# Maternal Diet in Pregnancy Paves the Way for Childhood Health, Neurological Function, and Weight Status

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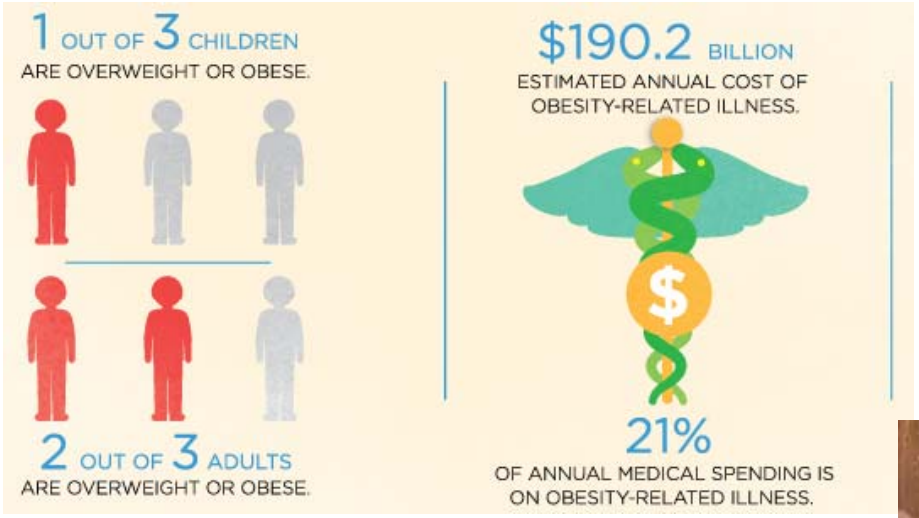
# Childhood and Adult Obesity



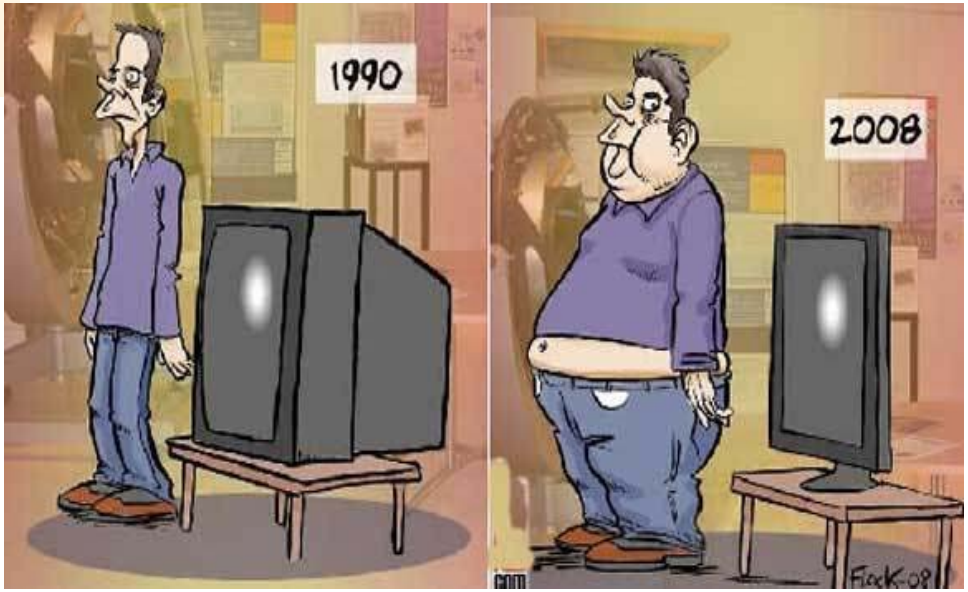

**By BMI (kg/m$^2$)**

Lean; 18.9 – 24.4

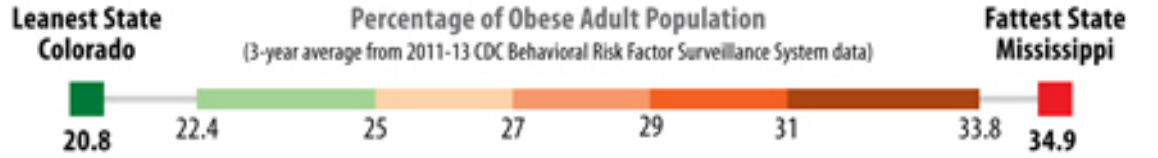
Overweight; 25 – 29.9

Obese $\geq$ 30

Diabetes
Metabolic syndrome
NAFLD & NASH



# Obesity *circa* 2014



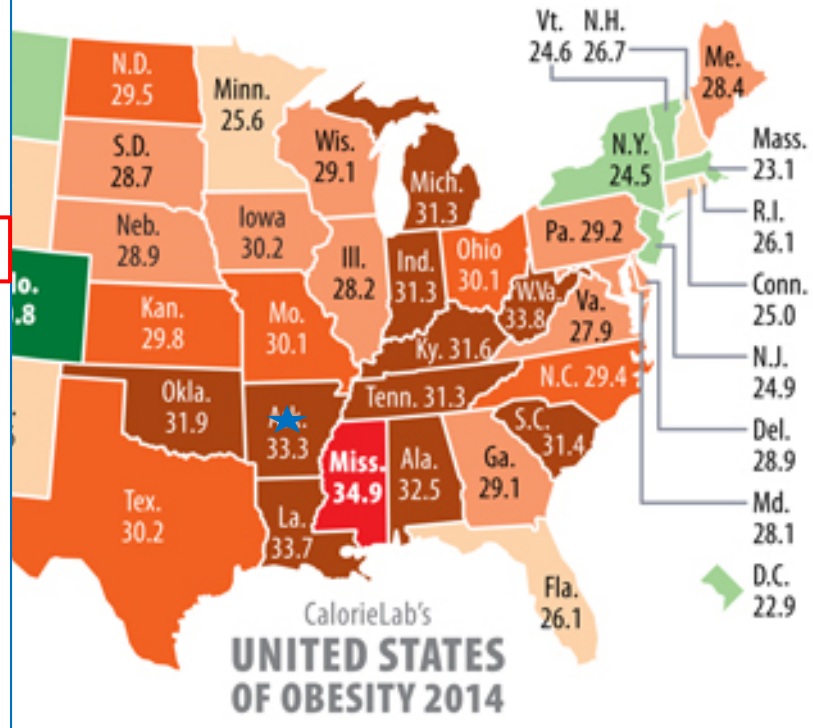

*2012 Most Obese Metro Areas*

Among the 189 U.S. Metropolitan Statistical Areas surveyed

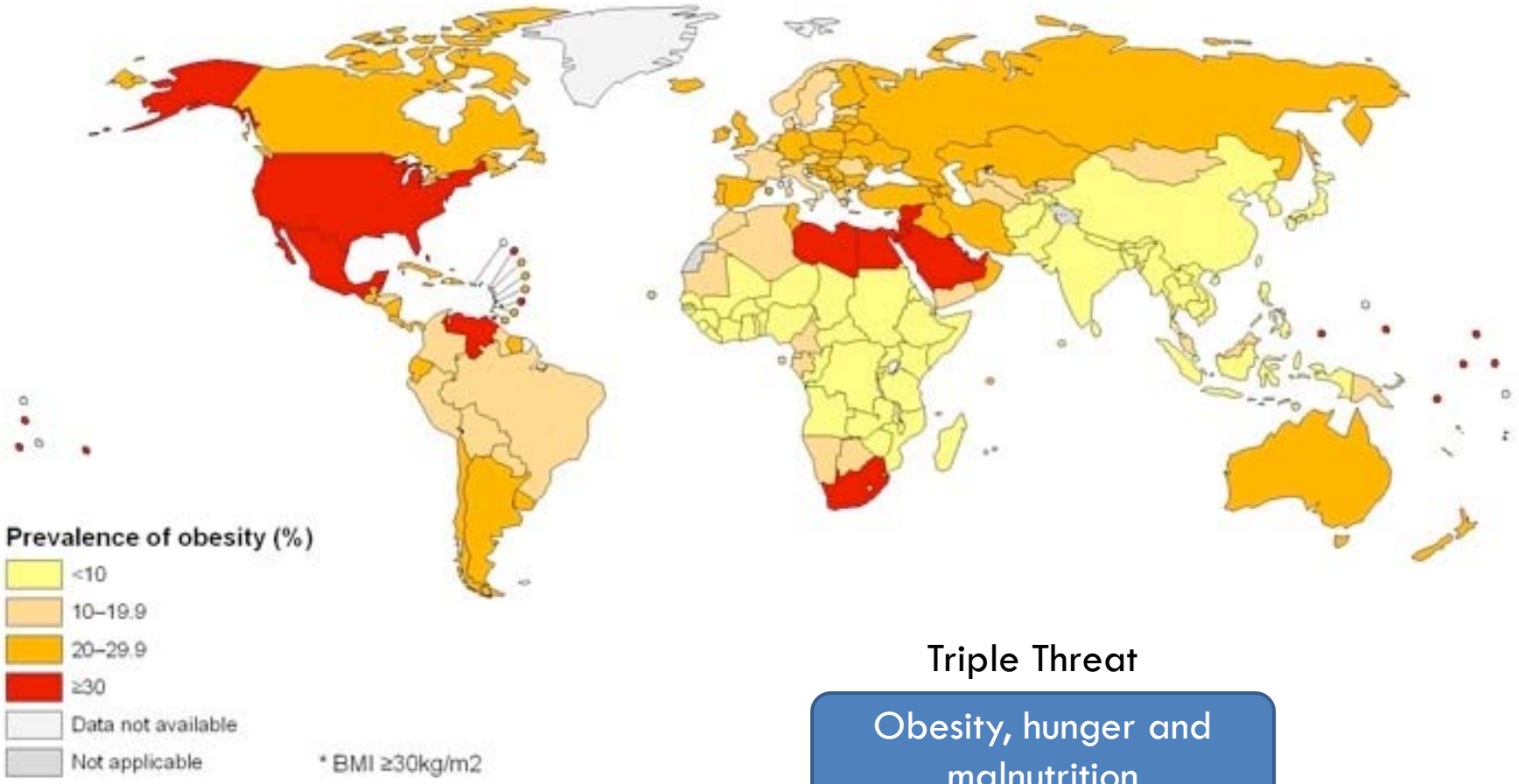
| Metropolitan area | % Obese |
|---|---|
| McAllen-Edinburg-Mission, TX | 38.5 |
| Huntington-Ashland, WV-KY-OH | 37.7 |
| Little Rock-N Little Rock-Conway, AR | 34.7 |
| Mobile, AL | 33.7 |
| Hagerstown-Martinsburg, MD-WV | 33.4 |
| Myrtle Beach-North Myrtle Beach-Conway, SC | 33.1 |
| Toledo, OH | 33.0 |
| Charleston, WV | 32.9 |
| Reading, PA | 32.8 |
| Erie, PA | 32.5 |
| Beaumont-Port Arthur, TX | 32.5 |

January-December 2012
Gallup-Healthways Well-Being Index

GALLUP



# The World is ~~Flat~~ Fat

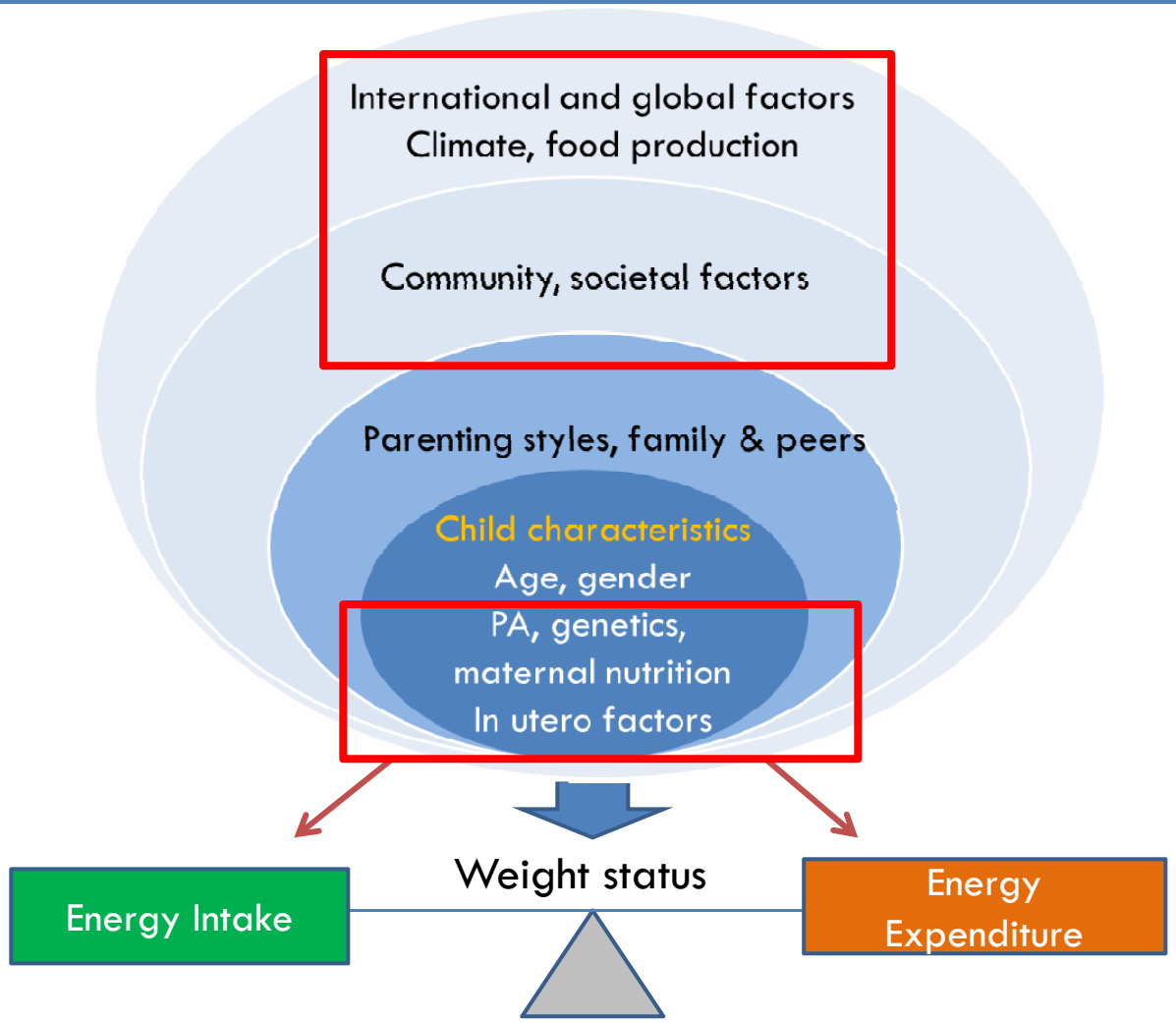


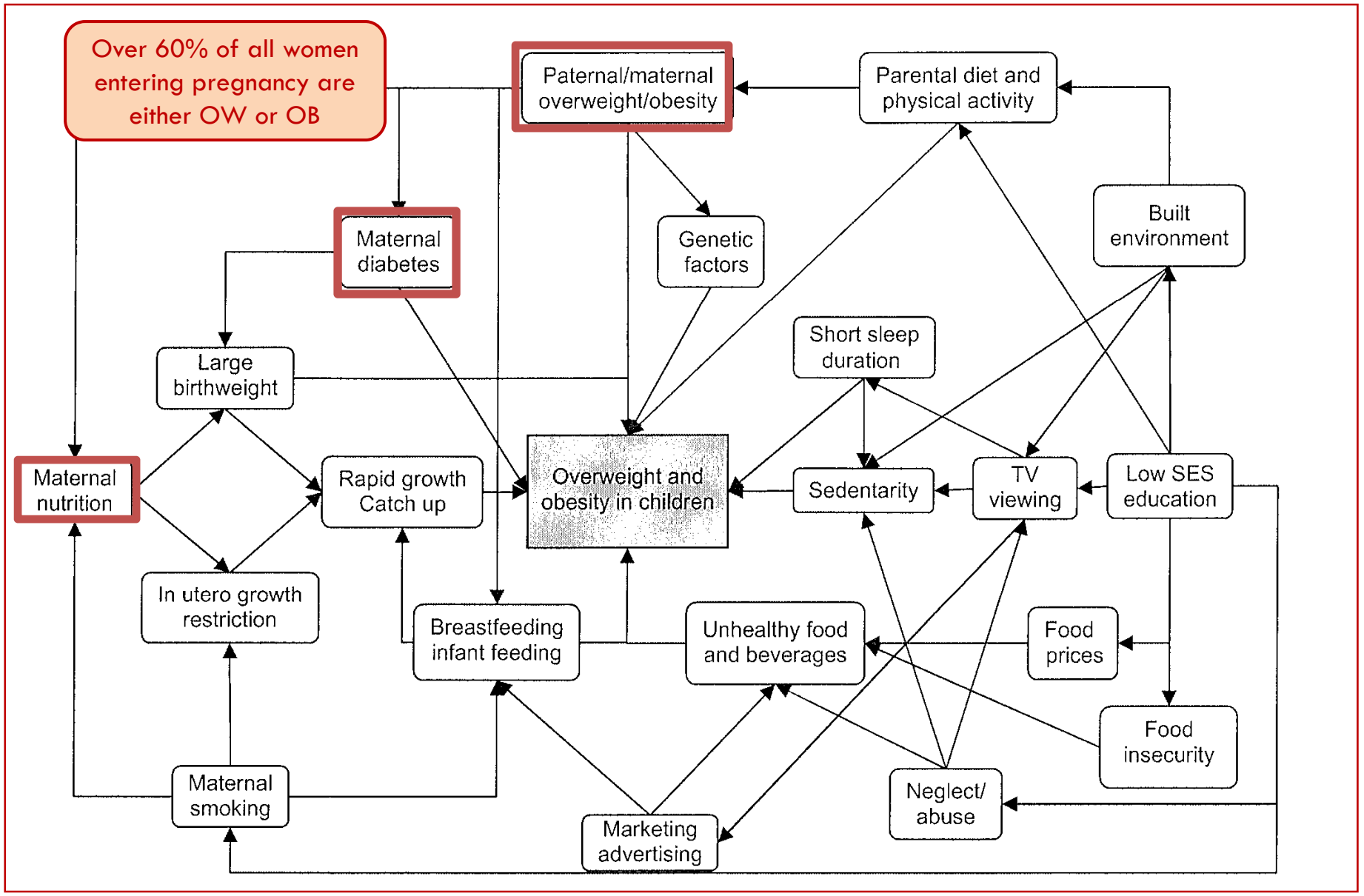

Triple Threat

Obesity, hunger and malnutrition

BOTERO

# Obesity Causes: A Systems Perspective






*Monasta L et al. Obesity reviews (2010) 11, 695–708*

# Developmental Origins of Health & Disease

## DoHAD 'Fetal Origins' Hypothesis

Undernutrition during critical windows in utero permanently alters body's structure, function and metabolism increasing risk of chronic diseases (CV, diabetes, obesity)

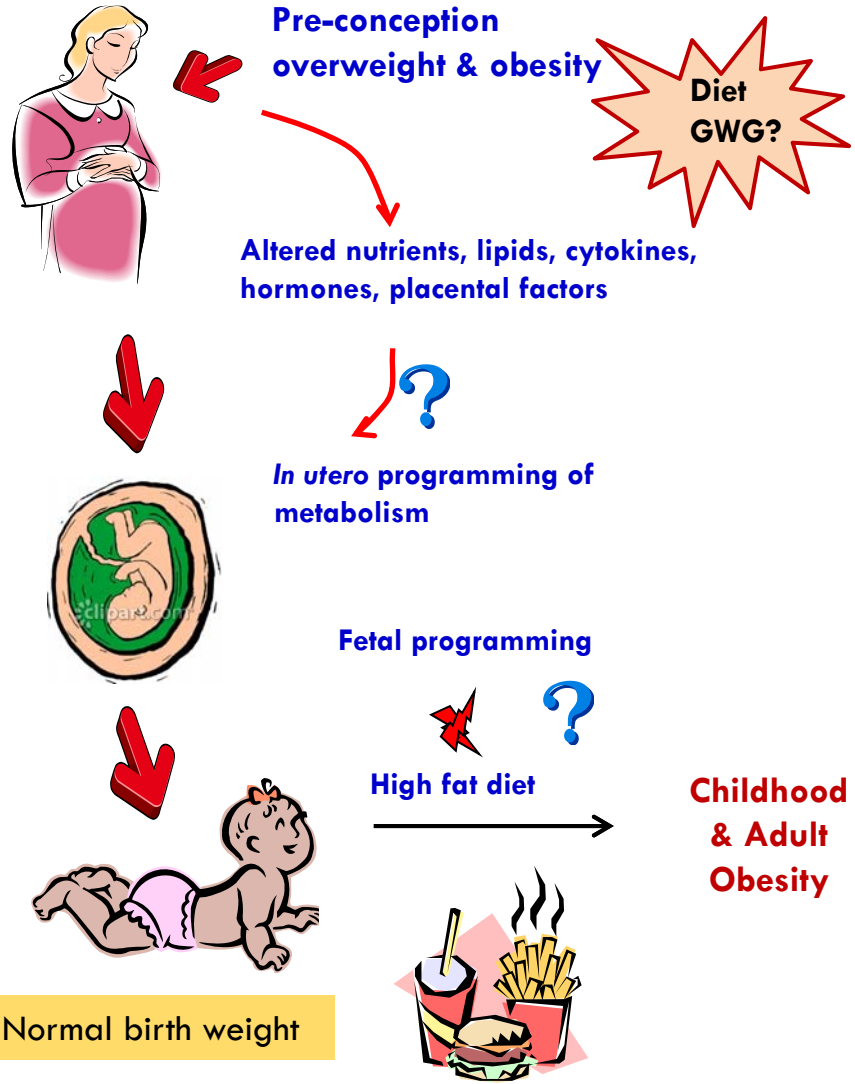
## Fetal Overnutrition Hypothesis

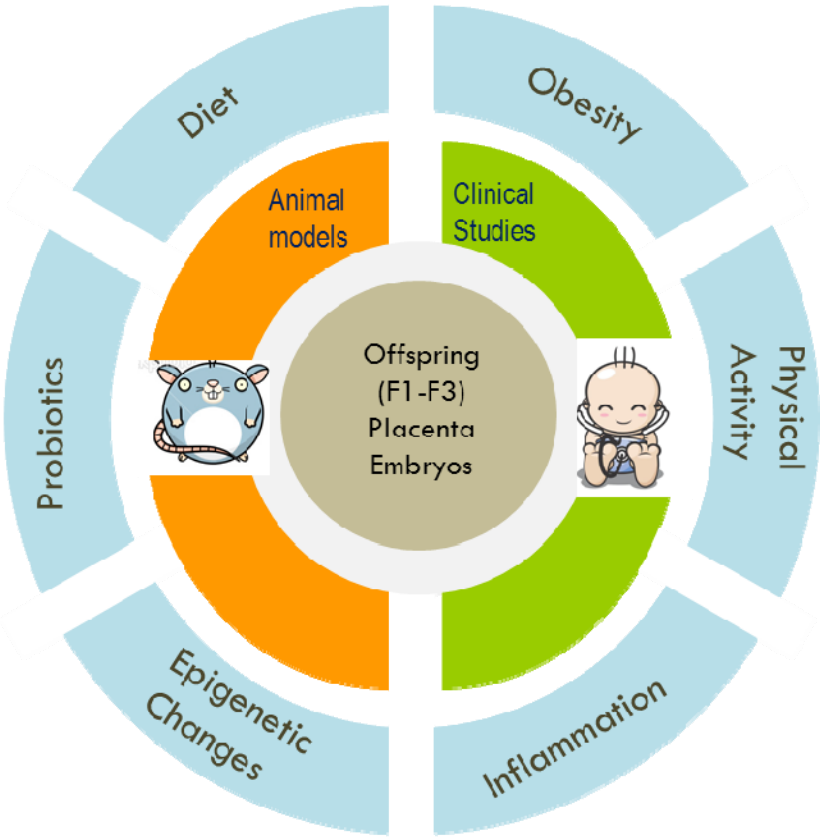
Armitage JA, 2008

Maternal obesity and overnutrition leads to programming of appetite and metabolism increasing risk of obesity in the offspring


Life-Course Paradigm

# Does Obesity Beget Obesity?




Understand the **nature** and **mechanisms** of maternal obesity-induced programming.

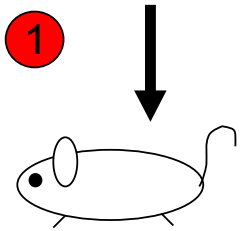
Multiple OMICs + Bioinformatics

Hypothesis-agnostic
Hypothesis-driven

# Modeling the *in utero* Exposure to Obesity

**Total Enteral Nutrition (TEN)**

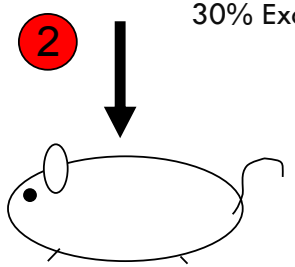
Normal caloric intake (155 Kcal/kg$^{3/4}$/d)




**Lean**

Overfed (220 Kcal/kg$^{3/4}$/d)

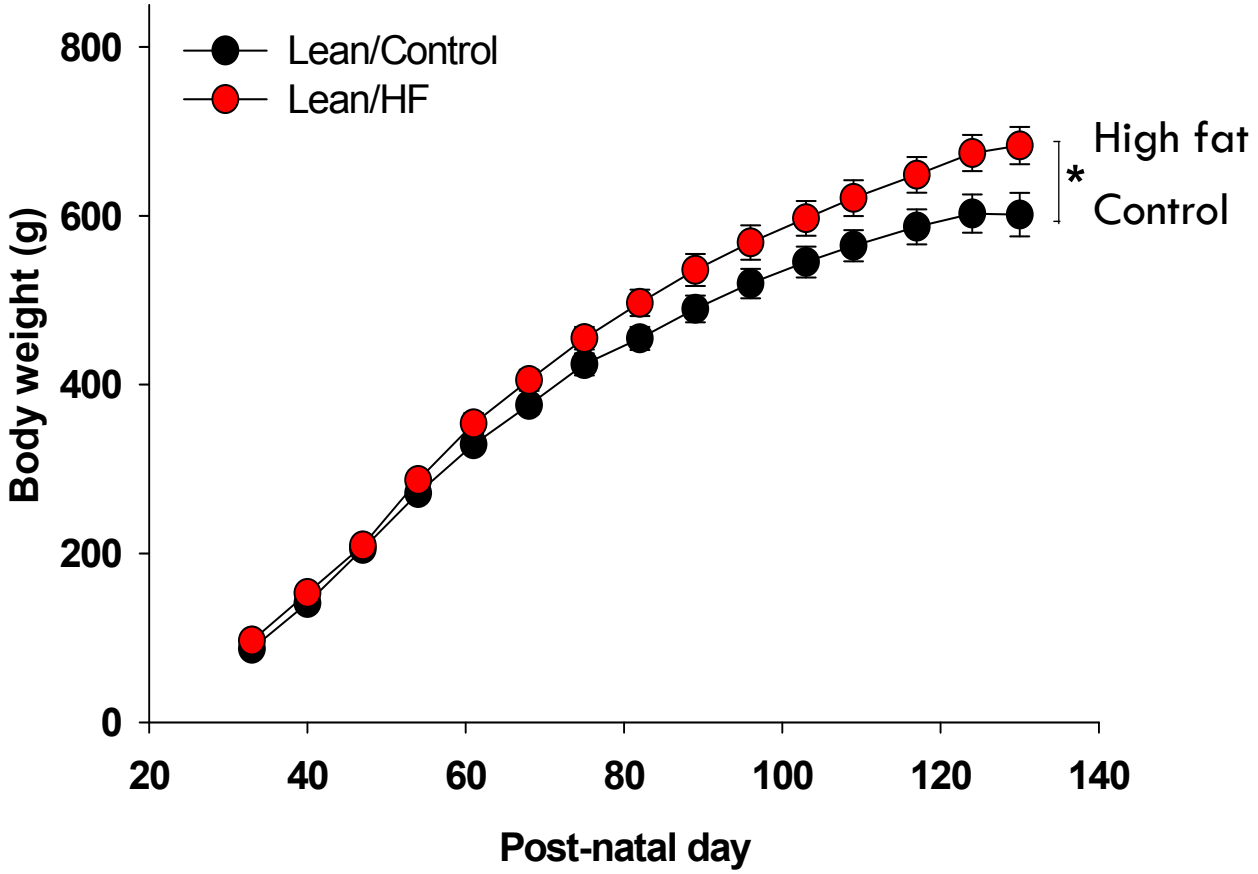
30% Excess




**Obese**

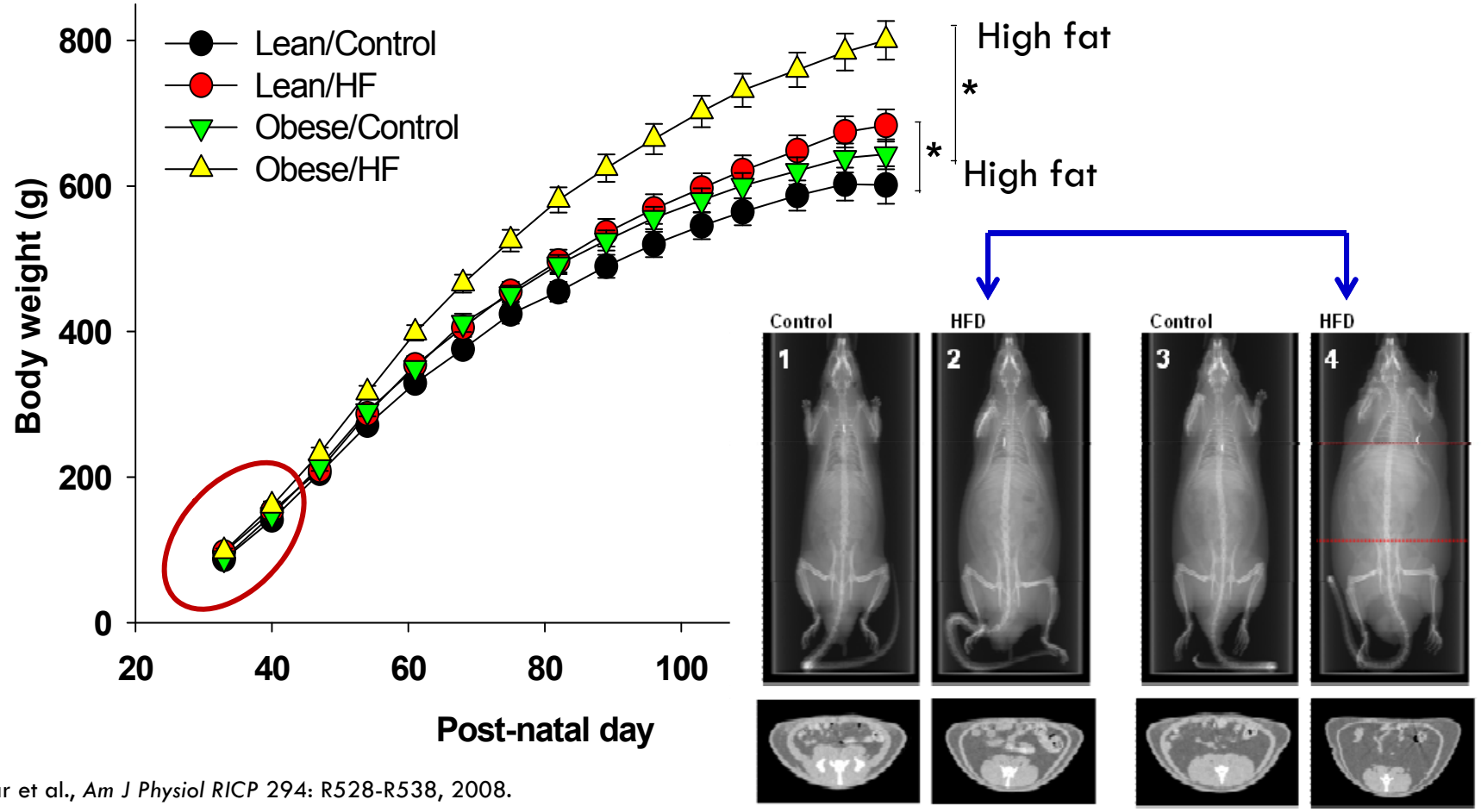
- Gestational weight gains of dams are matched
- Maternal obesity restricted to gestation via cross-fostering to lean dams.
- Body weights at birth or at weaning are unchanged.
- At weaning offspring receive either control or high fat diet (HFD) till PND130

# Offspring Body Weights



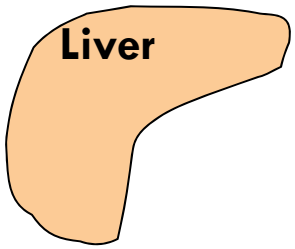

Shankar et al., *Am J Physiol RICP* 294: R528-R538, 2008.

# Offspring Body Weights

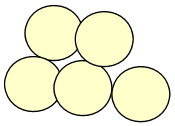



Shankar et al., *Am J Physiol RICP* 294: R528-R538, 2008.

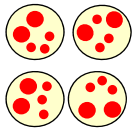
# Summary: Offspring Outcomes

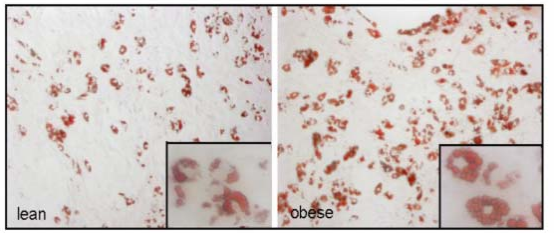


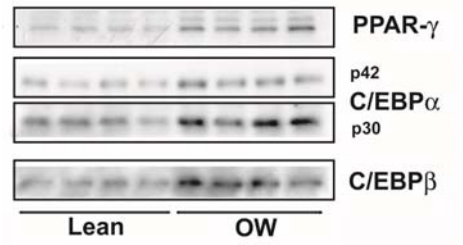

**White Fat**



**Brown fat**



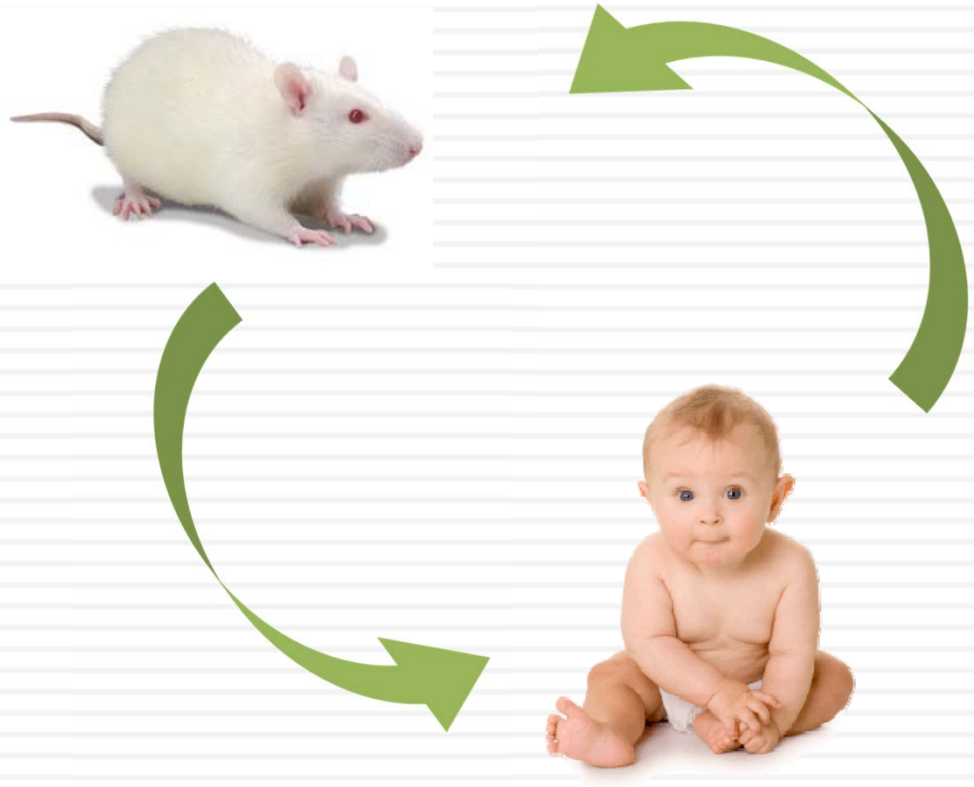



- LIVER
  - Reprogramming of lipid handling pathways.[1]
  - Decreased OXHPHOS and mitochondrial plasticity. [2,6]
  - Altered circadian rhythms.[5]
- White Adipose
  - Increased lipogenesis. [4]
  - Increased adipogenic differentiation of stem cells.[5]
  - Altered DNA methylation at key adipogenic genes.
- Brown Adipose
  - Decreased UCP proteins in liver, muscle & BAT.[7]


1. Shankar et al., *Endocrinology*, 151: 2277, 2010.
2. Shankar et al, *Endocrinology*, 152: 4158, 2011.
3. Borengasser et al., *PLoS One*, 6: e24068, 2011.
4. Borengasser et al., *Endocrinology, 154:4113*, 2013.
5. Borengasser et al., *PLoS One*, 9: e84209, 2014.
6. Borengasser et al., *Phy Gen.*, 46: 841, 2014.
7. Saben et al, *Placenta*, 35: 1013, 2014.

# Are these findings relevant in humans?



# Gestational Environment and Offspring Obesity

- Intrauterine exposure to maternal diabetes increases offspring's risk of diabetes and obesity (*Dabelea et al., Diabetes, 2000*) ; Pima Indians Discordant Sibships.
- Increasing trends of LGA birth were strongly associated with maternal BMI (*Surkan et al. Obs Gynecol, 2004*); large population based cohort.
- LGA offspring born to GDM mothers have 2-fold higher risk of MetS at 11 y of age (*Boney et al. Pediatrics, 2004*); also identified an independent risk of maternal OB.

# Maternal Obesity and The Neonate

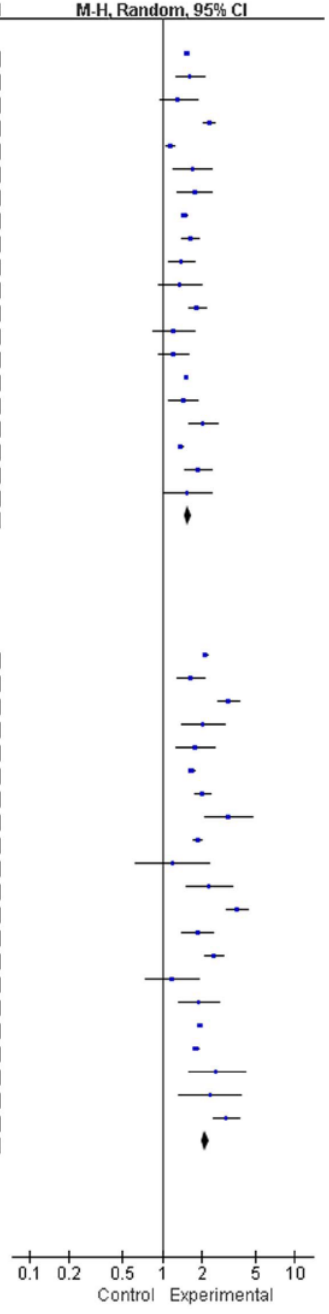
- Fetuses of obese mothers have greater adiposity and insulin resistance (*Catalano et al. Diabetes Care, 2009*); abdominal skin-folds at birth in obese women with NGT

- Maternal triceps skinfold positively associated with increased neonatal fat mass via DEXA (*Harvey et al., JCEM, 2007*). Southampton Women's Survey, UK.

- Maternal BMI independent of glycemia is strongly associated with excess fetal growth and adiposity (*HAPO study, BJOG, 2010; Catalano et al. Diabetes Care, 2012*). Maternal OB without GDM - OR 1.98 for fat > 90 percentile

# Meta-Analysis of Maternal BMI and LGA risk

| Study or Subgroup | Experimental Events | Experimental Total | Control Events | Control Total | Weight | Odds Ratio M-H, Random, 95% CI |
|---|---|---|---|---|---|---|
| **1 Overweight vs. Normal** | | | | | | |
| Sebire 2001 | 10596 | 79014 | 15976 | 176923 | 9.3% | 1.56 [1.52, 1.60] |
| Jensen 2003 | 504 | 642 | 425 | 614 | 3.6% | 1.62 [1.26, 2.10] |
| Gilboa 2008 | 51 | 451 | 195 | 2218 | 2.6% | 1.32 [0.95, 1.83] |
| Leung 2008 | 631 | 3956 | 1694 | 22041 | 7.6% | 2.28 [2.07, 2.52] |
| Khashan 2009 | 1064 | 24219 | 1648 | 43095 | 8.1% | 1.16 [1.07, 1.25] |
| Kalk 2009 | 53 | 309 | 156 | 1446 | 2.4% | 1.71 [1.22, 2.40] |
| Oken 2009 | 82 | 456 | 135 | 1227 | 2.9% | 1.77 [1.32, 2.39] |
| Dietz 2009 | 1589 | 12918 | 4934 | 56718 | 8.6% | 1.47 [1.39, 1.56] |
| Aydin 2010 | 315 | 2214 | 521 | 5685 | 6.0% | 1.64 [1.42, 1.91] |
| Margerison Zilko 2010 | 107 | 706 | 350 | 3108 | 4.0% | 1.41 [1.11, 1.78] |
| Athukorala 2010 | 48 | 446 | 76 | 943 | 2.0% | 1.38 [0.94, 2.01] |
| Narchi 2010 | 345 | 1537 | 450 | 3322 | 5.8% | 1.85 [1.58, 2.16] |
| Chen 2010 | 41 | 341 | 176 | 1744 | 2.2% | 1.22 [0.85, 1.75] |
| Tabatabaei 2011 | 82 | 682 | 352 | 3488 | 3.6% | 1.22 [0.94, 1.57] |
| Park 2011 | 15597 | 135668 | 23808 | 305295 | 9.3% | 1.54 [1.50, 1.57] |
| Liu 2011 | 94 | 926 | 231 | 3200 | 3.6% | 1.45 [1.13, 1.87] |
| Jeric 2012 | 95 | 550 | 342 | 3688 | 3.7% | 2.04 [1.59, 2.62] |
| Hunt 2012 | 4128 | 51594 | 5260 | 89146 | 9.0% | 1.39 [1.33, 1.45] |
| Ferraro 2012 | 143 | 1025 | 194 | 2428 | 4.0% | 1.87 [1.48, 2.35] |
| Heude 2012 | 33 | 311 | 83 | 1172 | 1.7% | 1.56 [1.02, 2.38] |
| **Subtotal (95% CI)** | | **317965** | | **727501** | **100.0%** | **1.56 [1.46, 1.65]** |
| Total events | 35598 | | 57006 | | | |
| Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 158.60$, df = 19 ($P < 0.00001$); $I^2 = 88\%$ | | | | | | |
| Test for overall effect: $Z = 14.17$ ($P < 0.00001$) | | | | | | |
| **2 Obesity vs. Normal** | | | | | | |
| Sebire 2001 | 5461 | 31276 | 15976 | 176923 | 9.0% | 2.13 [2.06, 2.20] |
| Jensen 2003 | 593 | 753 | 425 | 614 | 4.3% | 1.65 [1.29, 2.11] |
| Leung 2008 | 142 | 677 | 1694 | 22041 | 5.5% | 3.19 [2.63, 3.86] |
| Gilboa 2008 | 39 | 236 | 195 | 2218 | 2.5% | 2.05 [1.41, 2.98] |
| Oken 2009 | 59 | 328 | 135 | 1227 | 3.0% | 1.77 [1.27, 2.48] |
| Dietz 2009 | 2654 | 19235 | 4934 | 56718 | 8.8% | 1.68 [1.60, 1.77] |
| Joy 2009 | 461 | 3744 | 596 | 9171 | 7.1% | 2.02 [1.78, 2.30] |
| Kalk 2009 | 35 | 126 | 156 | 1446 | 2.1% | 3.18 [2.08, 4.86] |
| Khashan 2009 | 1052 | 15271 | 1648 | 43095 | 8.2% | 1.86 [1.72, 2.02] |
| Chen 2010 | 11 | 93 | 176 | 1744 | 1.0% | 1.20 [0.62, 2.29] |
| Athukorala 2010 | 45 | 272 | 76 | 943 | 2.3% | 2.26 [1.52, 3.36] |
| Aydin 2010 | 214 | 1213 | 310 | 5685 | 5.6% | 3.71 [3.08, 4.48] |
| Margerison Zilko 2010 | 72 | 376 | 350 | 3108 | 3.7% | 1.87 [1.41, 2.47] |
| Narchi 2010 | 353 | 1266 | 450 | 3322 | 6.3% | 2.47 [2.11, 2.89] |
| Tabatabaei 2011 | 22 | 186 | 352 | 3488 | 1.9% | 1.20 [0.76, 1.89] |
| Liu 2011 | 44 | 342 | 231 | 3200 | 2.9% | 1.90 [1.35, 2.68] |
| Park 2011 | 14357 | 101590 | 23808 | 305295 | 9.1% | 1.95 [1.90, 1.99] |
| Hunt 2012 | 5953 | 58367 | 5260 | 89146 | 9.0% | 1.81 [1.74, 1.88] |
| Heude 2012 | 25 | 152 | 83 | 1172 | 1.7% | 2.58 [1.59, 4.19] |
| Jeric 2012 | 17 | 89 | 342 | 3688 | 1.4% | 2.31 [1.35, 3.96] |
| Ferraro 2012 | 147 | 699 | 194 | 2428 | 4.5% | 3.07 [2.43, 3.87] |
| **Subtotal (95% CI)** | | **236291** | | **736672** | **100.0%** | **2.11 [1.97, 2.27]** |
| Total events | 31756 | | 57391 | | | |
| Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 186.88$, df = 20 ($P < 0.00001$); $I^2 = 89\%$ | | | | | | |
| Test for overall effect: $Z = 20.97$ ($P < 0.00001$) | | | | | | |




Yu et al, PLoS One, 2013

# Maternal Obesity and Childhood Obesity

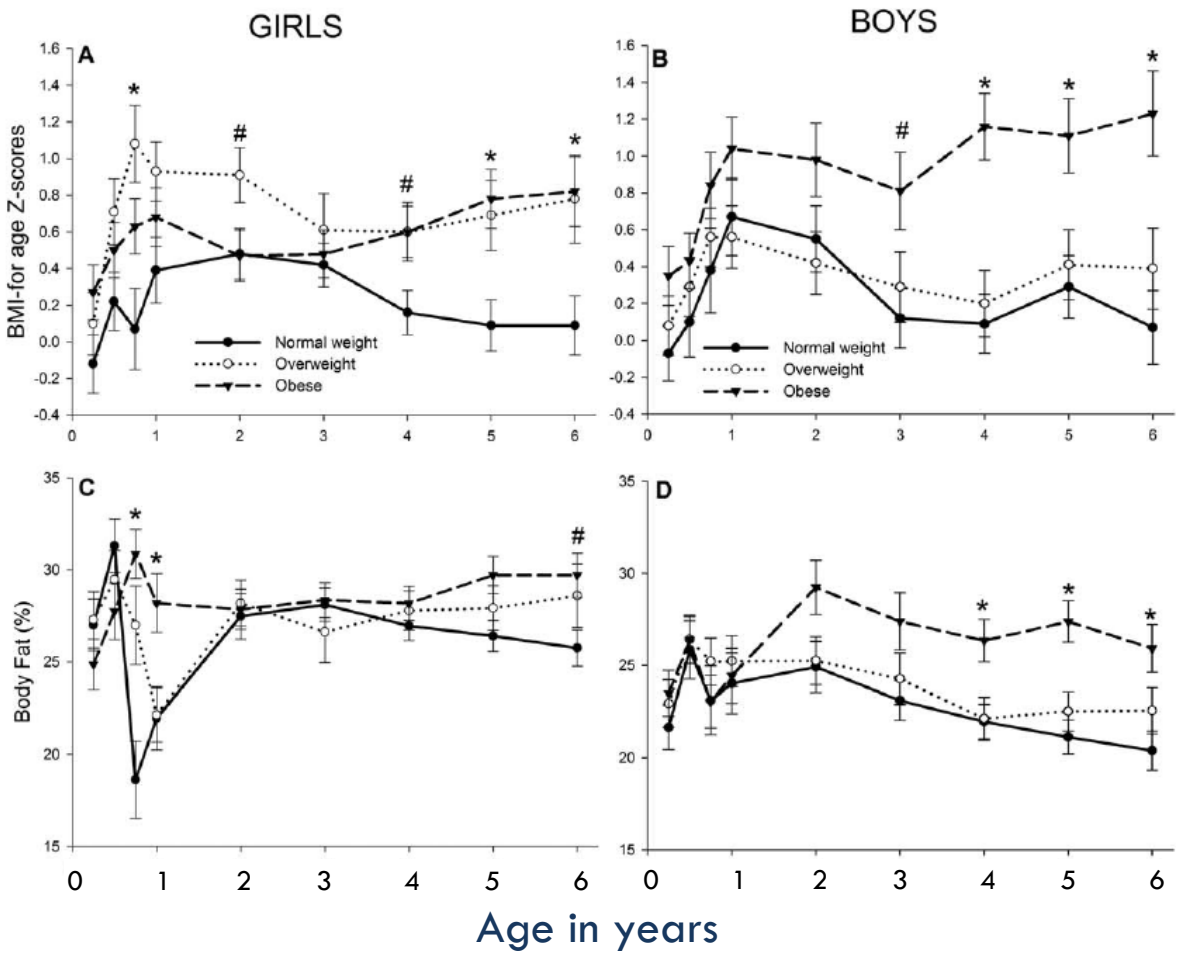
- Among 2-4 y olds maternal obesity, doubled the risk of obesity (*Whitaker et al. Pediatrics, 2004*); ~8500 low income children from the WIC program
- Offspring of obese mothers showed 4.6 times the risk of being obese at 7 y of age (*Reilly et al., 2005*). Data from the ALSPAC study examined 21 risk factors.
- Data from 313 mother-child pairs of the EPOC Study, 10 y olds had 2.5 times the risk of being obese (*Kaar JL, 2014*); risk diminished with lower GWG.
- Mater-University study confirmed that maternal BMI was associated with increased offspring BMI at 14 y of age (*Lawlor et al., Am J Epi, 2006*). Maternal stronger than paternal BMI

# Longitudinal Body Composition of Children Born to Mothers with Normal Weight, Overweight, and Obesity

*Aline Andres[1,2], Holly R. Hull[3], Kartik Shankar[1,2], Patrick H. Casey[2], Mario A. Cleves[1,2], and Thomas M. Badger[1,2]*

## The *Beginnings* Study

- Longitudinal body composition from 3 mo to 6 y of age (DEXA)
- 325 infants (51% female).
- Detailed infant diet information.
- Sex differences.




Andres et al., Obesity, 2015

# Maternal Obesity and Obesity in Adulthood

- Women whose mothers were obese had 6.1-fold greater risk of obesity at 18 y of age (*Stuebe et al. Int J Ob, 2009*); ~26,000 subjects Nurses Health Study II
- Greater % body fat at 30 y of age (*Reynolds et al. J DoHAD, 2009*). Motherwell birth cohort, UK
- Similar findings from the Pelotas trial, Brazil at 23 y of age increased offspring BMI and WHtR (*Tequeanes et al., J Nutr, 2009*).
- The decreased risk of obesity in children born to obese women following weight loss after bariatric surgery (*Kral JG et al., Pediatrics, 2006*).

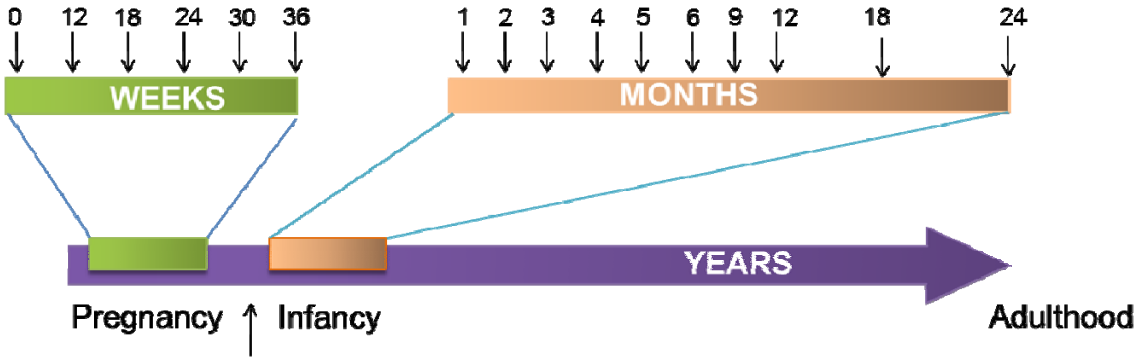
# Excessive GWG on Offspring Obesity Risk

- Clear evidence that excessive GWG in both normal and OW/ OB women increases offspring obesity
  - (*Oken et al. Obstetrics, 2007*); Project Viva at 3 y of age
  - (*Hull et al, Am J Ob Gyncol*);
  - (*Crozier et al. AJCN, 2011*); ALSPAC study 16 y of age
  - (*Badon et al, Obesity, 2014*); HAPO study, GWG independent of glycemic status increases neonatal fat mass.
  - (*Starling AP, AJCN 2015*), Healthy Start study, excessive GWG increases neonatal adiposity.

- Few studies have examined the effects of maternal diet
  - Majority of evidence for HF – rodent and non-human primate studies
- Specific dietary components, Fructose, LC-PUFA, protein:non-protein, DHA

# The *Glowing* Study

- N = 320 women, recruited 4-10 wk of pregnancy
- BMI Lean 18.5-24.9, Overweight/Obese 25-35
- Second parity, singleton pregnancy.




Andres, Shankar & Badger

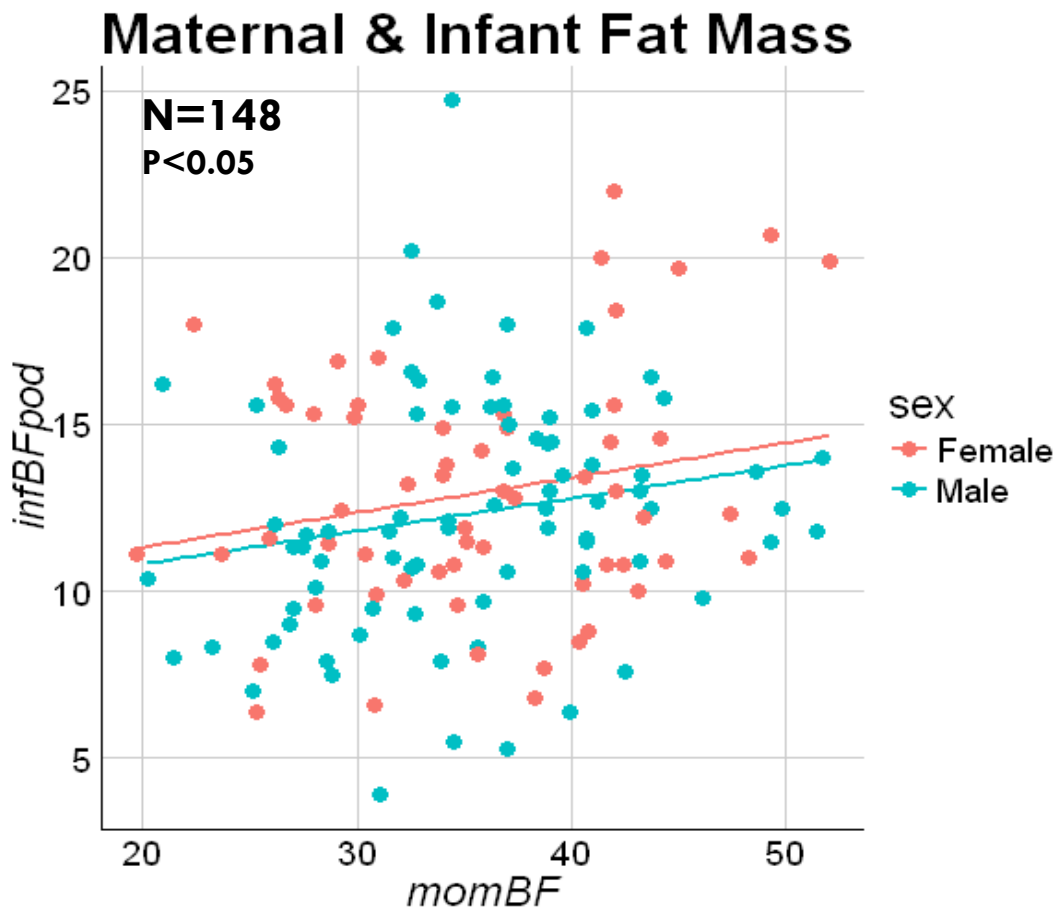
**Funding: USDA**

- Longitudinal assessment of body composition, diet intake & composition, energy expenditure in both mother and child
- Placenta, cord, cord blood (N= 150) and cord-matrix stem cells

glowing STUDY
Growing Life. Optimizing Wellness.

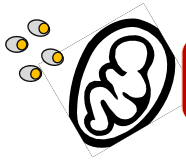
A prospective longitudinal study of mothers and infants throughout pregnancy to age 2 y

# Maternal and Infant Fat Mass



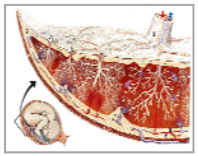
# Examining Mechanisms of Programming

Maternal Obesity / Diet



Early Development

- Epigenetic changes
- Programming of stem cells



Placental changes

- Alterations in nutrient transport
- Inflammation & Lipotoxicity

Offspring 'Neonatal programming'

- Microbiome
- Milk composition

Programming of metabolism & adiposity → Downstream Sequelae (Fatty liver, T2D)

Genome-wide DNA methylation

Transcriptome and Epigenomic analysis

Microbial Ecology Metabolomics

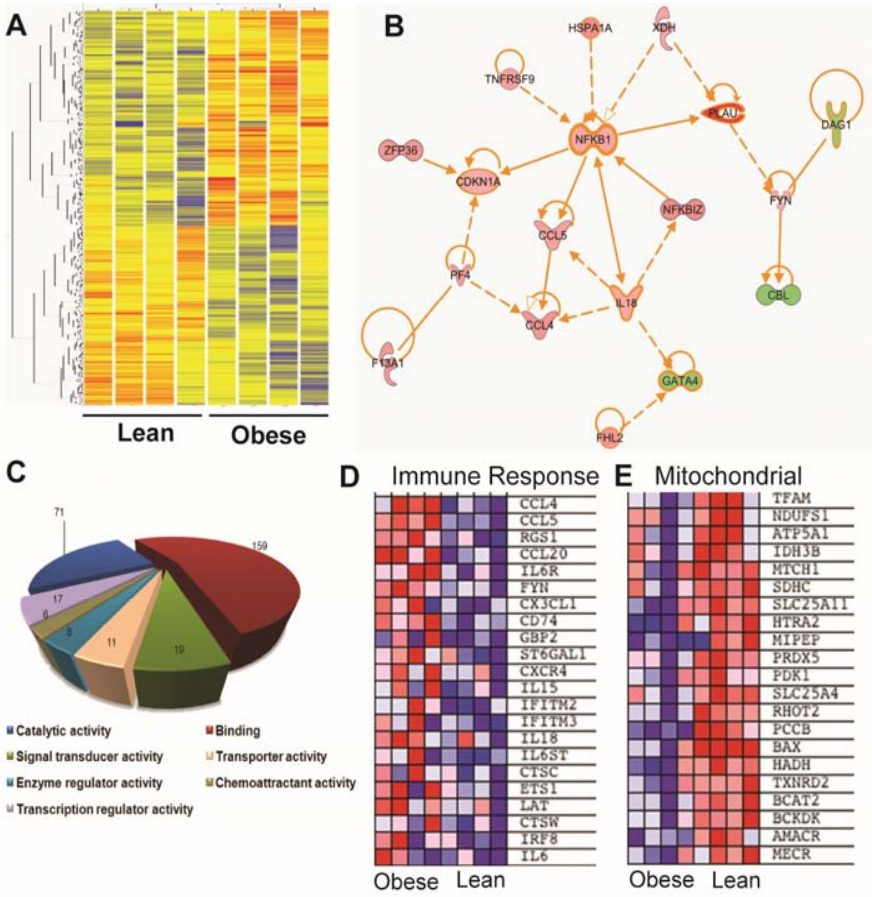
Energy expenditure Stable isotopes

# Maternal Pre- and Peri-conception Environment

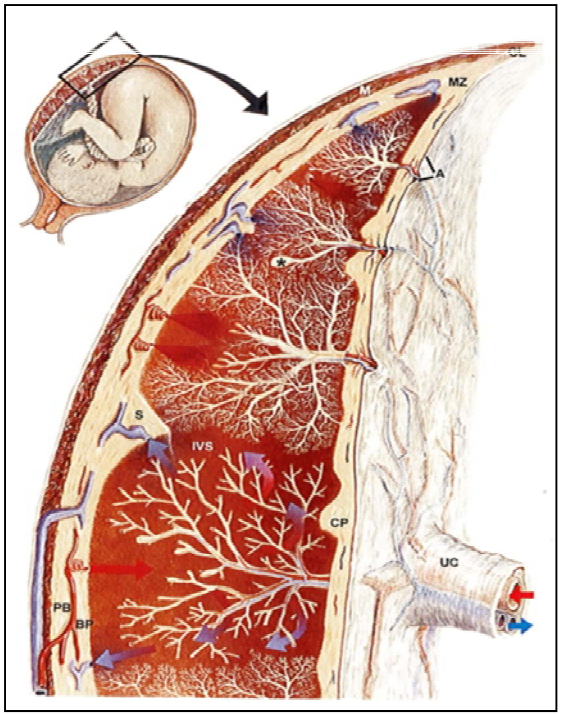
- Maternal influences can be programmed in the oocyte and early embryo.
- Critical period of epigenetic malleability.
- Whether germ-line specific exposure are sufficient remains unknown.

**Studies by Dr. Aline Andres (ACNC)**
Human Oocytes from Lean and Obese using global transcriptomic analyses (RNA-seq).

Blastocysts at dpc 4.5



# The Placenta is a Key Mediator



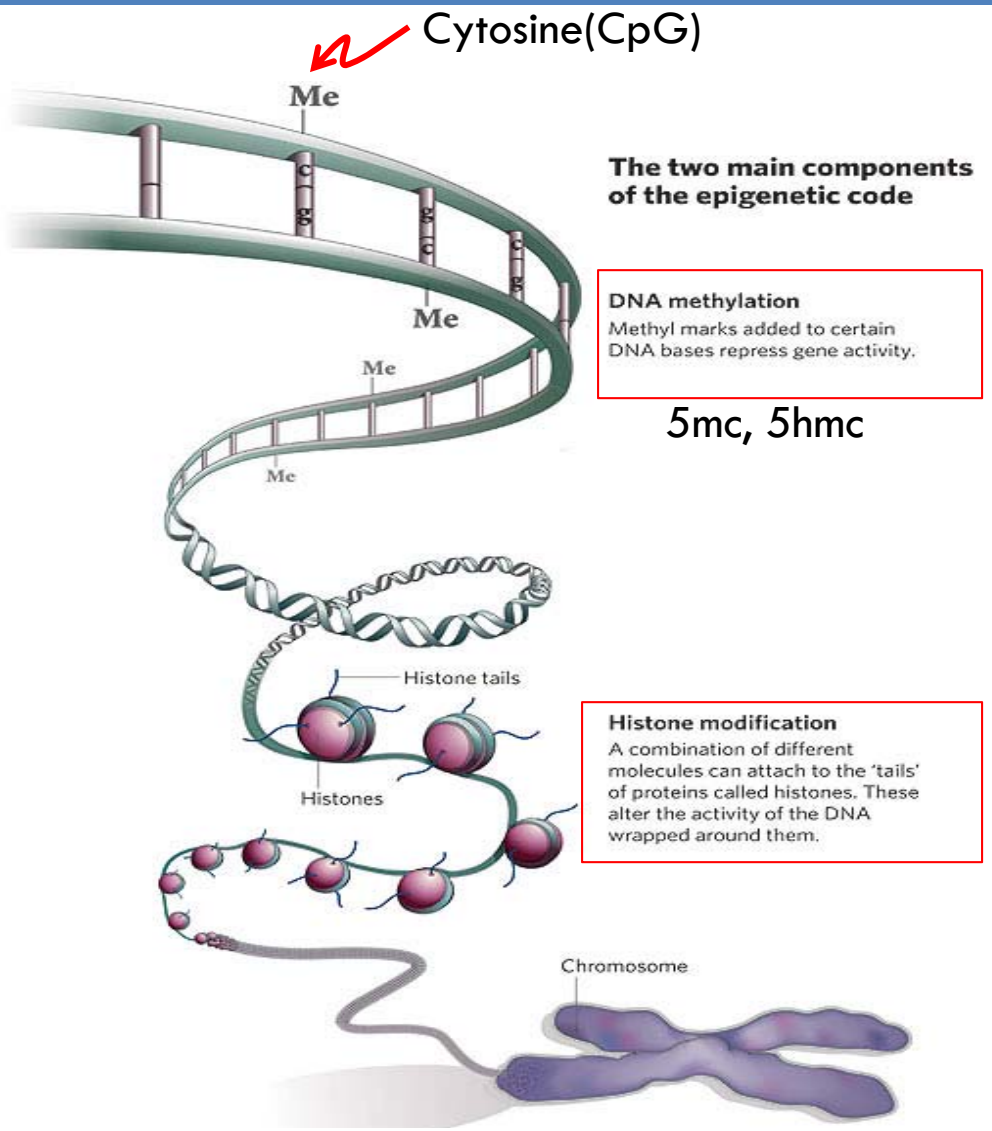

Obesity and maternal nutrition → Placental changes → Offspring development → Long-term programming

- Maternal obesity promotes lipotoxicity (JNK/Egr-1) in the placenta.
- Effect of obesity is sex and placental site-specific.
- Maternal obesity affects thyroid hormone signaling in the placenta.
- The placenta acts as a 'nutrient-sensor' and manages fetal demand (*Jansson and Powell, 2013*).


1. Saben et al., *AJP Endo & Met,* 305:E1, 2013
2. Saben et al., *Placenta*, 35:171, 2014
3. Saben et al, *Placenta*, 35: 1013, 2014.
4. Saben et al., Placenta, 35: 125, 2014.
5. Shankar et al., *Placenta.*, In press, 2015.

# Why Focus of Epigenetic Changes?

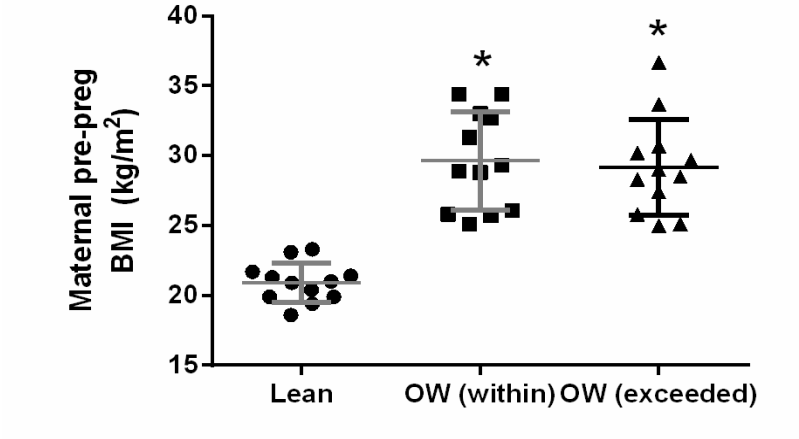
- Epigenetic mechanisms are key in development and differentiation
- Early development is associated with changes in the epigenetic landscape
- Nutritional and environmental challenges may alter epigenetic patterns



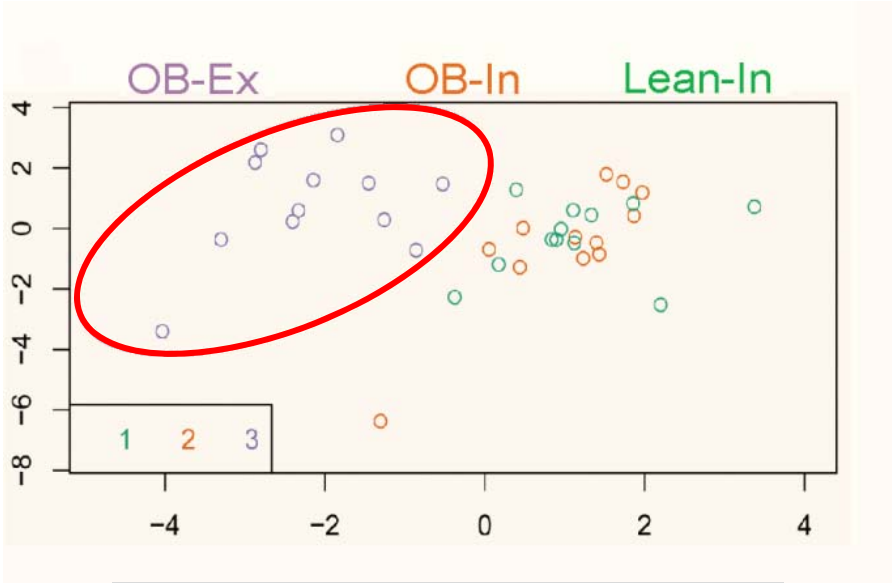

Epigenome : complete array of covalent modifications on the chromosomes

# Umbilical Cord DNA Methylation Analysis

- Pilot analysis of UC samples
- Lean or Obese women with either appropriate or excessive GWG
- **Infinium 450K** Genome-wide coverage: > 480,000 CpG sites

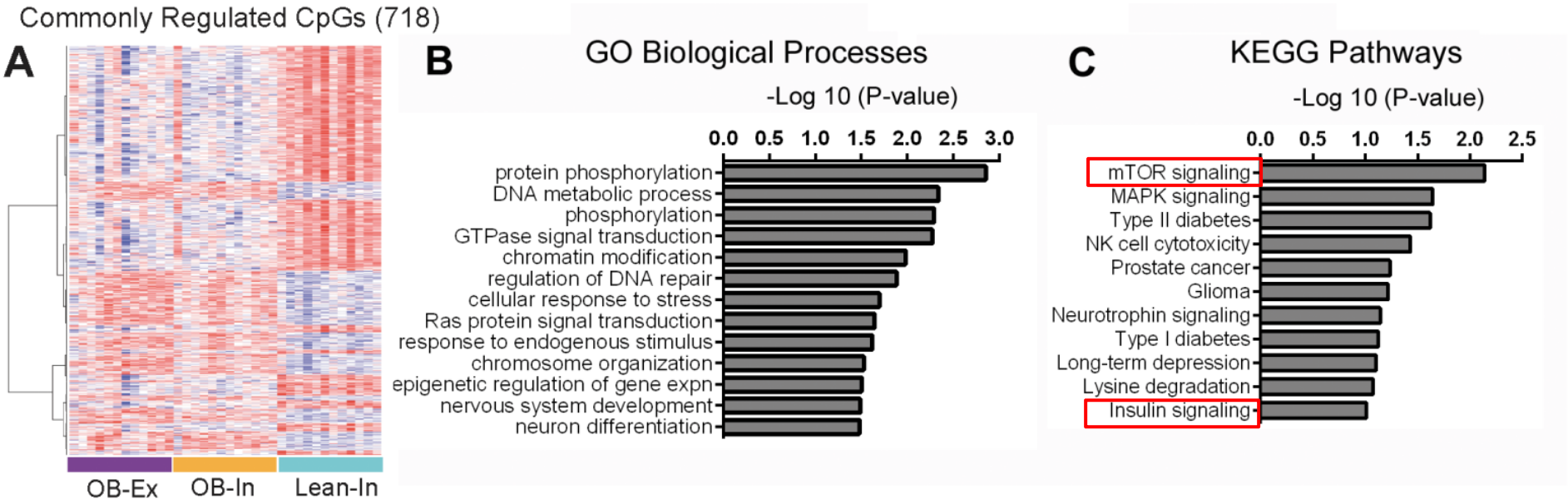



PCA analysis

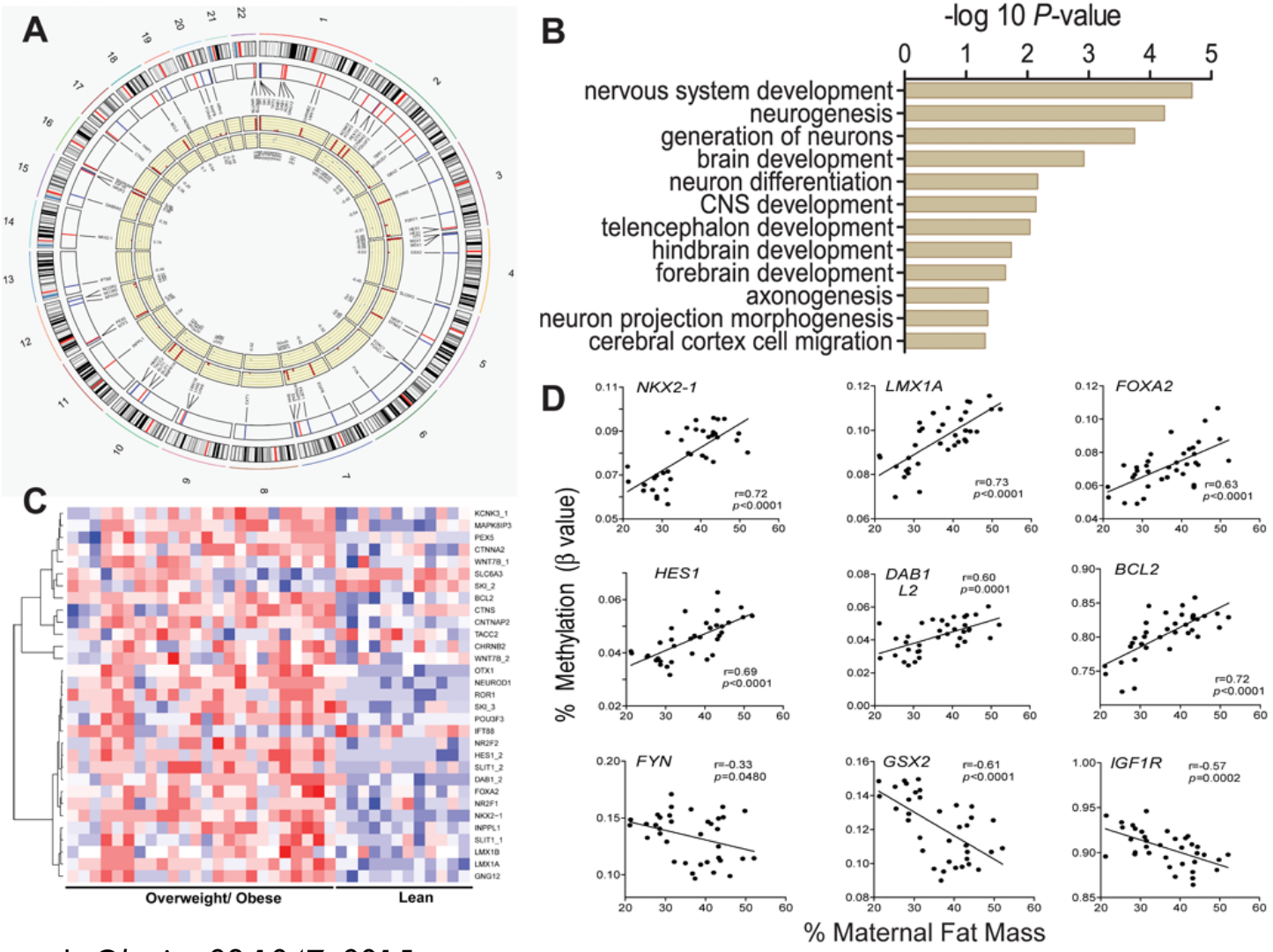



Obese women with excessive GWG cluster separately

# Nutrient Signaling is Affected Both by MatFM and GWG



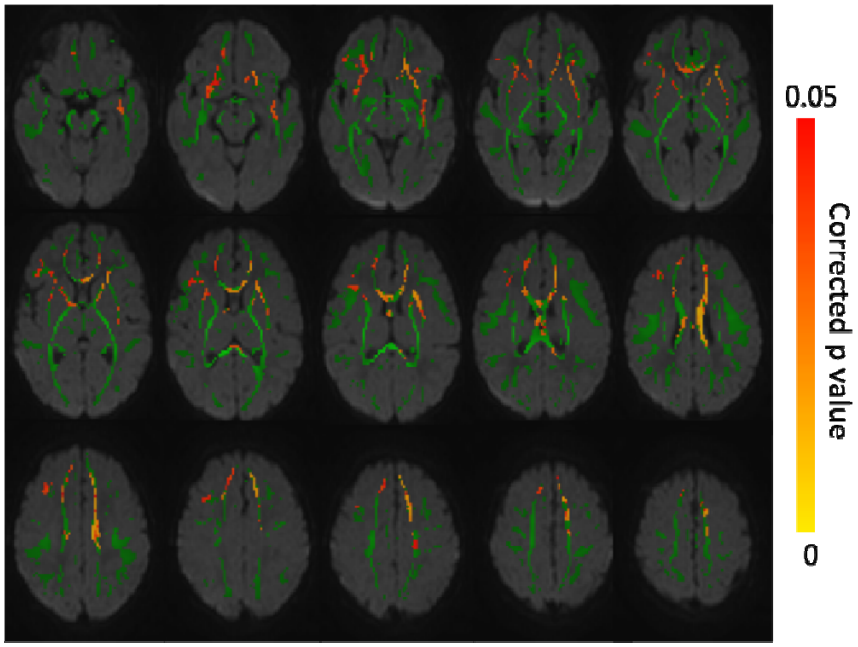
# Neurodevelopment Related Genes




Ou et al., *Obesity*, 23:1047, 2015.

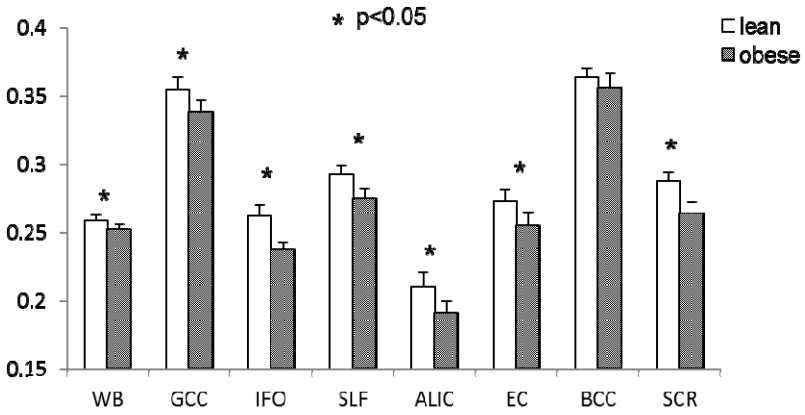
# Maternal Obesity Decreases Infant White Matter

Age 2 wk
Lean =17; Obese = 11




**Diffusion Tensor Imaging- Fractional Anisotropy (FA) values**
**Voxel-wise Tract-based Spatial Statistics analysis**

Whole Brain and Specific Regions




whole brain; WB
genu of corpus callosum; GCC
inferior frontal-occipital fasciculus; IFO
superior longitudinal fasciculus; SLF
anterior limb of internal capsule; ALIC
external capsule; EC
body of corpus callosum; BCC
superior corona radiata; SCR

Ou et al., *Obesity*, 23:1047, 2015.

Xiawei Ou, PhD

# Maternal Obesity & Offspring Neurological Function

- Maternal pre-pregnancy BMI is associated with ADHD symptoms (Swedish population-based cohort - 5 years old children, ***N=1,714***). (*Rodriguez, 2010*)
- Children of women who were both overweight and gained a excessive weight during pregnancy had a 2-fold risk of ADHD symptoms compared to normal-weight women. (*Rodriguez, 2008*). Teacher rated ***12,556*** school-aged children.
- Maternal obesity also increases risk of autism-spectrum disorder in children *(Krakowiak, 2012, Reynolds 2014, Moss and Chugani, 2014)*
- Non-human primates studies show maternal HFD induces anxiety-like behavior in offspring. (*Sullivan 2010).*

# Summary Messages

- Maternal nutritional status has persistent effects with significant public health importance in addressing child health.
- Maternal diet and obesity programs offspring's metabolism and brain development and risk of obesity.
- Other aspects of diet and lifestyle maybe have positive programming effects (physical activity, Mediterranean style diets).

- The role of diet and specific macronutrients (fat and carbs) is not clear.
- The interaction of genetic mediators for epigenetic changes.
- Microbiome and post-natal interactive factors

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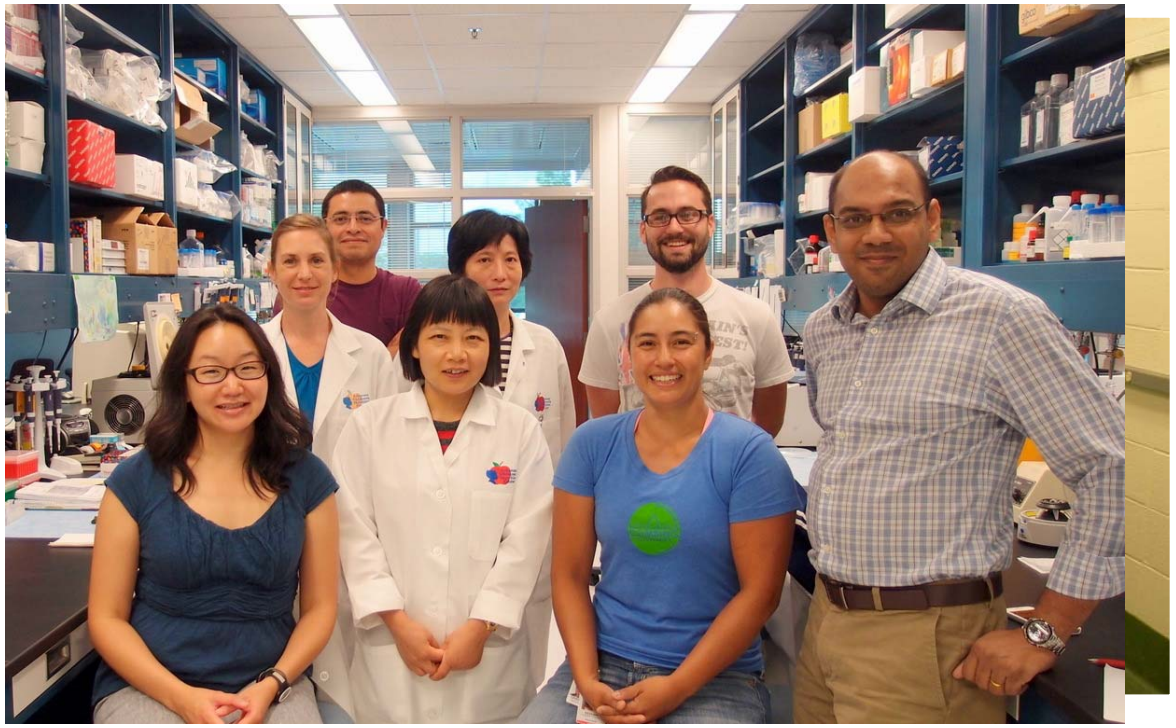
Matt Ferguson
& team members

**ACNC Human Core**
Jill Harsch & team

**Past Lab Members**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# Placental Metabolome in Obesity

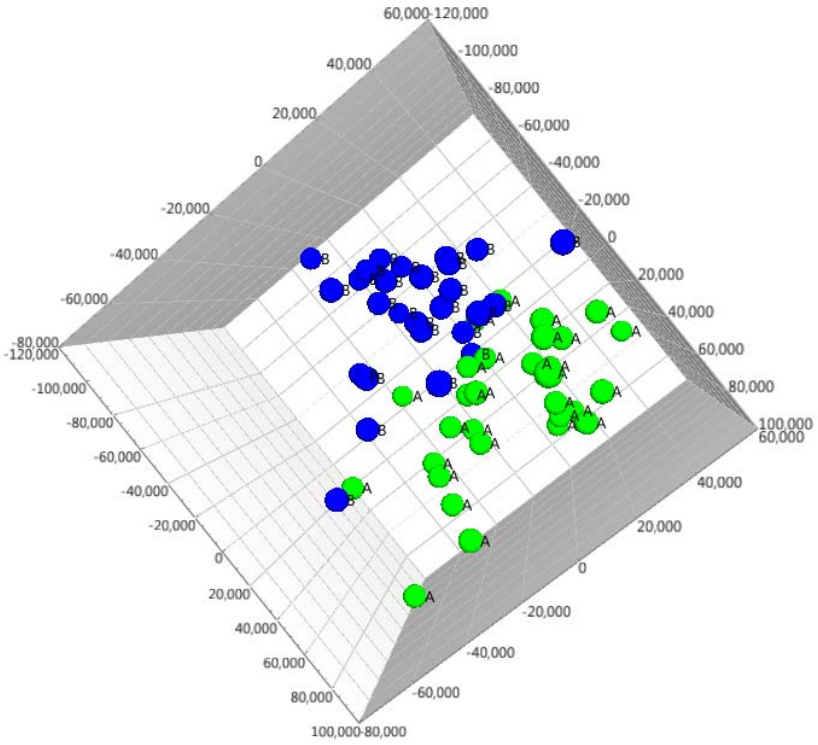
SIEVE ® (software for extracting putative biomarkers)

SIMCA-P (PCA, PLSDA, Cross validation, Permutation)

Pathway identification (MetaboLyzer, MetaboAnalyst…)

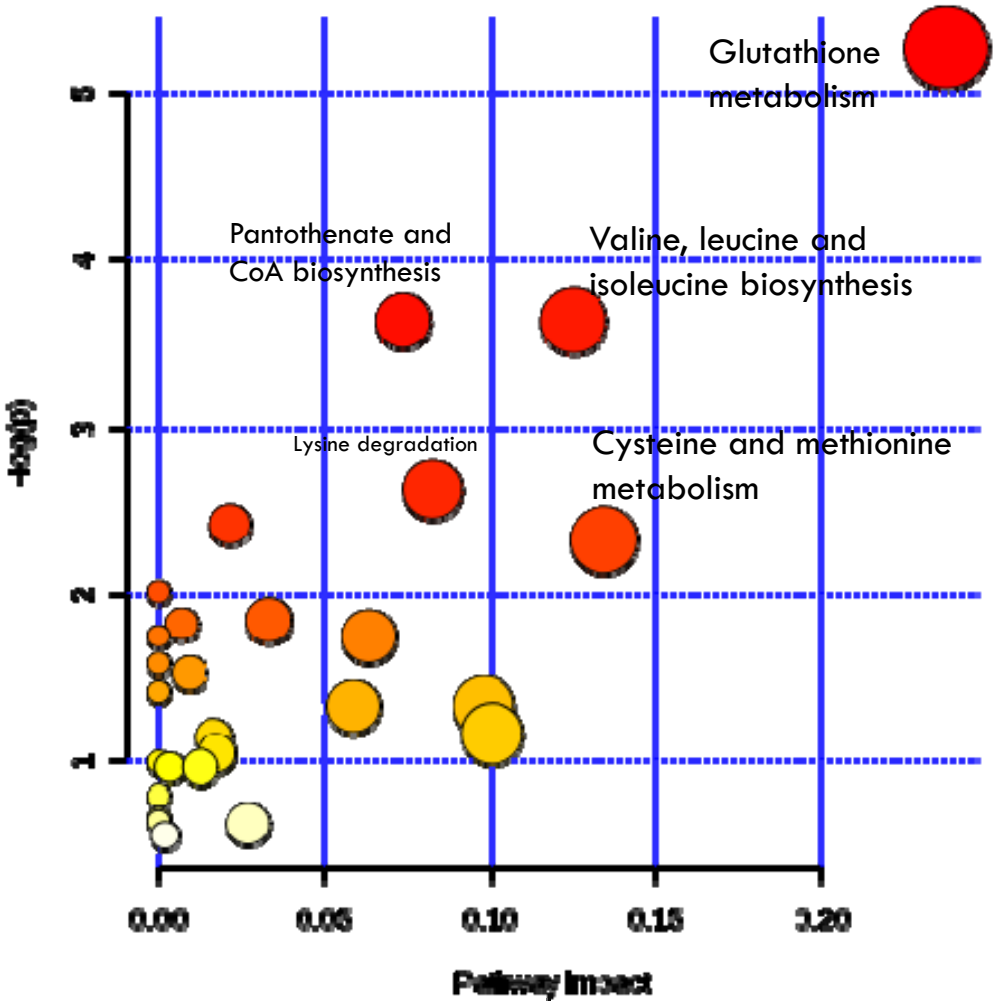
Validation LC-MS/MS detection and Quantification

PCA of significantly altered placental metabolites




~200 metabolites

# Putative Pathways (MetaboAnalyst)



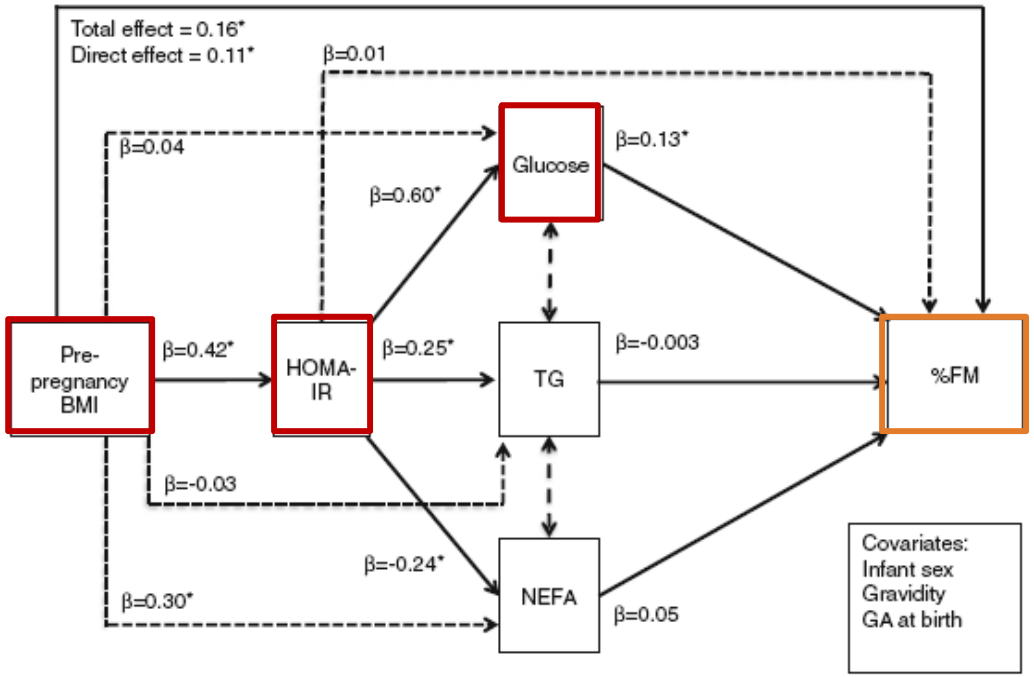
# Fuel-Mediated Hypothesis (Healthy Start study)

| 1st half | 2nd half of pregnancy | |
|---|---|---|
| <20 wk | >20 wk | N=804 |

| | |
|---|---|
| Strong association of neonatal fat with maternal insulin resistance | Strong association of neonatal fat mass with glucose levels |

Independent of pre-pregnancy BMI

Path analysis shows that maternal insulin resistance and glucose account for 21% of total effect of maternal BMI and offspring FM




*Crume et al., JCEM 2015*
*Shapiro et al., 2015*