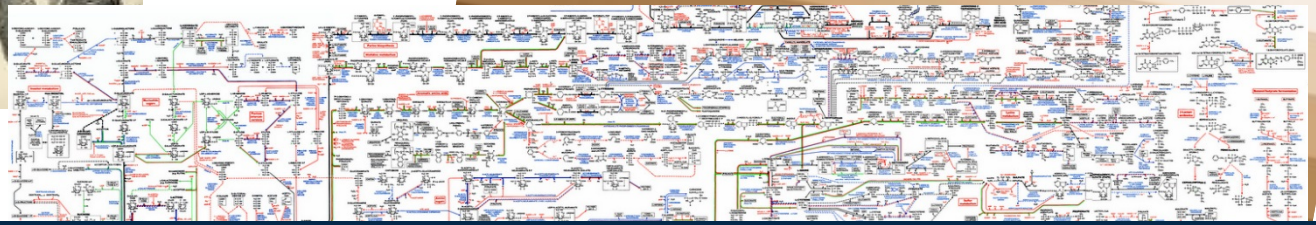
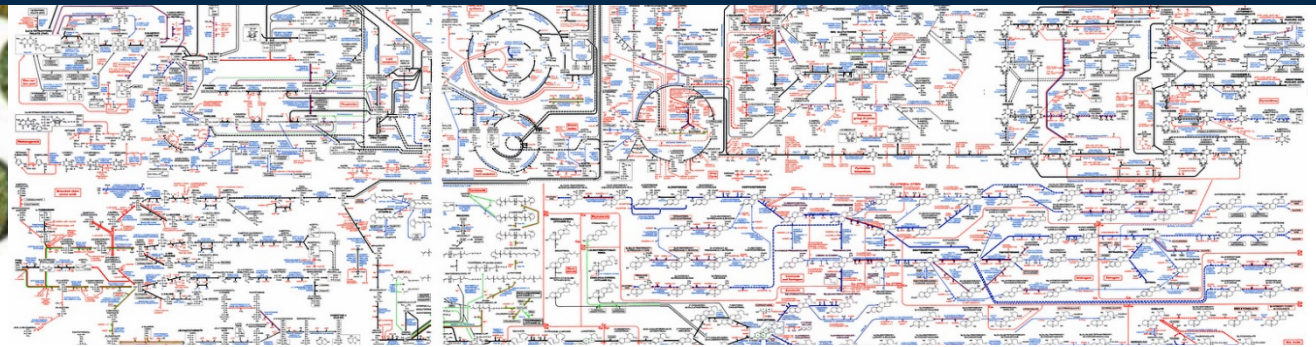
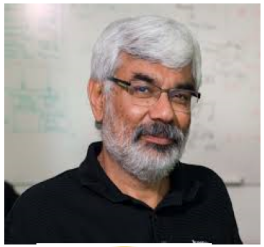
UNC Nutrition Research Institute



Using Biochemical Fingerprints to Understand Health



NIH Common Fund Metabolomics Centers



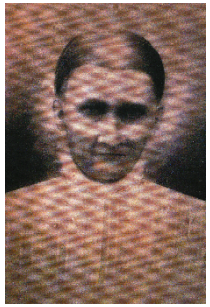
NIH Metabolomics Centers Ramp Up | November 4, 2013 Issue - Vol. 91 Issue 44 | Chemical & Engineering News. by Jyllian Kemsley

My Genetics!

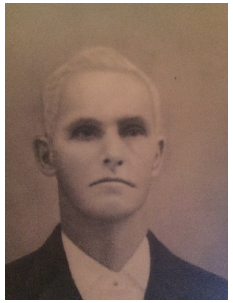
23&me, Ancestry

Emma **Kellis**
Troy, NC

E. Coleman **Ledbetter**
Albemarle, NC



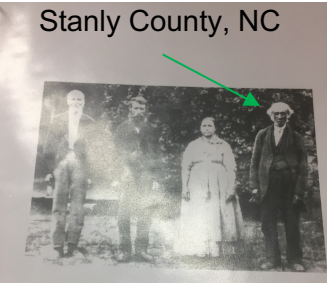
Cynthia **Thompson**
Mt Gilead, NC



Silas Aaron **Jenkins**
Midland, NC

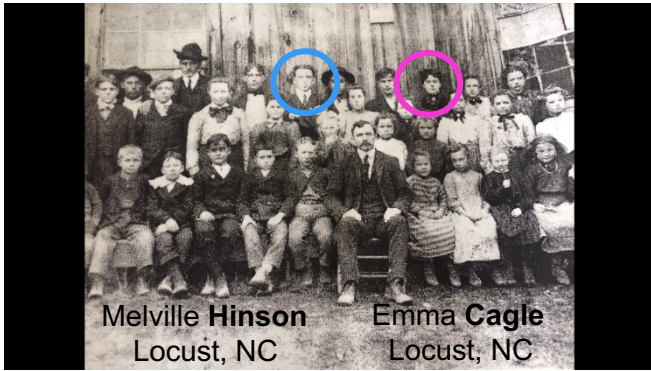
Honeycutt
Lambert
Green
Smith
Burelyson
Campbell
Wicker
Carpenter
Hughes

Allen Burris
Stanly County, NC



John and Victoria **Tucker**
Concord, NC

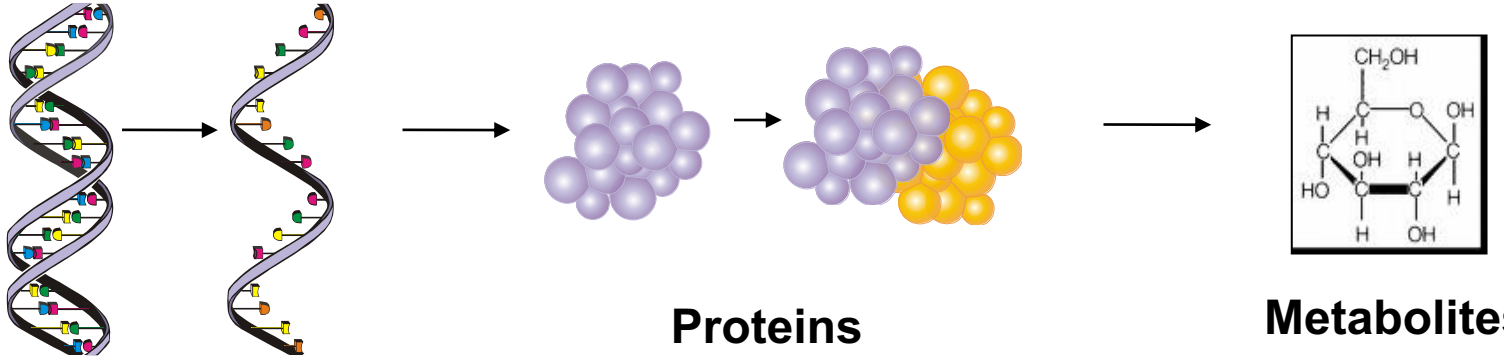
Mazeriah **Whitley**
Oakboro, NC



Melville **Hinson**
Locust, NC

Emma **Cagle**
Locust, NC

Genes, Proteins, and Metabolites



DNA

DNA contains genetic instructions:

- make components of cells
- regulate the use of these components

Proteins

- Proteins are made of sequences of amino acids; the sequence defined by the gene.
- Proteins are the enzymes that catalyze or accelerate chemical reactions in metabolism

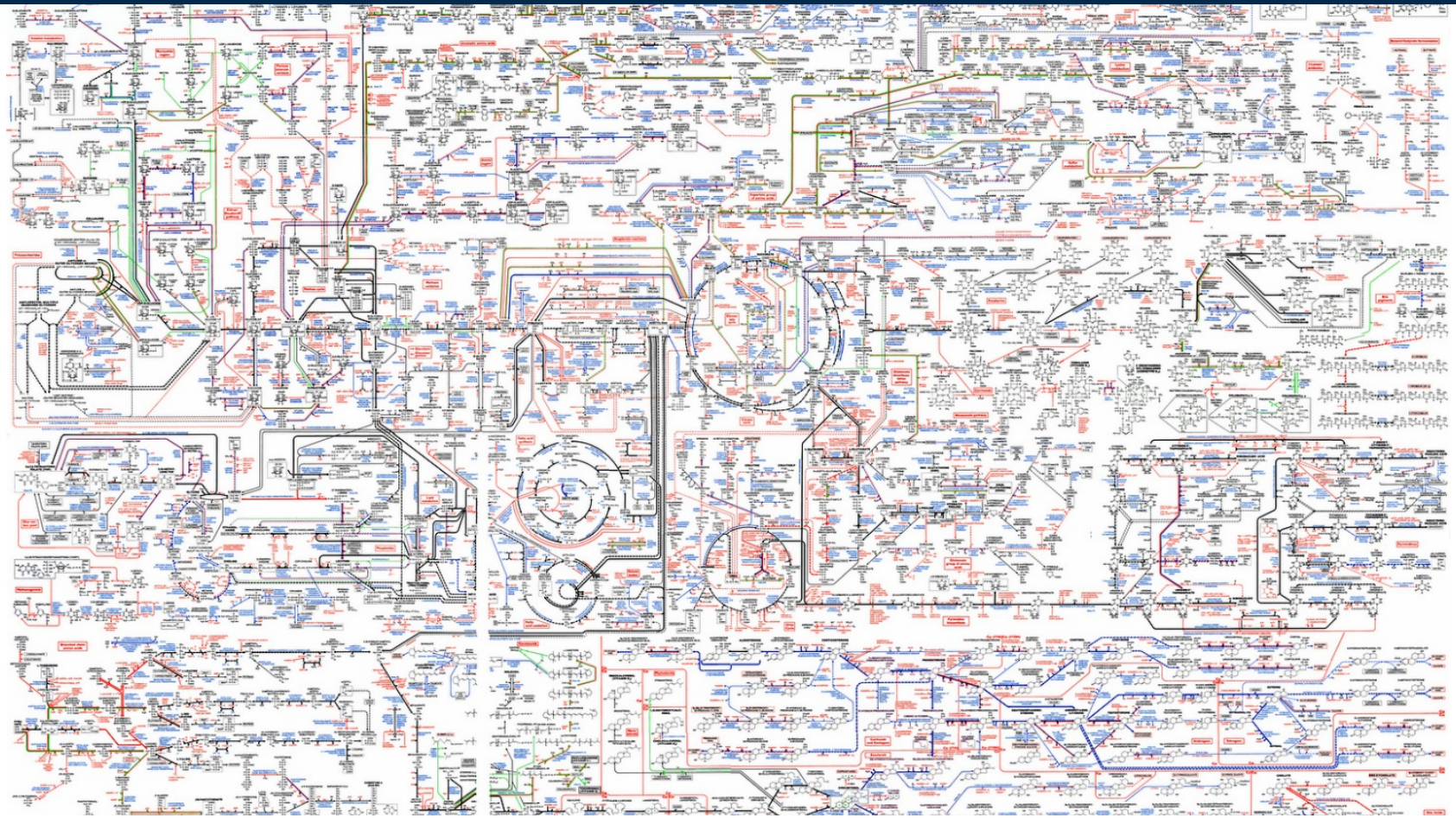
Metabolites

Metabolites are intermediates and products of metabolism.

Catabolism: the processes to breaks down large molecules.

Anabolism: the process to use catabolism energy to synthesize molecules

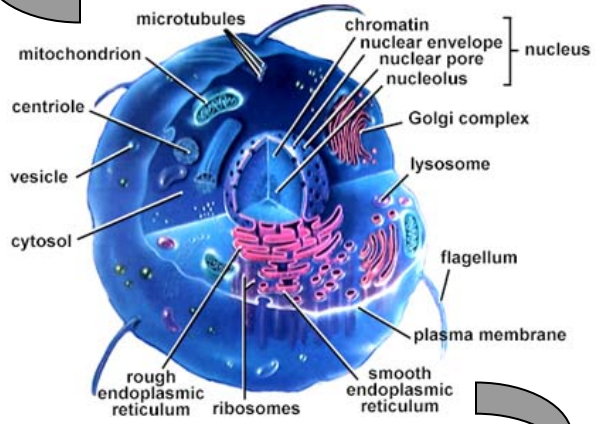
Pathways



Biochemicals are in our Cells, Tissues, and Biological Fluids

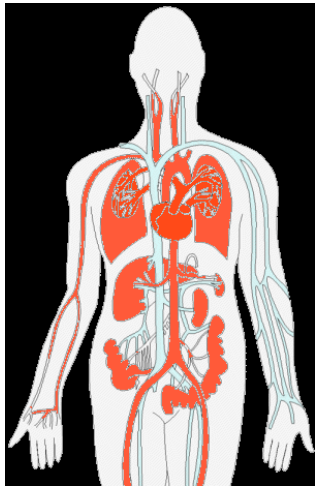
Cells / Organ

Released metabolites



Cytosolic metabolites

System

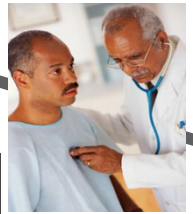


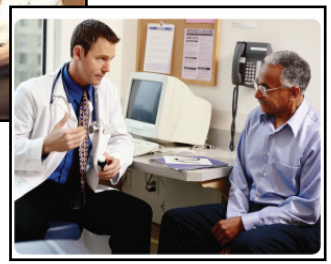
Serum
Urine
Saliva
Breath
Plasma
CSF

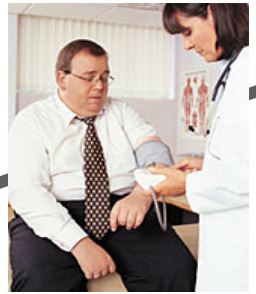
Signatures or Profiles

Discrete peaks ⇨ *Diagnostics*

Comparing States of Health



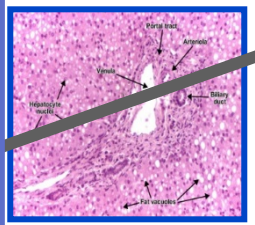


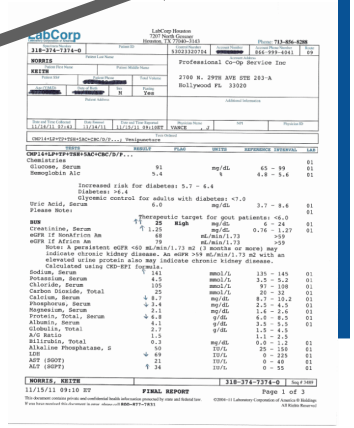


KADELPHIA, AR 71923 UPIN: J

Date and Time Collected	Date Entered	Date and Time Reported	Physician Name	NPI	Phys
/15/11 09:09	04/16/11	04/16/11 06:08ET	PLUNK, O	1104824069	1104

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL
eGFR If Africn Am	115		mL/min/1.73	>59
Note: A persistent eGFR <60 mL/min/1.73 m2 (3 months or more) may indicate chronic kidney disease. An eGFR >59 mL/min/1.73 m2 with an elevated urine protein also may indicate chronic kidney disease. Calculated using CKD-EPI formula.				
BUN/Creatinine Ratio	12			8-19
Sodium, Serum	137		mmol/L	135-145
Potassium, Serum	3.9		mmol/L	3.5-5.2
Chloride, Serum	102		mmol/L	97-108
Carbon Dioxide, Total	26		mmol/L	20-32
Calcium, Serum	10.2		mg/dL	8.7-10.2
Protein, Total, Serum	6.9		g/dL	6.0-8.5
Albumin, Serum	4.3		g/dL	3.5-5.5
Globulin, Total	2.6		g/dL	1.5-4.5
A/G Ratio	1.7			1.1-2.5
Bilirubin, Total	1.1		mg/dL	0.0-1.2
Alkaline Phosphatase, S	105		IU/L	25-150
AST (SGOT)	22		IU/L	0-40
ALT (SGPT)	25		IU/L	0-55
Testosterone, Serum	402		ng/dL	249-836
LH	<0.2	Low	mIU/mL	1.7-8.6
FSH		Low	mIU/mL	1.5-12.4
Estradiol	31.1		pg/mL	7.6-42.6





The NEEDS

More sensitive and early markers for disease detection and staging

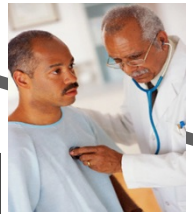
Markers to monitor treatment for

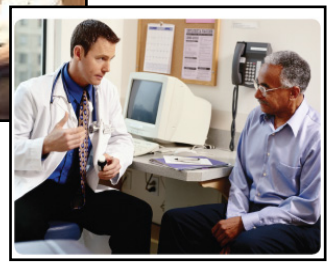
- **efficacy**
- **adverse response**
- **relapse**
- **transplantation**

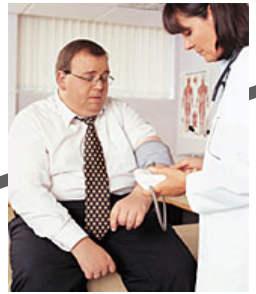
Determining the relationship of exposure to health outcomes

Mechanistic insights from pathway analysis

Comparing States of Health

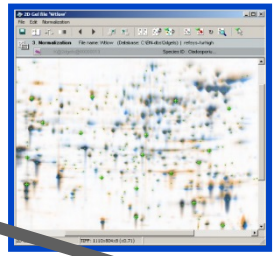




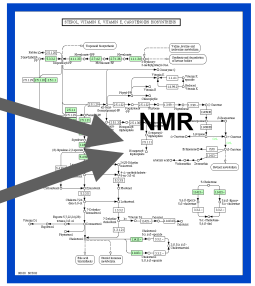




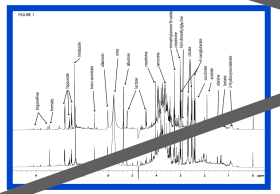
Protein Expression



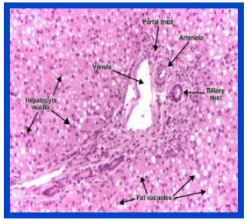
Pathways



Metabolite Levels



Traditional Clinical Parameters




Biomarkers

More sensitive and early markers for disease detection and staging

Markers to monitor treatment for

- efficacy
- adverse response
- relapse
- transplantation

Determining the relationship of exposure to health outcomes

Mechanistic insights from pathway analysis

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.

What we do

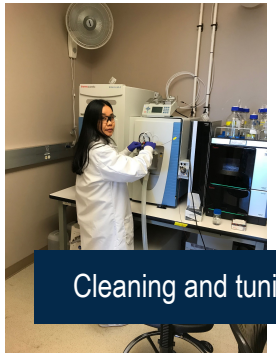
Calibration for sample preparation

Quality control

Running Standards

Reproducibility

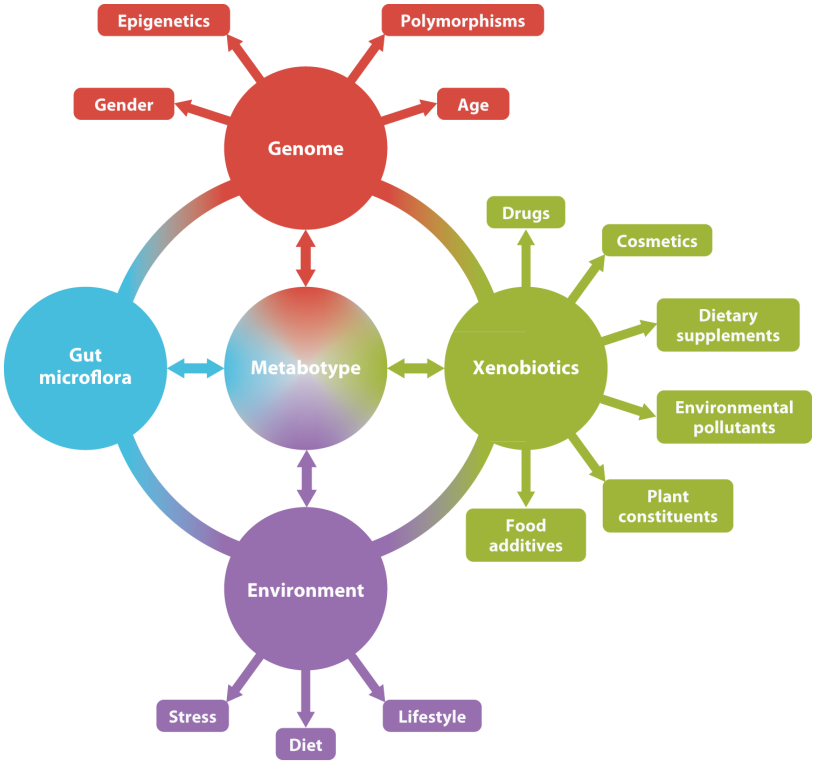
Loading autosampler



Cleaning and tuning

This is really complicated…..

Metabotype (1000s of signals)



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….

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*

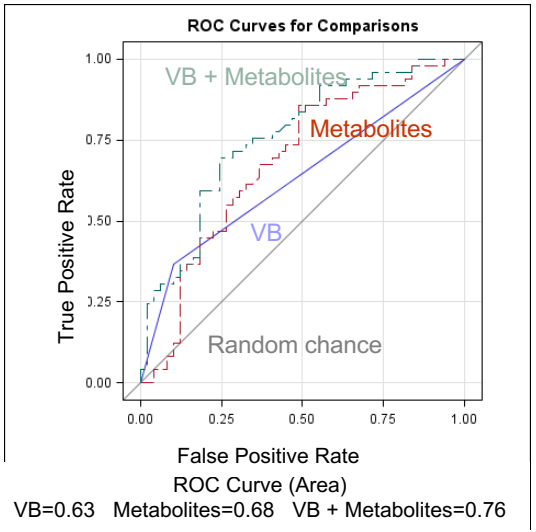
Collaboration with Michelle Williams and Bizu Gelaye (Harvard University)

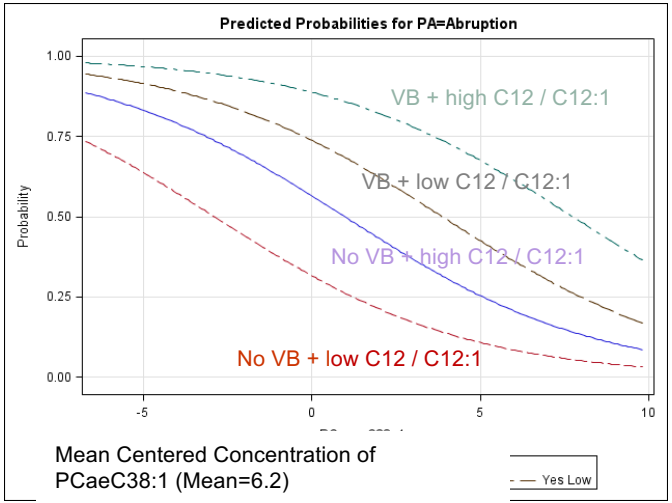
Only Nine Metabolites were Associated with PA

Classification	Metabolite Abbreviation	Metabolite	p-value
Acylcarnitines	C16-OH	Hydroxyhexadecanoyl-L-carnitine	0.021
Amino Acids	Arg	Arginine	0.029
Biogenic Amines	Histamine	Histamine	0.034
Custom Ratios	C12 / C12:1	Dodecanoyl-L-carnitine/Dodecenoyl-L-carnitine	0.045
Glycerophospholipids	PC aa C36:0	Phosphatidylcholine diacyl C 36:0	0.040
	PC aa C38:0	Phosphatidylcholine diacyl C 38:0	0.048
	PC aa C40:1	Phosphatidylcholine diacyl C 40:1	0.038
	PC ae C38:1	Phosphatidylcholine acyl-alkyl C 38:1	0.021
	lysoPC a C18:1	lysoPhosphatidylcholine acyl C18:1	0.040

Metabolites predicted PA better than traditional clinical measures

	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

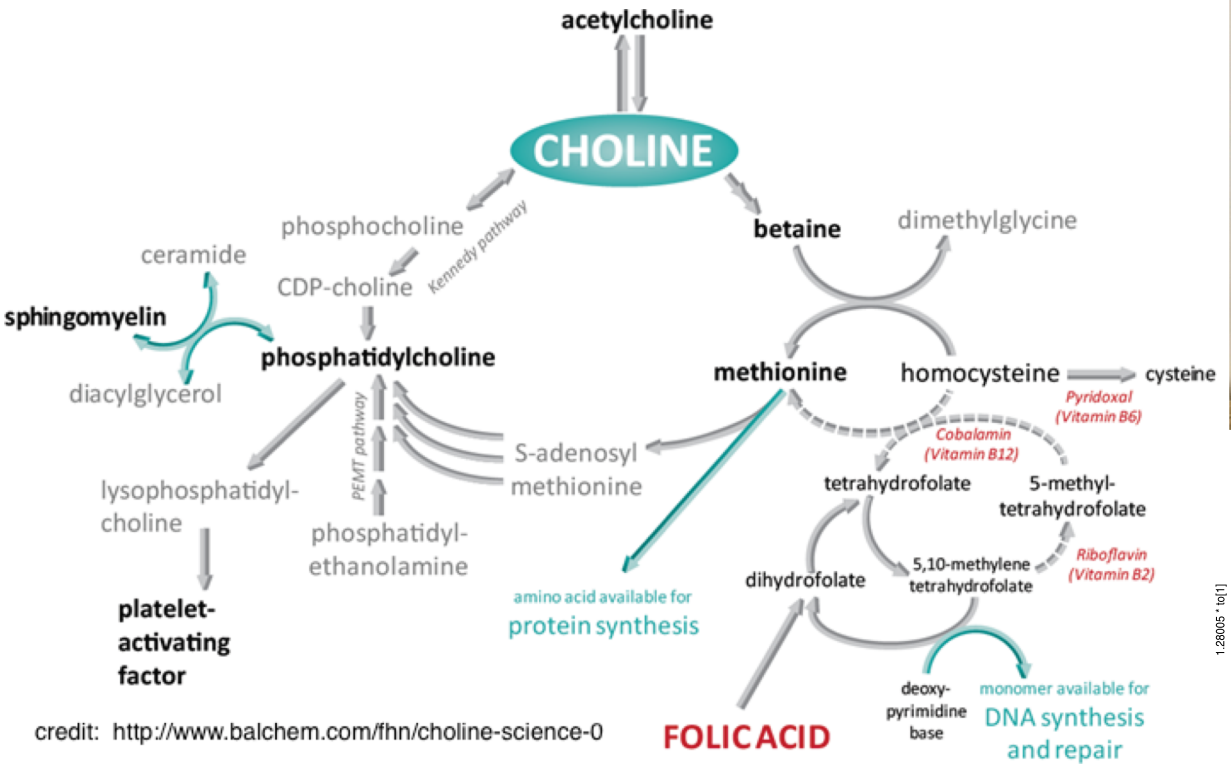




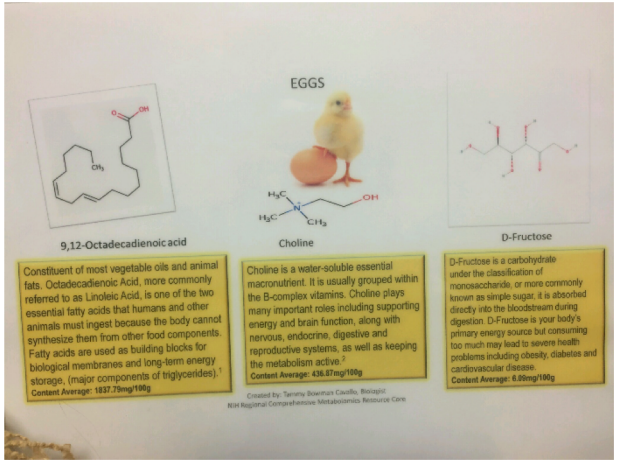
Gelaye et al., 2016. Maternal Early Pregnancy Serum Metabolomics Profile and Abnormal Vaginal Bleeding as Predictors of Placental Abruption: PlosOne 11(6).

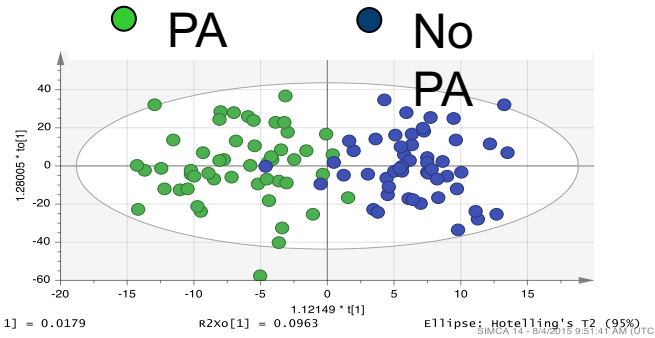
Pathways and Intervention

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



credit: http://www.balchem.com/fhn/choline-science-0





Larger cohort study currently being analyzed: ~230 samples

Metabolomics and Autism

Chinese Han population

3 - 6 years old

Discovery phase

- Autistic subjects n=73 (
- Neurotypical s (51 M/12 F)

Validation pha

- Autistic subjects n=100 (86 M/1
- Neurotypical subjects n

Exclusion:

- Asper
  d

Fastin **-MS**

agnosis Panels Used

- Autism Behav sm Rating Scales nt delay di

in the same city-
to assure no features of
or autistic traits.

very: 17 metabolites identified

alidation: 11 metabolites validated :

sphingosine 1-phosphate (S1P)

docosahexaenoic acid (DHA)

DHA is a supplement used in ASD

Sphingomyelin metabolism and fatty acid metabolism associated with ASD

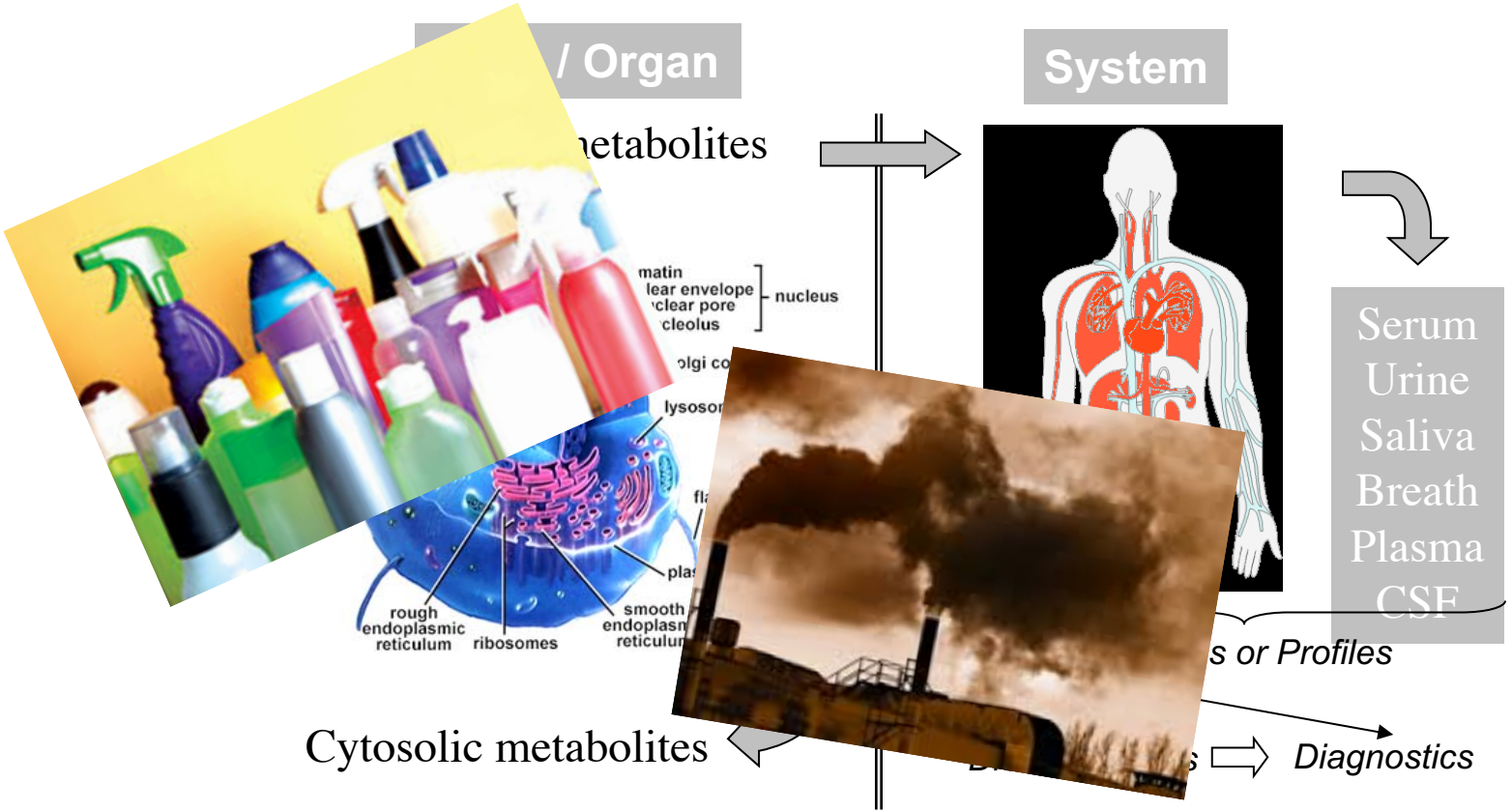
Biological psychiatry 2004;55(3):323-6.

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 .

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

Biochemicals are in our Cells, Tissues, and Biological Fluids



Environmental Chemicals and our Health

- Particulate matter mass
- Volatile organic compounds
- Pyrethroids
- Non-persistent pollut
- Pl
- Flame retardants
- Metabolites of PCB
- ...ldenyde and other carbonyls
- Organochlorine

NIEHS recently established the Children's Health Exposure Analysis Resource (CHEAR) to add or expand the inclusion of environmental exposures in studies of children's health.

Placenta, brain, CSF, urine, serum, ….

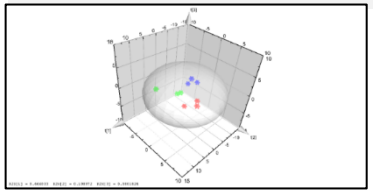
V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Pb, Sn, Hg

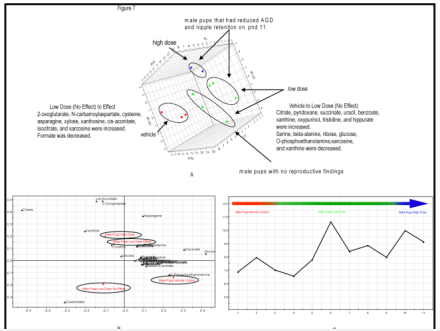
- Phthalates, ubiquitous in the enviro ent, have been characterized as endocrine disruptors.
- Pregnant rat during gesta 8-21): control, low dose (25 dose (250 mg
- Urine was collected from dams gd 18 an 21, and from pups after weaning but before puberty (pnd 26), and organ tissues were collected at study termination.

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

- Nanoparticles
- Arsenic
- PAHs

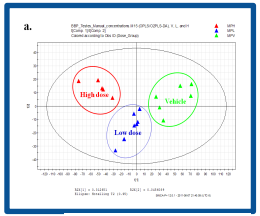
Dams that received the high dose, the low dose, or the vehicle at 21 days past exposure were distinguished



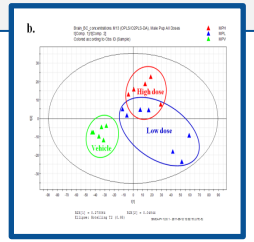


Urine from pups, on pnd 26, were distinguished by the dose the dam received during gestation.

Differences (control vs BBP dose groups) were noted in levels of metabolites in the brain (male and female pups, not dams), testes, and uterus (dam but not pup).



Testes



Male pup brain

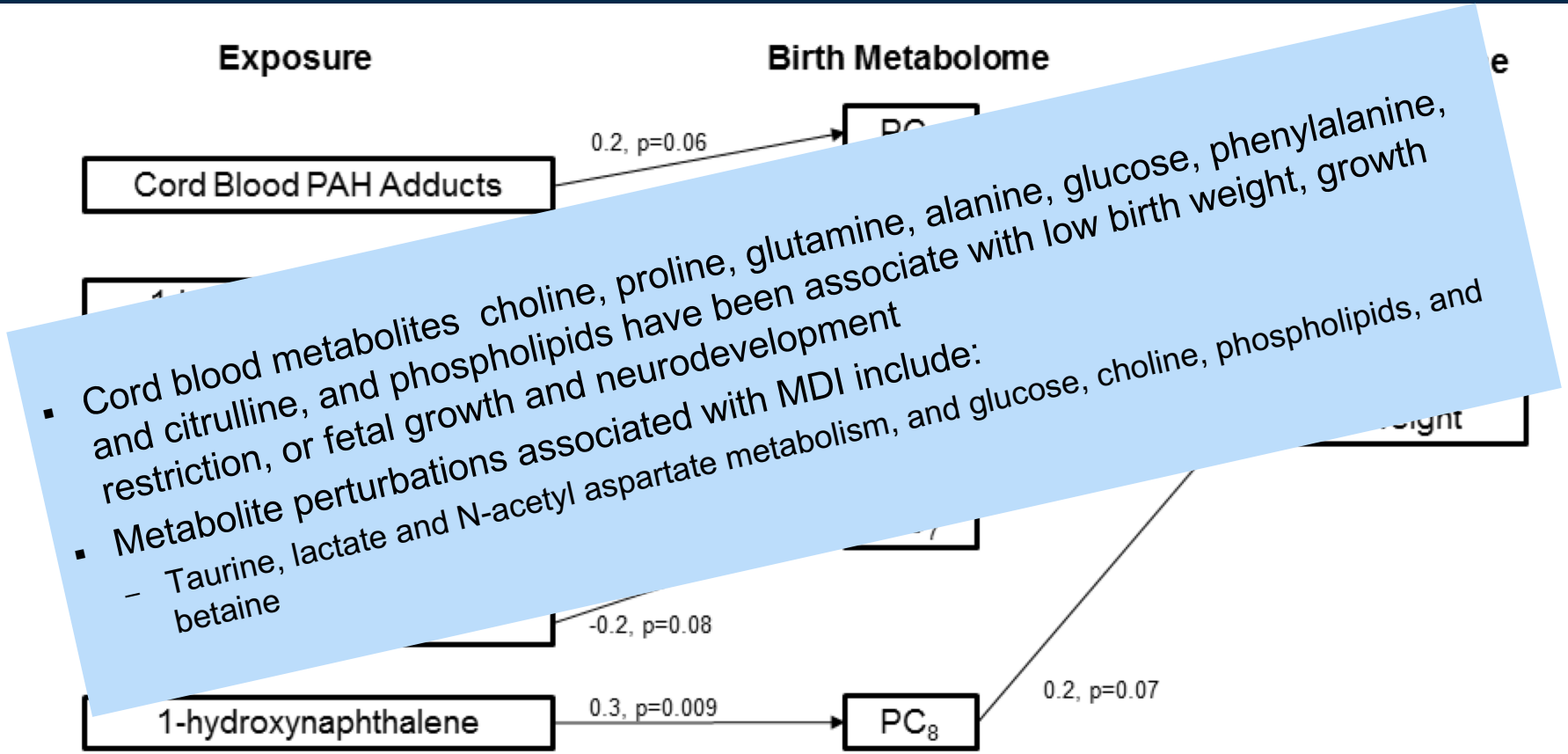
Sumner et al., 2009. Metabolomics in the assessment of chemical-induced reproductive and developmental outcomes using non-invasive biological fluids: application to the study of butylbenzyl phthalate. *Journal of Applied Toxicology* and **Banerjee et al., 2012**. Metabolomics of brain and reproductive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

Birth and Early Childhood Outcomes

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 used Structural equation modeling (SEM) to conduct pathway analysis between environmental exposures, the metabotype of cord blood, and birth and early developmental health outcomes.

Pathway Analysis of PAH Exposure to the Birth Metabolome and Birthweight



- Cord blood metabolites choline, proline, glutamine, alanine, glucose, phenylalanine, and citrulline, and phospholipids have been associate with low birth weight, growth restriction, or fetal growth and neurodevelopment
- Metabolite perturbations associated with MDI include:
  - Taurine, lactate and N-acetyl aspartate metabolism, and glucose, choline, phospholipids, and betaine

Kidney Disease

Pediatric Glomerular Disease

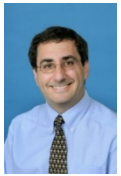
Bill Smoyer

Larry Greenbaum

Jon Klein

Neonatal Acute Kidney Injury

Pat Brophy



David Askenazi

CKD and ESRD

Snezanna Petrovic

Glomerular Nephritus Model System

Jan Novak

Meprin and A-C-K CKD, AA

Robert Newman
New award

Moige Onger

Probiotics and CKD

Subodh Saggi

Maternal and Child Health

Rebecca Fry

Arsenic, *in utero* exposure, diabetes

Fredrica Perea

PAH, *in utero* exposure, birth outcomes, MDI

Pregnancy Complications

Michelle Williams

NYU Medical Center

Leo Transnade

Phthalates and Pediatric Obesity

Columbia Center For Children's Environmental Health

Mailman School of Public Health
Columbia University

Childhood Infections

Margaret Karagas

Dartmouth

Vox Clamantis in Deserto

1769

Pediatric Obesity

David Collier

East Carolina University

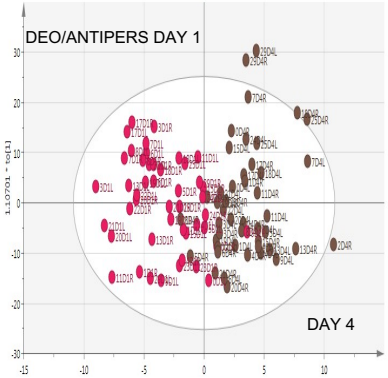
in utero exposure, obesity

Catherine Hoyo

Community Science

Sweat Metabolomics

Julie Horvath, Ph.D.
NC Museum of Sciences



- Twenty-three adult male and female participants were recruited that fell within three groups.
  - no deodorant or no antiperspirant
  - used antiperspirant
  - used deodorant

- Day 1: Sweat collected from participants
- Days 2-4: Participants used no products and wore t-shirt provided by museum.
- Day 4: Sweat collected from participants

- GCTOF-MS broad spectrum metabolomics differentiated the biochemical profiles of sweat in the different phenotypic groups.
- The microbial diversity and the metabolite profiles of individuals are being accessed.

ERCMRC at UNC Chapel Hill

UNC NUTRITION OBESITY RESEARCH CENTER

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LC-MS/MS
LC-TOF-MS

Wimal Pathmasiri
NMR & GC-TOF-MS

Delisha Stewart
NMR and LC-TOF-MS

Reza Ghanbari
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Graduate Studies

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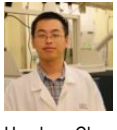
6500 Sciex
Triple Quad

DHMRI

Kevin Knagge
700 and 950 MHz NMR

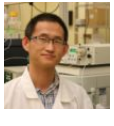
Jason Winnike
NMR
2D-GC-TOF-MS

David Kirchner
LC-MS/MS

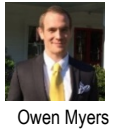


Huadong Chen
LC-MS
LC-TOF-MS

Huiyuan Chen
GC-MS
GC-TOF-MS



UNC Charlotte Bioinformatics



Owen Myers

XiuXia Du

Aleksandr
Smirnov

UNC-G
Q-Exactive

Metabolomics on the North Carolina Research Campus