Metals, EDCs and biomarkers of metabolic syndrome risk in adolescence

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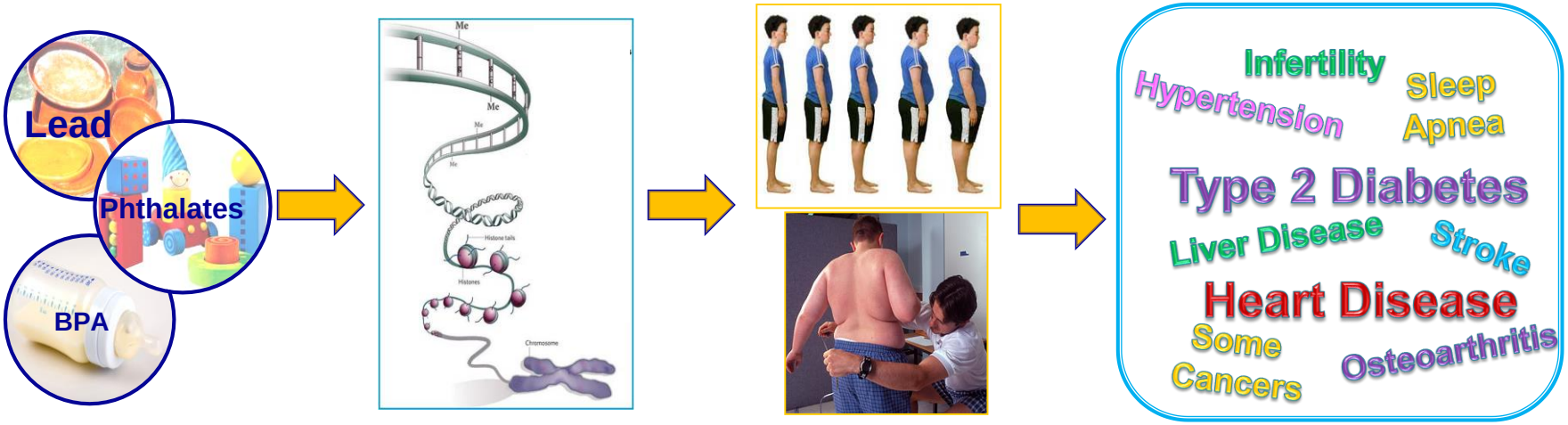
Research Goal

To explore the epigenetic mechanisms by which **perinatal** and **peripubertal** exposures to representative toxicants and interactions with diet affect the **development of obesity**, the tempo of sexual maturation and **metabolic homeostasis** in human and animal models.



Environmental Exposure | **Modification of Epigenome** | **Weight Status Maturational Tempo** | **Chronic Health Conditions**

Research Setting

Early **L**ife **E**xposures in **M**exico to **EN**vironmental **T**oxicants

20-year collaboration with *Instituto Nacional de Salud Pública (INSP)*

EDCs & Metabolic Syndrome

- **Metabolic syndrome** affects **30 % of obese adolescents**
- **Risk factors** predispose to persons to **CVD and T2DM**
  - Waist circumference $\geq$ 90th percentile **AND** $\geq$ 2 criteria:
    - Systolic Blood Pressure $\geq$ 90th percentile
    - Triglycerides $\geq$110 mg/dL
    - HDL cholesterol $\leq$ 40mg/dL
    - Fasting glucose $\geq$ 110 mg/dL
- **EDCs** have been shown to alter weight, glucose homeostasis, lipid profile, free fatty acid (FFA) balance and transport, adipogenesis and oxidative stress
- **Metabolomics** reflect cellular biochemistry; could be used as **early predictors** and/or **clinical biomarkers of disease**

Background: ELEMENT

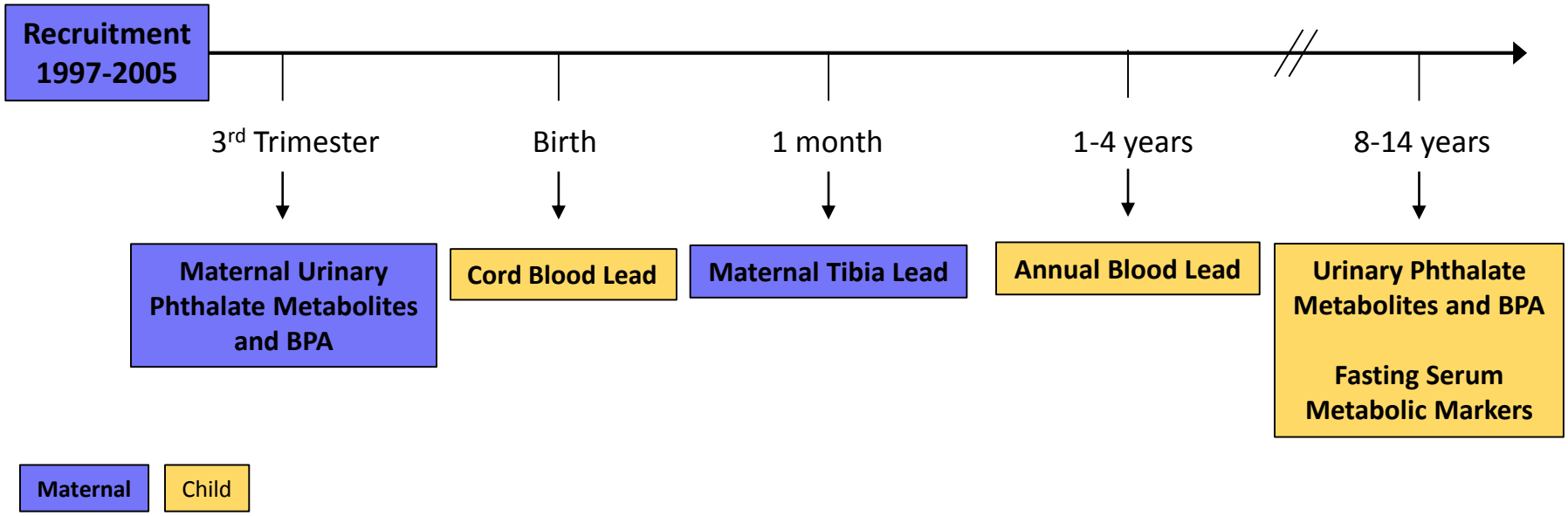
- **Lead exposure in GIRLS 8-14 yr**
  - Maternal patella bone lead (µg/g) associated with **-0.037 lower BMI** (p=.01) (Peterson ISEE 2011)
  - IQR increase in **maternal tibia lead** (13 µg/g) at 1 mo postpartum associated with **2.11-mmHg increase in SBP** (95% CI: 0.69, 3.52) and **1.60-mmHg increase in DBP** (95% CI: 0.28, 2.91) (Zhang EHP 2012)
- **BPA and phthalates**
  - **BOYS: *in utero*** exposures associated with **increases** but **concurrent** exposure with **decreases in fat distribution** measures, e.g., waist (WC), triceps skinfold (TSF))
  - **GIRLS: concurrent** BPA and MEP associated with **increases** but HMW, MBP, MCPP, and MIBP with **decreases** in WC, TSF (Yang TC, ISEE 2013)

Specific Aims

- Examine the impact of *in utero* and peripubertal exposures to **BPA, phthalates and lead (Pb)** on fasting serum measures of lipid metabolism, glucose and leptin among boys and girls ages 8-14 yr
- Explore the relationship of early childhood Pb exposures with **untargeted metabolomic** features in adolescence

Study Population

- 248 children whose mothers were recruited from 1997-2005 were re-recruited in 2011-2012 at ages 8-14 yr
- Measures of EDC exposures *in utero* and pre/adolescence
- Biological samples, anthropometry and questionnaires obtained at recruitment and repeat visits in early childhood (birth-5 yr) and pre/adolescence (8-14 yr)



Measures

Exposure Periods

- **Lead**
  - **Maternal bone lead**: 1 month postpartum (cumulative *in utero* exposure) (n=137)
  - **Cord blood lead** (n=78)
  - **Child blood lead**

    Cumulative from age 1-4 yr (n=245)

    Concurrent (8-14 yr) (n=246)
- **Phthalate metabolites & BPA** (n=248)
  - 3rd trimester urine
  - Concurrent urine sample (8-14 yr)

Outcomes

- **Fasting serum (8-14 yr)** (n=248)
  - Total cholesterol
  - LDL cholesterol
  - HDL cholesterol
  - Triglycerides
  - Glucose
  - Leptin

Statistical Analysis

- Multivariable linear regression used to estimate effects of continuous exposures in sensitive developmental periods on serum outcome measures at 8-14 yr
- Covariates
  - Phthalate and BPA models: child age, BMI Z-score, urinary specific gravity
  - Lead (Pb) models: child age, maternal schooling, and cohort; BMI Z-score (girls)
- Outcomes quantified as **% change** in outcome measure associated with an **interquartile range (IQR) increase** in exposure

Sample Characteristics

	Males (n=117)	Females (n=131)
	Mean±SD	Mean±SD
Age (yr)	10.35±1.61	10.30±1.72
BMI (kg/m^2)	19.09±3.13	19.71±3.94
BMI Z scpre	0.88±1.19	0.84±1.27
Maternal schooling (yr)	11.24±2.80	10.83±2.79
Maternal Bone Lead (ug/g bone)		
Patella	8.43±9.79	9.40±10.59
Tibia	6.20±10.12	8.57±9.25
Child's blood lead (ug/dL)		
Umbilical cord	3.39±2.15	4.01±1.99
Cumulative 1-4 yr	14.50±1.45	14.24±1.49
Concurrent	3.48±3.07	3.21±2.48
Lipids at 8-14 yr (mg/dL)		
Total Cholesterol	151±28	159±28
LDL	76±24	82±22
HDL	60±12	58±12
Triglycerides	77±38	97±47
Metabolic Measures at 8-14 yr		
Leptin (ng/mL)	8.3±6.4	14±10
Glucose (mg/dL)	88±7.9	86±10

Association of Urinary BPA and Serum Metabolic Markers at 8-14 yr

Exposure Timing	Total Cholesterol % Change	Total Cholesterol (95% CI)	LDL % Change	LDL (95% CI)	Triglycerides % Change	Triglycerides (95% CI)	Glucose % Change	Glucose (95% CI)	Leptin % Change	Leptin (95% CI)
Boys										
3rd Trimester	-0.1	(-5.0, 4.7)	-3.4	(-11.4, 4.5)	1.2	(-10.5, 14.4)	-0.04	(-3.3, 3.3)	-2.4	(-16.8, 14.4)
8-14 years	-0.1	(-5.0, 4.7)	-3.4	(-11.4, 4.5)	1.2	(-10.5, 14.4)	0.2	(-2.4, 2.9)	**16.3**	**(3.8, 30.3)**
Girls										
3rd Trimester	**7.3**	**(0.8, 13.7)**	8.1	(-1.9, 18.1)	**22.3**	**(4.0, 43.8)**	2.0	(-2.4, 6.6)	7.9	(-5.2, 22.9)
8-14 years	0.0	(-4.5, 4.6)	-0.4	(-7.5, 6.8)	8.0	(-3.5, 20.8)	-0.9	(-3.9, 2.2)	5.3	(-4.0, 15.4)

Percent change per IQR increase in exposure; adjusted for age, BMI Z-score, and urinary specific gravity

Boys: Associations of Urinary Phthalates and Serum Metabolic Markers at 8-14 yr

		Total Cholesterol		LDL		Glucose		Leptin	
Exposure	Timing	% Change	(95% CI)	% Change	(95% CI)	% Change	(95% CI)	% Change	(95% CI)
ΣDEHP	3rd T	-1.4	(-7.3, 4.5)	-3.6	(-13.4, 6.2)	-0.2	(-3.1, 2.7)	3.8	(-9.9, 19.6)
	8-14	-0.1	(-4.0, 3.8)	-0.6	(-7.1, 5.8)	0.7	(-1.4, 2.9)	5.0	(-4.4, 15.4)
MnBP	3rd T	-2.2	(-7.2, 2.8)	-1.6	(-10.1, 6.8)	-0.5	(-2.9, 2.1)	3.8	(-8.1, 17.2)
	8-14	**-7.5**	**(-12.3, -2.6)**	**-11.1**	**(-19.1, -3.1)**	-0.9	(-3.6, 1.9)	1.4	(-10.3, 14.5)
MiBP	3rd T	-0.8	(-5.7, 4.2)	-1.0	(-9.2, 7.3)	0.2	(-2.2, 2.7)	-3.8	(-14.6, 8.3)
	8-14	1.8	(-3.0, 6.6)	2.4	(-5.4, 10.2)	0.6	(-2.0, 3.3)	7.6	(-4.1, 20.6)
MBzP	3rd T	-1.2	(-5.5, 3.1)	-0.9	(-8.1, 6.3)	-0.7	(-2.8, 1.4)	3.8	(-6.4, 15.2)
	8-14	-0.02	(-5.4, 5.3)	-5.9	(-14.6, 2.8)	0.0	(-2.9, 3.0)	-0.1	(-12.2, 13.7)
MCPP	3rd T	1.7	(-7.1, 3.6)	-1.4	(-10.4, 7.5)	-1.5	(-4.1, 1.2)	3.7	(-8.9, 17.9)
	8-14	**-6.2**	**(-11.4, -1.0)**	**-10.5**	**(-19.1, -2.0)**	-1.0	(-3.9, 1.9)	2.3	(-10.1, 16.4)
MEP	3rd T	-0.2	(-4.7, 4.3)	-0.03	(-7.6, 7.5)	0.8	(-1.4, 3.1)	2.0	(-8.5, 13.7)
	8-14	**-5.4**	**(-9.9, -0.9)**	**-10.3**	**(-17.5, -3.0)**	-1.6	(-4.0, 0.9)	-1.7	(-12.0, 9.9)

Percent change per IQR increase in exposure; Adjusted for age, BMI Z-score, and urinary specific gravity

Girls: Associations of Urinary Phthalates and Serum Metabolic Markers at 8-14 yr

Exposure Timing		Total Cholesterol		LDL		Glucose		Leptin	
		% Change	(95% CI)	% Change	(95% CI)	% Change	(95% CI)	% Change	(95% CI)
ΣDEHP	3rd T	-0.7	(-5.7, 4.3)	-0.4	(-8.1, 7.3)	2.0	(-1.3, 5.5)	-7.0	(-15.7, 2.6)
	8-14	-4.3	(-9.1, 0.5)	**-8.1**	**(-15.6, -0.7)**	0.03	(-3.2, 3.4)	-1.2	(-10.3, 8.9)
MnBP	3rd T	-0.9	(-5.5, 3.7)	0.1	(-7.0, 7.1)	1.0	(-2.0, 4.2)	-2.3	(-10.8, 6.9)
	8-14	-1.1	(-5.3, 3.0)	-5.0	(-11.4, 1.4)	-0.02	(-2.8, 2.8)	-2.5	(-10.2, 5.9)
MiBP	3rd T	-0.3	(-5.3, 4.7)	0.8	(-6.9, 8.5)	**3.6**	**(0.3, 7.1)**	1.9	(-7.7, 12.6)
	8-14	1.5	(-3.1, 6.0)	1.0	(-6.2, 8.1)	1.7	(-1.4, 4.9)	-3.1	(-11.6, 6.2)
MBzP	3rd T	1.5	(-2.7, 5.7)	3.0	(-3.3, 9.4)	0.1	(-2.6, 2.9)	-2.6	(-10.3, 5.7)
	8-14	-0.8	(-5.5, 3.9)	-0.7	(-8.0, 6.6)	0.2	(-2.9, 3.4)	-5.6	(-14.0, 3.7)
MCPP	3rd T	-1.6	(-6.9, 3.8)	-0.2	(-8.4, 7.9)	0.8	(-2.7, 4.4)	-6.3	(-15.6, 4.0)
	8-14	-1.1	(-4.9, 2.8)	-3.4	(-9.4, 2.6)	1.5	(-1.1, 4.2)	4.1	(-3.7, 12.4)
MEP	3rd T	-1.3	(-5.1, 2.5)	-1.6	(-7.4, 4.3)	1.3	(-1.2, 3.9)	**8.0**	**(0.3, 16.4)**
	8-14	-1.9	(-5.8, 2.0)	-5.1	(-11.1, 0.9)	-0.2	(-2.8, 2.4)	-5.5	(-12.5, 2.1)

Percent change per IQR increase in exposure; Adjusted for age, BMI Z-score, and urinary specific gravity

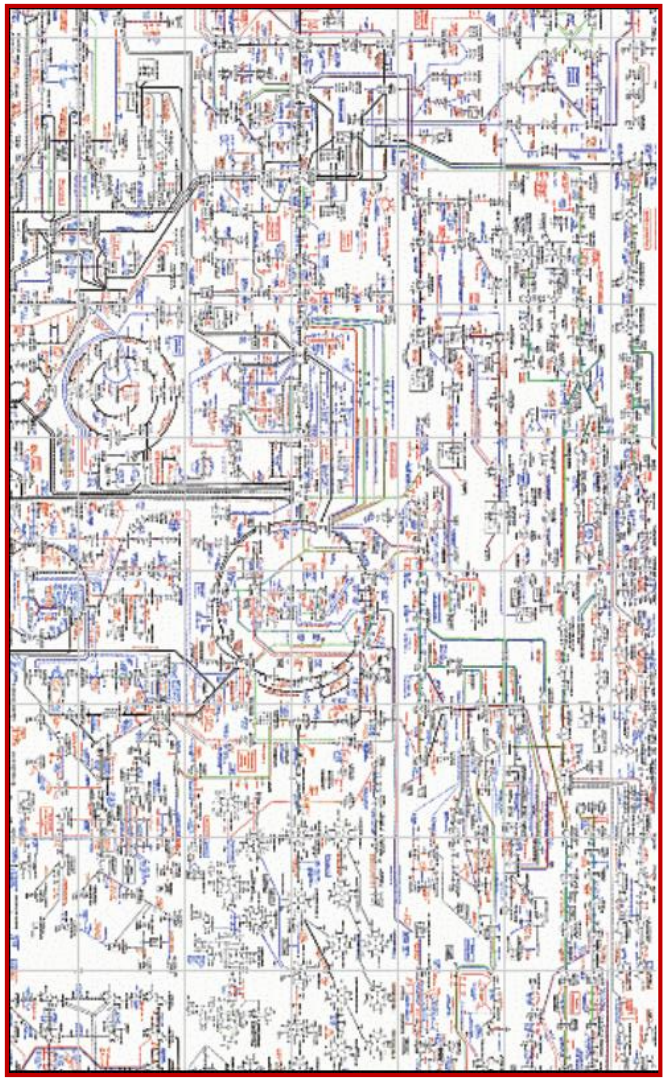
Association of Lead Exposure and Serum Metabolic Markers at 8-14 yr

Exposure Timing	Total Cholesterol % Change	Total Cholesterol (95% CI)	HDL % Change	HDL (95% CI)	Triglycerides % Change	Triglycerides (95% CI)	Glucose % Change	Glucose (95% CI)	Leptin % Change	Leptin (95% CI)
Boys										
Maternal Tibia	-5.23	(-12.8, 2.99)	-6.42	(-13.2, 0.89)	-2.65	(-20.6, 19.3)	-2.91	(-6.65, 0.97)	1.28	(-28.3, 43.1)
Cord Blood	1.37	(-8.92, 12.8)	3.33	(-6.25, 13.9)	-3.67	(-23.9, 22.0)	-3.60	(-7.89, 0.88)	-15.8	(-44.4, 27.5)
Blood 8-14 yr	0.35	(-3.09, 3.91)	1.87	(-1.77, 5.65)	-2.59	(-10.5, 6.08)	**2.47**	**(0.79, 4.17)**	-5.46	(-17.2, 7.95)
Girls										
Maternal Tibia	0.11	(-4.17, 4.57)	2.46	(-2.21, 7.37)	-9.45	(-19.2, 1.53)	1.35	(-1.24, 4.02)	-4.16	(-19.2, 13.7)
Cord Blood	8.90	(-1.84, 20.8)	-2.18	(-12.4, 9.27)	**32.4**	**(1.58, 72.7)**	0.89	(-4.41, 6.49)	11.2	(-28.9, 74.0)
Blood 8-14 yr	-0.17	(-3.41, 3.17)	**4.25**	**(0.18, 8.49)**	-7.07	(-145, 1.57)	-0.95	(-3.14, 1.28)	**-11.2**	**(-22.4, 1.65)**

Percent change per IQR increase in exposure; adjusted for age, maternal schooling, cohort

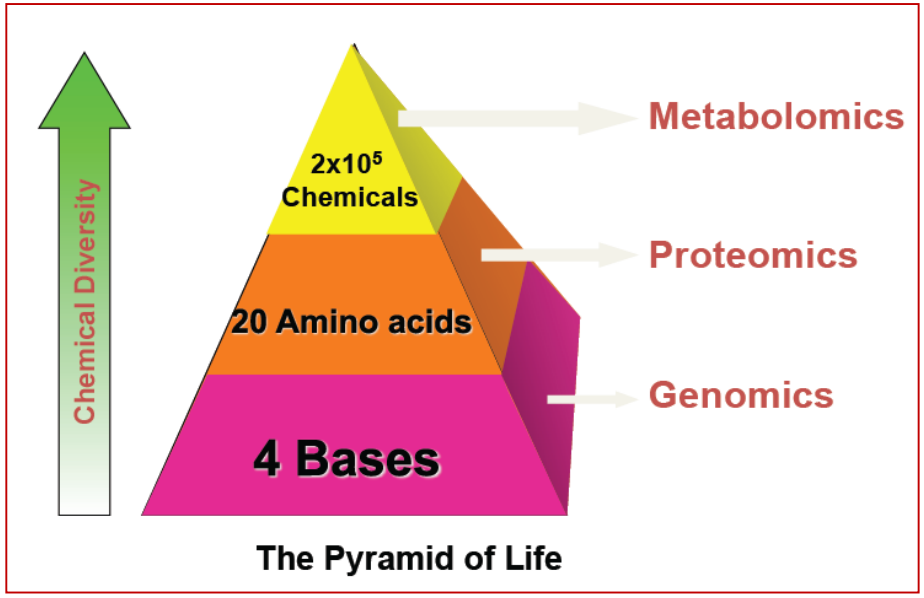
Rationale for Expanding to Metabolomics

- >95% of all diagnostic clinical assays test for small molecules
- 89% of all known drugs are small molecules
- 50% of all drugs are derived from pre-existing metabolites
- 30% of identified genetic disorders involve diseases of small molecule metabolism
- Metabolites are cofactors & signaling molecules to 1000's of proteins
- Metabolic pathways are well understood

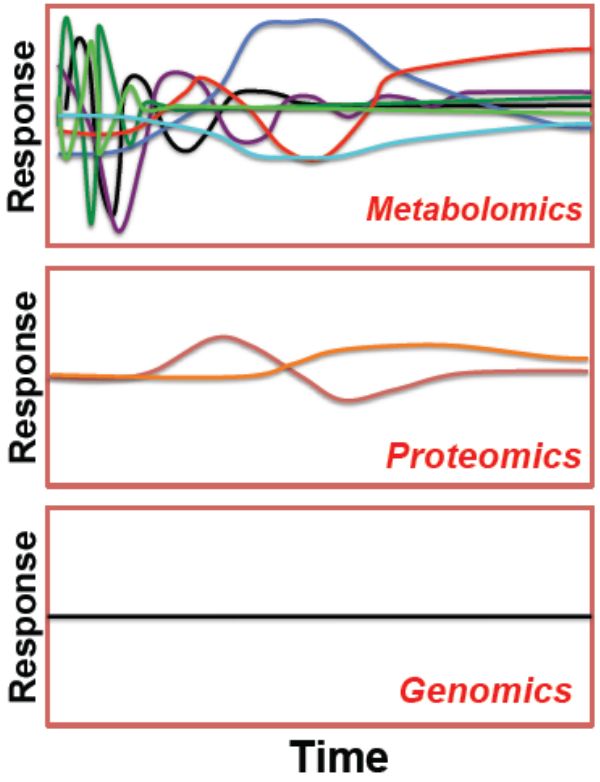


Use of Metabolomics in Environmental Exposure Research

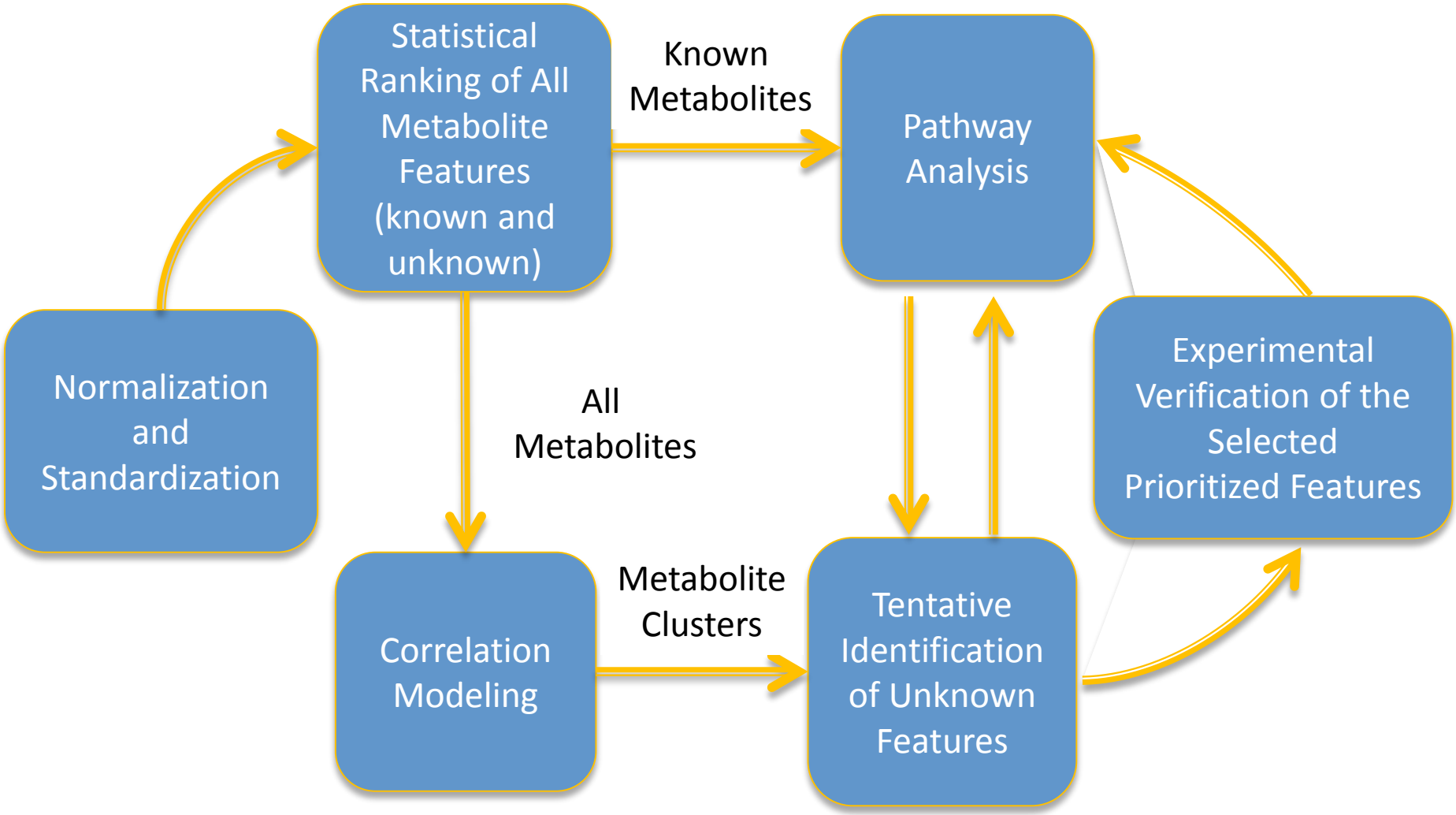
Metabolomes of exposed vs. unexposed groups have not been characterized → benefit of integrating exposure science with metabolomics



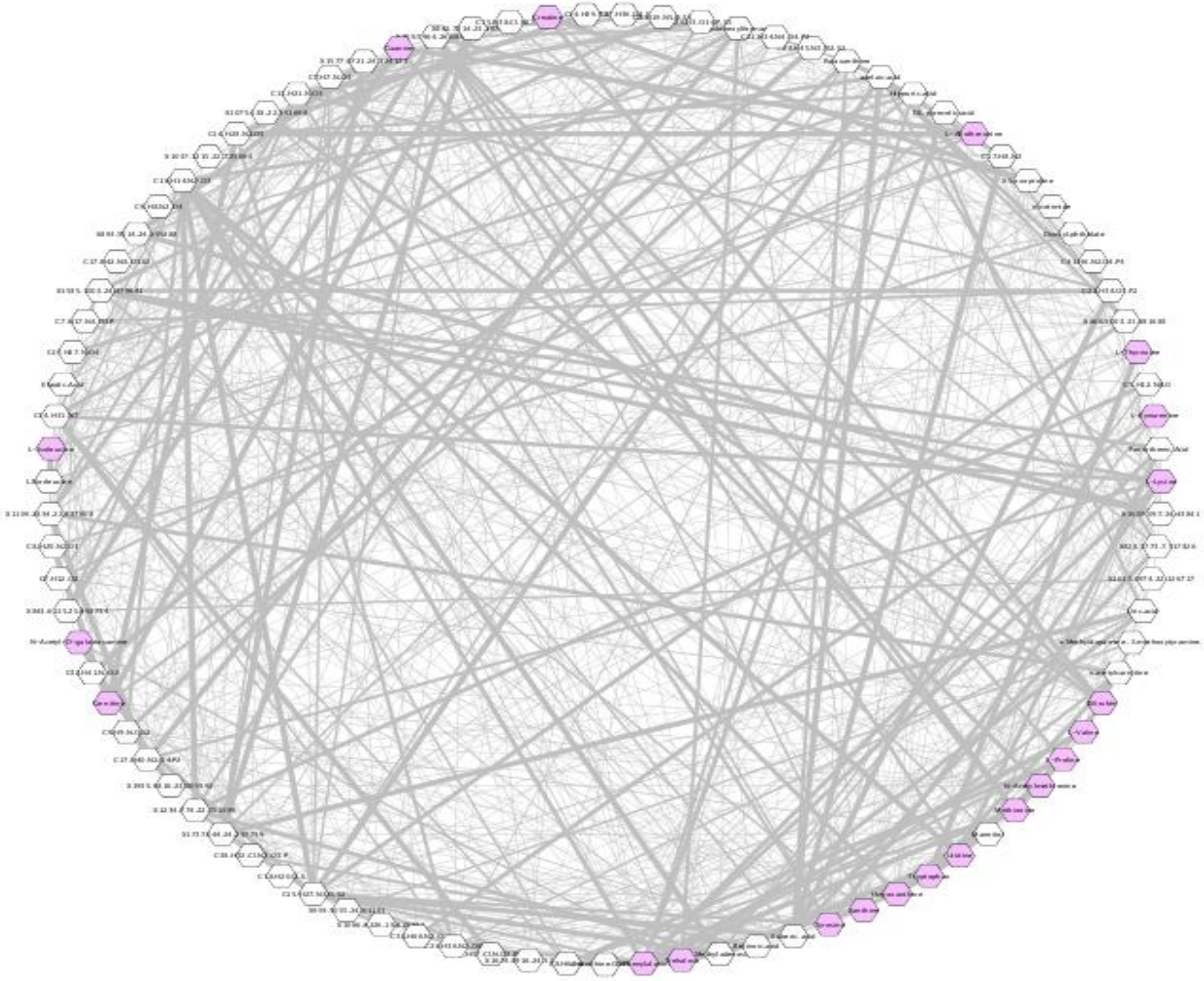
Metabolomics is more time-sensitive (and environmentally sensitive) than other 'omics



Untargeted Metabolomics Data Analysis Workflow



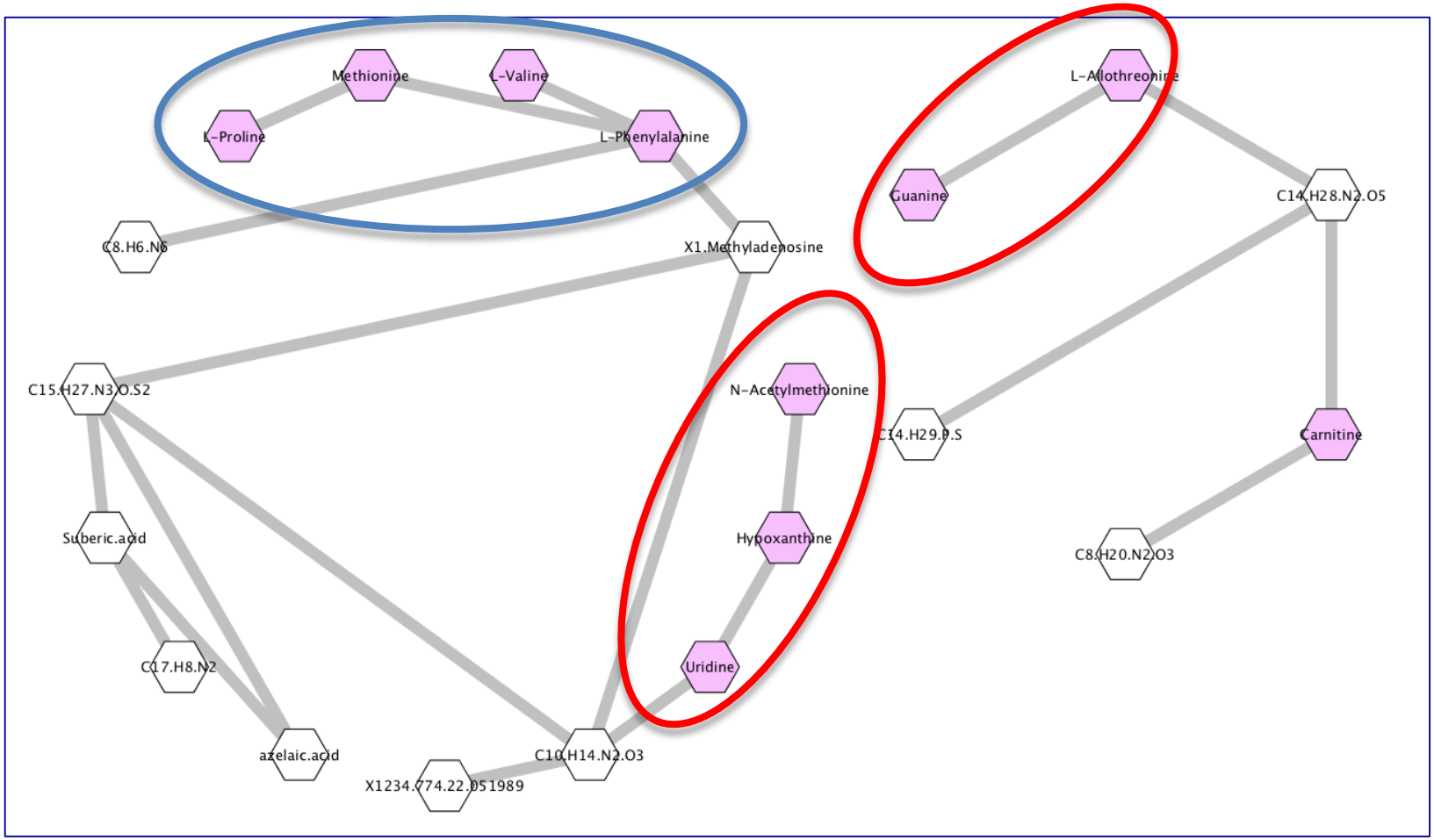
Using Correlations between Metabolites to Build Networks



Using Correlations between Metabolites to Build Networks

Amino acid metabolism

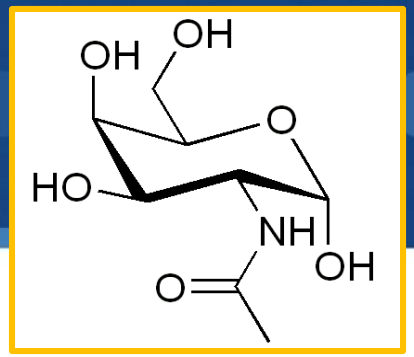
Nucleotide metabolism



The p values for lead exposure are shown in **pink**

The partial correlation q values used for the edges are shown outlined in **black** (for the unknown compounds)

N-acetyl galactosamine (GalNAc)



Relevance

- Inversely correlated with tibia bone lead levels

Mechanism

- Participates in protein O-glycosylation (added to serine and threonine residues by N-acetylgalactosaminyl transferase)

Function

- Plays an important role in sensory neuron conduction in brain
- Is a proteoglycan core protein component in vascular smooth muscle cells
- Lead and cadmium have been shown to affect the synthesis of these proteins in cultured vascular smooth muscle cells

(Fujiwara et al., J Health Sci 01/2003; 49(6):534-540)

Summary

- **Effects of EDCs** on glucose, lipids and leptin in adolescence **vary by sex and timing of exposure**
- Childhood **lead** exposure related to higher fasting glucose in boys and higher HDL cholesterol in girls. Cord blood lead related to higher triglycerides in girls, but small sample size.
- In utero **BPA** associated with elevated total cholesterol and triglycerides in girls; concurrent **BPA** associated with higher leptin in boys.
- Concurrent but not *in utero* **MCPP, MEP, and MBP** exposures associated with lower total and LDL cholesterol in boys.
- In girls, concurrent **DEHP** metabolites associated with decreased LDL, while in utero **MEP** was related to higher leptin, and in utero **MBP** was associated with higher glucose.

Limitations and Future Work

- Limitations
  - Longitudinal observational design limits causal inferences
  - Small sample size within strata, statistical power
  - Single spot urine from each developmental period
- Future work
  - EDC mixtures, repeated measures: Pb, Cd, BPA, phthalates
  - Expand sample size, obtain longitudinal measures of metabolic perturbations (metabolomics) and risk factors for metabolic syndrome in adolescence
  - Examine role of diet in modifying effect of toxicants on metabolic homeostasis *in utero* and pre/adolescence
  - Epigenetic regulation of toxicant and diet exposures
  - Test dietary intervention in isogenic mouse model of perinatal/peripubertal exposures

University of Michigan CEHC Team

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