Nutrition & metabolism are important at all stages of life ...especially pregnancy & early childhood.

Nutrition and metabolic status of both mother and father are important for health of offspring.
Diabetes Risk
What are the Earliest Molecular Mediators?

- Family History
- Genetics
- Shared Environment
- Intrauterine Environment
- Developmental Effects
- Epigenetics
- Obesity
- Inactivity
- Aging

Progressive Pathogenesis of Type 2 DM

- Insulin Resistance
- Abnormal Insulin Secretion

Type 2 DM
Diabetes Risk Increases Progressively Throughout the Lifespan

Genetics

Abnormal Intrauterine Environment

Early Postnatal Growth Patterns

↑ Adipose Mass

↓ Muscle Mass

Obesity↓ Fitness

Overnutrition Shared Family Environment

prenatal

Diabetes Risk

postnatal

Age
How the first nine months shape the rest of your life

The new science of fetal origins

BY ANNIE MURPHY PAUL
OVERVIEW

• Maternal nutrition and metabolism and developmental programming as a risk factor for adult obesity and type 2 DM

• Mouse model of metabolic risk induced by maternal undernutrition

• Metabolic disease can be transmitted through both the paternal & maternal lineage to subsequent generations, even without further experimental nutritional modulation

• Potential epigenetic & metabolic mediators of these phenotypes
The Original Fetal Origins Epidemiologist

Ethel Margaret Burnside

Chief Health Visitor and Lady Inspector of Midwives
Hertfordshire, England

- Sample ledger with birth weight and weight at 1 yr, 1911-1948
- Data accessed by David Barker, who demonstrated link between low birth weight, accelerated early postnatal growth, and adult disease risk

BMJ, 2003
The Dutch Hunger Winter

- Western Netherlands affected by acute famine by the end of World War II
- Official rations: 400-800 calories/day
- Women exposed to famine during the 2nd and 3rd trimester of pregnancy delivered small babies.
- These low birth weight babies (exposed to famine *in utero*) had a higher prevalence of adult diseases:
  - diabetes
  - cardiovascular disease (heart attack, stroke)
  - hypertension
  - obesity
What Did These and Other Studies Teach Us?
Both Low and High Birth Weight Associated with Risk of Metabolic Disease in Humans

<table>
<thead>
<tr>
<th>Low Birth Weight</th>
<th>Normal BW</th>
<th>High Birth Weight</th>
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<tbody>
<tr>
<td>RR 1.47</td>
<td>RR 1.36</td>
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- Higher Risk with LOW Birth Weight
  - Genes, Adverse Fetal Environment (UN or Obesity) → Poor Growth → LBW

- Higher Risk with HIGH Birth Weight
  - Maternal Gestational Diabetes & Excess Weight Gain
Birth Weight is a **Biomarker of Development**
Influenced by Nutrition, Environment, and Genetics

**Placenta**
- Hypertension
- Vascular anomalies
- Hypoxia

**Nutrition/Metabolism**
- Excess maternal weight gain & obesity
- Undernutrition
- Hyperglycemia
- Iron deficiency

**Stressors**
- Infection
- Glucocorticoids
- Maternal smoking

**Genes**
- Ethnicity
- Chromosomal anomalies
- DNA polymorphisms
  - GCK, PPAR-γ, TCF7L2, GR, HNF4
- Epigenetic regulation
  - Igf2/H19, Grb10

**BIRTH WEIGHT**
Postnatal Catch-Up Growth and Obesity During Adult Life Further Increase Risk

San Antonio Heart Study, 1994
“DEVELOPMENTAL PROGRAMMING”

Alterations during critical periods of development influence risk of adult disease

Adverse Intrauterine & Early Postnatal Environment → Adult Disease

Potential Mechanisms?
Patti Lab Models of Developmental Programming and DM Risk

Rodent models:

- Exposure to maternal undernutrition
- Exposure to genetically-determined maternal insulin resistance – even without maternal diabetes or obesity

Human studies:

- Humans with history of low birth weight
Protocol: Exposure to Maternal Undernutrition

Virgin ICR (Outbred) -> Gestation 19 days -> CON

50% global food restriction pregnancy d12.5-19 -> UN

Effects in Offspring

Reduced Fetal Growth & Birth Weight

E16.5

Newborn

Body weight (g)

Control

UN

Isganaitis & Patti 2009.
Woo & Patti 2011.

Litter size equalized at birth
Effects similar with cross-fostering
Undernutrition-Exposed Mice Have “Catch-Up” Growth & Maintain Normal Body Weight during Adult Life

![Graph showing body weight (g) over weeks after birth for control and undernutrition-exposed mice. The graph indicates "Catch-Up" growth and normal body weight during adult life.]
Exposure to Maternal Undernutrition Results in Impaired Glucose Tolerance and DM in Offspring Mice

5X Increase in Risk of Diabetes in UN Mice

Normal mice, outbred ICR; normal chow during postnatal life (Purina 9F) Intraperitoneal GTT; 2g glucose/ kg body weight
Adiposity is Increased in Mice Exposed to Maternal UN

**DEXA Scan, Age 12 Months Chow Diet**

- More abdominal fat
- Larger Adipocytes
- Lipogenic Gene Expression

**Gonadal Adipose Tissue Histology, 3 wks**

- No change in food intake

Aris Lytras
Multiple Tissues Are Affected by Exposure to Maternal UN

- Age-Related Insulin Resistance
-↓ Insulin Clearance
-↓ Glucose-Stimulated Insulin Secretion
-↓ Muscle Mass
-↓ Skeletal Muscle Stem Cells
-↓ Regeneration

Metabolic Risk

Altered Body Composition
↑ Abdominal Fat
↓ Muscle Mass

Dysregulation of Neural Control of Appetite and/or Metabolism
When & How Are these Developmentally-Mediated Phenotypes “Programmed”? 
When & How Are these Developmentally-Mediated Phenotypes “Programmed”?

Potential Molecular Mediators

Potential Physiological Mediators

**PRENATAL**

- Intrauterine insults:
  - Undernutrition
  - Placental dysfunction
  - Hypoxia
  - Decreased blood flow
  - Δ Parental metabolism

- Altering Development
- Altered Hormonal/Metabolic Milieu
- Stem Cell Effects

**POSTNATAL**

- Catch-up Growth
- Overnutrition
  - Inactivity
  - Obesity
  - Aging

- Obesity & DM Risk

**EPIGENETIC MECHANISMS**

- DNA Methylation & Hydroxymethylation
- Histone Modification
- miRNA
When & How Are these Developmentally-Mediated Phenotypes “Programmed”? 

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Obesity & DM Risk

**EPIGENETIC MECHANISMS**
- DNA Methylation & Hydroxymethylation
- Histone Modification
- miRNA

“Memory” of Nutritional History?
Hypothesis: Epigenetic Alterations Contribute to Metabolic Disease Risk

Epi-genetics:
- Changes in gene expression NOT due to changes in DNA sequence
- Can be influenced by nutritional or other environmental stimuli
- May be “permanent” and affect cellular function long after the causal nutritional or other stressor is gone
- Can be inherited

[Diagram of DNA and histone modification]

DNA CLOSED = INACTIVE  DNA OPEN = ACTIVE
Sequence can be read
Evidence for Epigenetic Contribution to Metabolic Disease

Fetal programming – birth weight and adult disease effects in humans and mice

Human obesity:
  • Prader-Willi syndrome – disruption at imprinted locus causes obesity and diabetes
  • therapy with valproate (HDAC inhibitor) increases obesity

Altered maternal nutrition at conception associated with reduced methylation at IGF2 locus (Dutch hunger winter population)

Disruption in H3K9 demethylase JHDM2a causes obesity

*Intergenerational transmission*
Multigenerational Effects

• Developmental & nutrition-associated phenotypes can progress to subsequent generations, even in the setting of normal nutrition during pregnancy.
  • *Paternal GF food supply associated with premature mortality in grandsons*
  • *Paternal GM food supply associated with mortality of granddaughter*

• Insulin resistance can be transmitted to offspring of IUGR rats despite embryo transfer and cross-fostering

• Can we observe multigenerational effects in our model?
• Can we use these data to dissect epigenetic vs. other molecular mechanisms?

Food abundance for the grandfather associated with lifespan effects in grandchildren
Overkalix, Sweden
Multigenerational Effects of Nutritional History

**Birth Weight – F1 Generation**

![Graph showing birth weight comparison between F1-C and F1-UN groups](image)

**Birth Weight – F2 Generation**

![Graph showing birth weight comparison between CF-CM, CF-UM, UF-CM, and UF-UM groups](image)

*Normal Nutrition During 2nd Generation Pregnancy*

**Paternal Effects Dominant**

Jimenez, Patti. Diabetes 2009
Multigenerational Metabolic Disease in Our Model

A. *Fat Mass (DEXA)*

B. *ITT*

C. *Glucose Tolerance*


- Obesity
- Altered Gene Expression
- ↓ adipose Dlk1 in both paternal and maternal
- Insulin Resistance
- Impaired Glucose Tolerance
- ↓ GSIS
Potential Mechanisms Mediating Multigenerational Metabolic Disease?

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Paternally-Mediated Multigenerational Effects: Test the Hypothesis that Epigenetic Mechanisms Contribute to Offspring Phenotypes

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Isolated Paternal Effects:
- Birth Weight: ↓
- Glucose Tolerance: ↓
- Obesity: ↑
“You are What Your Father Ate”

History of UN Exposure in Fathers

\[\text{Breed with Control Females}\]

\text{Obesity & Glucose Intolerance in Offspring}

\text{Exposure during Development}

HFD-Induced Obesity in Fathers

\[\text{Breed with Control Females}\]

\text{Mild Glucose Intolerance in Daughters}

\text{Exposure during Adult Life}

Low Protein/High Sucrose Diet in Fathers

\[\text{Breed with Control Females}\]

\[\uparrow\text{Expression of Lipid Synthesis Genes in Offspring Liver}\]

\text{Jimenez & Patti, Diabetes 2009}

\text{Ng & Morris, Nature 2010}

\text{Carone & Rando, Cell 2010}
Multigenerational Phenotypes via Paternal Lineage Implicates Epigenetic Modification of Sperm

Compare Sperm Epigenome: C vs. UN Father Does DNA Methylation Differ?
Multigenerational Phenotypes via Paternal Lineage Implicates Epigenetic Modification of Sperm

Compare Sperm Epigenome: C vs. UN Father Does DNA Methylation Differ?

F0 Pregnancy

± Caloric Restriction

F0

F1 Females
F1 Males

F1 UN

F1 C

F1 C

F1 C

C-UN

C-C

F1 Mating sperm

Normal nutrition during pregnancy

F2 Adult

Obesity Glucose Intolerance
History of Nutritional Exposure During Development Alters DNA Methylation in Sperm

Unsupervised clustering of methylation patterns
Multigenerational Transmission via Paternal Lineage Implicates Epigenetic Modification of Sperm

- F0 Pregnancy
- F0 Adult
- + Caloric Restriction

SPERM: 
- Altered Methylation?
- Other Modifications?
- miRNA?
- Altered maintenance of histones at key developmental loci?

Are there effects on fetal development and patterns of gene expression?
- ED 16.5 LIVER & PLACENTA

Obesity Glucose Intolerance
Fetal Liver Gene Expression is Altered in F2 Offspring of Males Exposed to Maternal UN

Downregulated in offspring of males with history of prenatal UN exposure:

- Cell Cycle
- Cytoskeleton

Upregulated in offspring of males with history of prenatal UN exposure:

- Lipid β-Oxidation
- Microsome

DAVID Ontology (FDR <0.05):

- Downregulated in C x U:
  - Cell Cycle
  - Cytoskeleton

- Upregulated in C x U:
  - Lipid β-Oxidation
  - Microsome

\( n = 4949 \text{ genes with } q < 0.05 \)

\( N = 5 \text{ pools per group, each with 2-3 litters} \)
Placental Gene Expression is Altered in F2 Offspring of Males Exposed to Maternal UN

**DAVID Ontology (FDR <0.05):**

**Downregulated in C x U:**
Symporter Activity
Transmembrane Transporters (e.g. GLUT3)

**Upregulated in C x U:**
Inflammatory Genes

Downregulated in F2 offspring of males with history of prenatal UN exposure

Upregulated in offspring of males with history of prenatal UN exposure

\[ n=479 \text{ genes with } q<0.05 \]

\[ N=5 \text{ pools per group, each with } 2-3 \text{ litters} \]
A Vicious Cycle of Risk?

Effects on Germ Cells

Abnormal Intrauterine Environment

Epigenetic

Non-Epigenetic

Germ Cells

Genetics

Postnatal Growth Patterns

↑ Adipose Mass

↓ Muscle Mass

Adult Obesity

↓ Fitness

Overnutrition

↓ Fitness

Age

Diabetes Risk

Prenatal

Postnatal
Let’s Be Optimistic: Opportunities to Reduce Risk!

- Effects on Germ Cells
- Abnormal Intrauterine Environment
- Epigenetic
- Non-Epigenetic
- Germ Cells
- Genetics
- Foster Healthy Postnatal Growth Patterns
- Diabetes Risk
  - ↑ Fitness
  - Improve Nutrition
  - ↓ Obesity

Age

prenatal  postnatal
Nutrition & metabolism are important at all stages of life ... *especially* pregnancy & early childhood.

Nutrition and metabolic status of both mother and father are important for health of offspring.

I’m happy because now I can blame my father AND my mother AND my grandparents for everything!