Epigenetic Mechanisms for Obesity Risk

Jacob E. (Jed) Friedman, PhD
Professor of Pediatrics, Biochemistry & Molecular Genetics
Reproductive Sciences & Endocrinology
Director, Colorado Program in Nutrition & Healthy Development
University of Colorado Anschutz Medical Center, Aurora CO
Objectives

1) Highlight the role of Prenatal exposure-maternal obesity, diet, and inflammation on neonatal fat accretion and epigenetic risk for subsequent childhood obesity and NAFLD.

1) Highlight Postnatal growth Influences from Breast Milk and maternal diet on Infant Microbiome and the Metagenome.

1) Future challenges/directions for research-food for thought.
Maternal Obesity in U.S.
(20-39 yr, 2011-12)

How could this not impact infant outcomes?

Ogden, et al, JAMA, 2014
Obesity Begets Obesity

• Clear association between maternal obesity and:
  – Early onset (<6yo) metabolic syndrome in offspring
  – Fatty liver (NAFLD)
  – Childhood obesity

• Animal and human studies:
  – Signs of aberrant methylation and fatty liver can be seen in utero
  – Mitochondrial dysfunction, NAFLD, Adiposity persist through 1st year (despite weaning)

Smith et al, 2009
Borrengasser et al, 2013
Lawler et al, 2011
Feinberg et al, 2010
Shock et al, 2009
Brumbaugh et al, 2013
A recent report has found an association between the methylation status of specific genes in human fetal tissue and the subsequent development of childhood adiposity in two longitudinal cohorts. Would epigenetic analysis at birth, therefore, have utility in identifying future risk of obesity?
Figure 1 Medical problems and epigenetic changes (in placenta and blood) at birth and later in life, that have been associated with intrauterine exposure to diabetes mellitus, maternal obesity, and famine.

2015: Omics applied to Humans

MOTHER
Metabolome
MICROBIOME
Epigenome

NEONATE in Utero
Metabolome
Epigenome
Imprinted & Non-imprinted Genes

Infant Outcomes
Metabolome
Epigenome
= Proteome

Metabolome
Glucose → Pyruvate → Acetyl-CoA → TCA Cycle
Glycolysis
Lactate
Gluconeogenesis

Placental Transcriptome
CD14 selected cells
Cell metabolism and signaling
Matrix remodeling
Immune regulation
Laosocyte adhesion

Micorbiome
“Thinner” MB
“Fatter” MB

Proteome
Tissue Function

Our Approach in Moms & Infants –Work in Progress-

Can we observe physiologic differences in fat deposition that predate Influence of diet and lifestyle?
Umbilical Cords and Stem Cells

• UC is fetal tissue-infant derived, exposed to trans-placental blood.
• Stem cells surrounding the vasculature are mesenchymal (MSC)
  • Pluripotent *in vivo* (Cset et al, 2001)
  • *In vitro* can differentiate into myocyte or adipocyte (Janderova et al, 2003; Gang et al, 2004)
  • Expose to variety of nutrient rich conditions
• Non-invasive, plentiful
• Novel application: Pediatric Obesity
## Maternal Characteristics

Kristen Boyle, PhD.

<table>
<thead>
<tr>
<th></th>
<th>NW (n=13)</th>
<th>Obese (n=14)</th>
<th>t-test p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y), mean (SD)</td>
<td>28.8 (5.5)</td>
<td>26.7 (7.2)</td>
<td>0.41</td>
</tr>
<tr>
<td>Pre-Pregnancy BMI (kg/m(^2)), mean (SD)</td>
<td>21.2 (1.1)</td>
<td>34.6 (3.7)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Primiparous, n (%)</td>
<td>4 (30.8)</td>
<td>6 (42.9)</td>
<td>0.51</td>
</tr>
<tr>
<td>Glucose, mean (SD)</td>
<td>74.8 (3.7)</td>
<td>76.0 (6.4)</td>
<td>0.55</td>
</tr>
<tr>
<td>Insulin, mean (SD)</td>
<td>7.8 (1.5)</td>
<td>12.5 (6.0)</td>
<td>0.02*</td>
</tr>
<tr>
<td>HOMA-IR, mean (SD)</td>
<td>1.4 (0.3)</td>
<td>2.4 (1.3)</td>
<td>0.03*</td>
</tr>
<tr>
<td>Triglycerides (mg/dL), mean (SD)</td>
<td>129.2 (56.5)</td>
<td>138.7 (46.6)</td>
<td>0.67</td>
</tr>
<tr>
<td>Free Fatty Acids (mg/dL), mean (SD)</td>
<td>333.8 (117.0)</td>
<td>471.8 (159.3)</td>
<td>0.02*</td>
</tr>
<tr>
<td>Gestational Age at Delivery (wk), mean (SD)</td>
<td>40.1 (0.8)</td>
<td>39.6 (1.1)</td>
<td>0.24</td>
</tr>
<tr>
<td>Cesarean Delivery, n (%)</td>
<td>2 (15.4)</td>
<td>2 (14.3)</td>
<td>0.94</td>
</tr>
</tbody>
</table>
Hypothesis

Infants born to obese mothers are predisposed to early onset metabolic disease due to “programming” events before birth, resulting from fuel overload, that impair intramitochondrial pathways involved in oxidative metabolism and energy balance.

Specific Pathways:

• Fatty Acid Oxidation

• Amino Acid Metabolism
  • BCAA catabolism
  • Intermediary metabolism

Epigenetic Mechanisms Testable in offspring uMSC Model
Aim 3a: Epigenetics & Differentiation

*H3a*: MSC epigenetic signatures relevant to adipogenic and myogenic differentiation are associated with maternal BMI

![Bar chart showing DNMT1 and KDM6A mRNA content](chart.png)
Aim 3c: Epigenetics & Insulin Resistance

H3c: MSC epigenetic signatures relevant to insulin resistance are associated with maternal BMI
Metabolic Programming in the Fetus: is it a Matter of Fat?
Collaborative Research
Oregon National Primate Research Center,
University of Colorado

**Long-Term Goal:**

- To develop a Non-Human Primate Model to study the effects of Maternal Diet, Obesity and GDM on the development of metabolic systems (liver, muscle, fat, heart, brain) in utero and the effects on infant behavior and post-natal disease pathways.
Fetal Hepatic Lipid Accumulation—Early 3rd Trimester

Female Japanese Macaques

- Control Diet
- High Fat Diet

Maternal Diet Reversal

C-section

Fetal Liver Triglycerides, mg/g

Maternal Diet

Control

High Fat

Reversal to control

* p < 0.01

# p < 0

Does hepatic steatosis occur in infants of obese-GDM Mothers?

Infant “Papoose” for whole body MRI
MRI for Neonatal Fat Measurement

- Magnetic Resonance Spectroscopy (MRS)
- Cohort of 25 infants
  - 13 born to normal weight controls
  - 12 born to obese mothers with GDM

• 72% increase in hepatic fat in neonates born to obese GDM mothers.

Visceral fat = less than 0.1% of total fat and independent of subcutaneous fat. 
Brumbaugh, 2013.
Risks for NAFLD

Genetic Risk
- PNPLA3

Gestational Risk

Lifestyle Risk
- Excess Adiposity
- Metabolic syndrome
- Diet (Fructose, n-3 PUFA)

“First Hit”
Hepatic Lipid Accumulation

“Second Hit”
Oxidative Stress
Hepatocyte Injury
Inflammation
Fibrosis

Early Life Exposure to Maternal Insulin Resistance Has Persistent Effects on Hepatic NAFLD in Juvenile Nonhuman Primates

Female Japanese Macaques

Control Diet → Control or High Fat Diet

High Fat Diet → Control or High Fat Diet

Birth

Weaning 7 mo.

Mothers

Juvenile Livers-from IR mothers

Genes for De-novo Lipogenesis

Metagenomic signatures of dysbiosis in immune mediated diseases

Bacterial genes & genomes will be specific of patients’ microbiome.
The Early Neonatal Period

“a critical role for lactation on programming the immune system”

Major step for gut development-

- Exposure to novel nutrients, bioactive molecules, and bacteria
- First challenge of “diet composition”, Infants double fat mass.
- Establish brain neurocircuitry for gut/brain energy sensing systems.
- Establishing the symbiotic relationship with immunomodulatory microflora.
- Instruction in early life aspects of immune protection – maturation/education of immune cells in gut and liver may have persistent effects.
- Epigenetic priming can take place.

Milk composition can influence each aspect of this major step
Dysbiosis

Maternal Western Style Diet (Breast milk n6/n3)

Intestinal Epithelial cells

SCFA

n6/n3

Microbial Products (LPS)

“Leaky” Gut

Epigenetic programming

Macrophage activation

Portal Vein

NASH

Inflammation

Steatosis

Plasticity & Reversibility

PRE-CONCEPTION  GESTATION  INFANCY  EARLY CHILDHOOD  ADOLESCENCE  ADULTHOOD
Host remodeling of the gut microbiome and metabolic changes during pregnancy.

High-fat maternal diet during pregnancy persistently alters the offspring microbiome in a primate model

Maternal gut microbiome clusters according to diet

Offspring gut microbiome clusters according to maternal diet
Over-arching Hypothesis
maternal obesity will directly affect the development
of the infant’s microbiome and will be associated with
increased adiposity during the first 4 months of life.

Do your genes act on your microbiome, which
in turn promotes disease?
Objectives:
To determine how maternal obesity and diabetes act to colonize the microbiome of the mother-infant pairs

To establish how maternal characteristics and breast milk composition impact the infant microbiome and adipose tissue development at 1-yr post-partum
Breast feeding may be protective of offspring obesity in N1, Obese, and GDM women

Appetite Regulatory Hormones

Inflammatory Cytokines

Antioxidants

Fatty Acid Profiles?

Microbiome?

What is Really in Breast Milk???
Biochemical Analyses in Human Breast Milk

**Composition**
- Calories
  - Lactose
  - Protein
  - Fat
- Glucose
- Fatty Acid Profile*
  - Free Fatty Acids
  - Triglycerides

**Cytokines**
- IL-6*
- IL-8*
- IL-10*
- TNF-α*
- IFN-γ*
- CRP

**Hormones & Adipokines**
- Insulin
- Leptin
- Adiponectin
- Ghrelin (total)
- Ghrelin (acylated)

**Oxidative Stress**
- F2-Isoprostanes
- Oxidized-LDL
- TBARS
- 8-OH-dG
- 4-HNE

**Antioxidants**
- Total antioxidant capacity
- DPH Radical Scavenging Activity
- Catalase Activity

**Microbiota**
- Probiotic Content*

---

Bridget Young, PhD

Table 3. Maternal Breast Milk Microbiome Taxa (Phylum)

? Very Low Abundance
Maternal Obesity changes the bioactive components in human breast milk

Milk TG are not significantly different at 2-weeks from Obese Mothers

<table>
<thead>
<tr>
<th>Variable</th>
<th>NW</th>
<th>Ob</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>265.7 ± 89.7</td>
<td>1546.6 ± 573.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Insulin</td>
<td>9.3 ± 4.6</td>
<td>27.33 ± 14.9</td>
<td>0.03</td>
</tr>
<tr>
<td>n6/n3 ratio</td>
<td>6.7 ± 2.1</td>
<td>8.27 ± 1.6</td>
<td>0.05</td>
</tr>
</tbody>
</table>

\(\n = \text{Mother stopped breastfeeding; converted to formula}\)

-Courtesy of Bridget Young and Mike Rudolph
Figure 4. Human milk bioactive components that differ according to maternal BMI are associated with the composition of the infant microbiome (n=30) after adjusting for maternal BMI. (*) notes associations p<0.1.
Hypothesized mechanism(s) linking maternal obesity to “obesogenic” gut microbiome

**Human Milk**
- Leptin (+)
- Insulin (+)
- n6/n3 (+)

**Infant Gut Taxa**
- Bifidobacteraceae (+)
- Erysipelotrichaceae (+)
- Staphylococcaceae (+)

**Infant Gut Metagenome**
- Lipid metabolism
- Amino acid Synthesis
- Nucleic acid pathways

**Liver**
**Adipose**
**Muscle**
**Inflammation**
**Epigenetic Programming**
Community Transcriptomics: what we don’t know

• Microbial membership varies.
  - Early Colonization? Genetics?

• Over time, does the community “solves” for a habitat-specific metagenome?

• It then differentially regulates that metagenome?
  - These two types of regulation differ in time scale.
Molecular function in metagenomes

Phylum

Pathway Abundance

This is the "core" human microbiome,
Not this.
- Over 2/3 of its genes are uncharacterized, more than almost any single bacterial genome
- We don't know how its "cell types" communicate
- We don't know their physical structure or lineages

The convergence of carbohydrate active gene repertoires in human gut microbes
Final Thoughts:

- Humans share a core microbiome and yet differ by genes, species, ecology, and gene count/richness.
- The gut MB is dynamic and we don’t know the time scales.
- Changes in diet lead to short-term changes in the MB, yet it is not clear which are reversible.
- MB gene richness is a key stratifier for the response to dietary intervention in obese subjects, yet mice revert back.
- Some MB-derived metabolites can have a positive effect on anti-inflammatory activity or energy harvest, while others are toxic to the host.
- Can we identify specific species or patterns of the gut MB that are more relevant to serve as targets for obesity?
Environmental influences

Increased Obesity Risk
Low /neutral risk

Vaginal birth,
Breast feeding,
Hygiene,
Maternal Diet

C-section delivery,
Formula feeding, Hygiene,
Vaccination, altered diet

Microbiota

Normal B/F ratio?
Low LPS,
Butyrate-SCFA,
Microbial diversity, richness

Altered B/F ratio
Low Butyrate, SCFA
Low microbial diversity,
High omega 6:3 ratio
High leptin?

High *Lactobacillaceae*
Low *Staphylococcus aureus*
Other species?

Epigenetics- New genes

Exercise
Low inflammation,
Normoglycemia,
Normal GWG

Smoking, excessive GWG
Inflammation, high fat diet
Fasting glucose, HOMA-IR

Obesity development

“the undiscovered
Country within”

Rapid weight gain

First 1000 days of Life
Thank-You!

Home Team:
Lynn Barbour, M.D.
Teri Hernandez, Ph.D.
Karim el Kasimi, Ph.D.
Stephanie Thorn, Ph.D.
Margaret Heerwagen, B.S.
Becky DeLaHoussaye, M.S.
Rachel Van Pelt, Ph.D.
Sarah Borengasser, Ph.D.
Pete Baker M.D.
Kristen Boyle, Ph.D.
David Brumbaugh, M.D.
Rachel Janssen, M.S.
Rebecca Aiken, M.S.
Allison Buti, M.S.
Sean Newsom, Ph.D.
Dominick Lemas, PhD.
Cathy Chartiere-Logan, M.S.
Bridget Young, PhD.
Regina Reynolds, M.D.
Melanie Reece, PhD.
Nancy Krebs, M.D.
Dan Franks, PhD.
Molly and the Nutrition staff.

Oregon Health Sci Ctr & Novo-Nordisk
Kevin Grove, PhD., Dan Marks, M.D. PhD.
Kjersti Aagaard, M.D, PhD – Baylor Coll of Medicine

NIH-R01 DK060685, RO1 DK074643,
R24 DK90964, P30 DK048520, ADA
University of Colorado Nutrition & Obesity Research -Center (NORC); University of Colorado Maternal-Child Clinical Translational Research Center, Bill and Melinda Gates foundation