturated fatty acid** and reported to play a role in reduced heart disease risk.
  - Lower in AB than O
  - Blood type A, B, or AB had a higher risk for coronary heart disease when compared to those with blood type O
    - He et al., 2012. ABO Blood Group and Risk of Coronary Heart Disease in Two Prospective Cohort Studies. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *32*(9), 2314–2320.

STS Center

Wimal Pathmasiri
NMR & GC-MS

Jim Carlson
LC- and GC-MS

Jessica Gooding
LC-MS

Kelly Mercier
NMR

Susan McRitchie
Data Analysis

Bob Clark
Genetics

Zach Acuff
Biostatistics

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

Tim Fennell
Metabolism

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

Sue Clark
Administrative
Support

Melody Markley Model Systems

Funding

NIDDK: NIH Common Fund
1U24DK097193: NIDDK: Sumner, RTI, PI
"NIH Eastern Regional Comprehensive Metabolomics Resource Core"

NIGMS: NIH Common Fund
5R25GM103802: Martin Kohlmeier, UNC-CH, PI
"Online learning platform introducing clinicians and researchers to metabolomics."

NEI: NIH Common Fund
R01EY012224: Ellen Weiss, UNC-CH
"Desensitization of Cone Visualization Signaling Pathways."

NHLBI: NIH Common Fund
HHSN268201300021C:Herb Seltzman, RTI, PI
"Metabolite Standards Synthesis Core"

NIDDK: 1UMDK10086601: William Smoyer, Nationwide Children's Hospital & Larry Greenbaum, Emory, MPI
"Integrated Proteomics and Metabolomics for Pediatric Glomerular Disease"

NIEHS: U19ES019525: Timothy Fennell, RTI, PI
"Estimating Human Health Risks from Exposure to Nanoparticles"

NCATS: UL1TR00111: PI: John Buse and Timothy Carey, UNC-CH, MPI
"North Carolina Translational and Clinical Sciences Institute- NCTraCS"