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/m²)
Lean; 18.9 – 24.4
Overweight; 25 – 29.9
Obese ≥ 30

1 out of 3 children are overweight or obese.
21% of annual medical spending is on obesity-related illness.

Diabetes
Metabolic syndrome
NAFLD & NASH
Obesity *circa* 2014

**2012 Most Obese Metro Areas**

Among the 180 U.S. Metropolitan Statistical Areas surveyed

<table>
<thead>
<tr>
<th>Metropolitan area</th>
<th>% Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>McAllen-Edinburg-Mission, TX</td>
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<tr>
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<td>Little Rock-N Little Rock-Conway, AR</td>
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<td>Mobile, AL</td>
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</table>

January-December 2012
Gallup-Healthways Well-Being Index

GALLUP®
The World is Flat Fat

Triple Threat
Obesity, hunger and malnutrition
Obesity Causes: A Systems Perspective

- International and global factors
  - Climate, food production

- Community, societal factors

- Parenting styles, family & peers

  - Child characteristics
    - Age, gender
    - PA, genetics, maternal nutrition, In utero factors

- Energy Intake
- Energy Expenditure

Weight status
Over 60% of all women entering pregnancy are either OW or OB.
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?

Pre-conception overweight & obesity
- Altered nutrients, lipids, cytokines, hormones, placental factors
- In utero programming of metabolism
- Fetal programming
- High fat diet
  - Childhood & Adult Obesity
  - Normal birth weight

Diet GWG?

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)**

1. Normal caloric intake (155 Kcal/kg\(3/4\)/d)
   - Lean

2. 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

Offspring Body Weights

Summary: Offspring Outcomes

- **Liver**
  - Reprogramming of lipid handling pathways.\(^1\)
  - Decreased OXPHOS 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.\(^7\)

Are these findings relevant in humans?
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

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<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental</th>
<th>Control</th>
<th>Odds Ratio</th>
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<td>57006</td>
<td>100.0%</td>
<td>190.0%</td>
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**Heterogeneity:** \( I^2 = 0.01, \) **Chisq \( = 158.60, df = 19 (P < 0.00001), \) \( p = 88\% \)
Test for overall effect: \( Z = 14.17 (P < 0.00001) \)

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</table>

**Heterogeneity:** \( I^2 = 0.01, \) **Chisq \( = 166.68, df = 20 (P < 0.0001), \) \( p = 89\% \)
Test for overall effect: \( Z = 20.97 (P < 0.00001) \)

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 Andres¹,², Holly R. Hull³, Kartik Shankar¹,², Patrick H. Casey², Mario A. Cleves¹,², and Thomas M. Badger¹,²

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
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).
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

A prospective longitudinal study of mothers and infants throughout pregnancy to age 2 y
Maternal and Infant Fat Mass

Maternal & Infant Fat Mass

N=148
P<0.05

sex
- Female
- Male

momBF
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).
The Placenta is a Key Mediator

- 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
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.
PCA 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

Obese women with excessive GWG cluster separately

Umbilical Cord DNA Methylation Analysis
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

Whole Brain and Specific Regions

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

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.


- 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
Acknowledgments

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Umesh Wankhade, PhD
Ying Zhong
Ping Kang

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Thomas M Badger, Ph.D.
Sean H Adams, PhD
Keshari Thakali, PhD
Jin-Ran Chen, PhD
Sree Chintapalli

ACNC Animal Core
Matt Ferguson
& team members

ACNC Human Core
Jill Harsch & team

Past Lab Members

Funding Supported by NIH NIDDK R01-DK084225 and USDA-ARS CRIS 6026-51000-0010-05S

UAMS- Translational Research Institute (CTSA)
Placental Metabolome in Obesity

**Validation LC-MS/MS detection and Quantification**

**Pathway identification (MetaboLyzer, MetaboAnalyst...)**

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

**SIEVE® (software for extracting putative biomarkers)**

PCA of significantly altered placental metabolites

~200 metabolites
Putative Pathways (MetaboAnalyst)

- Glutathione metabolism
- Valine, leucine and isoleucine biosynthesis
- Lysine degradation
- Pantothenate and CoA biosynthesis
- Cysteine and methionine metabolism
Fuel-Mediated Hypothesis (Healthy Start study)

1\textsuperscript{st} half \quad 2\textsuperscript{nd} half of pregnancy
<20 wk \quad >20 wk \quad N=804

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

Strong association of neonatal fat with maternal insulin resistance
Strong association of neonatal fat mass with glucose levels
Independent of pre-pregnancy BMI

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