Developmental Programming: Effects of Diet, Exercise, and Polychlorinated Biphenyl Exposure during Pregnancy on Long-term Health in Offspring

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Outline

• Developmental origins of health and disease

• Maternal exercise during healthy pregnancy

• Maternal obesity and high fat diet consumption

• Polychlorinated biphenyl exposure

• Translational approach
Known for decades
David Barker
Neonatal and CVD mortality link

Coronary Heart Disease (1968-1978)  Infant Mortality (1901-1910)

Slide credit: Barbara Alexander
Adapted from Barker and Osmond, 1986
Low birth weight

• Low birth weight is defined as less than 5 ½ pounds.
• About 1 in every 13 babies in the US is born with LBW.
Causes

- Alcohol, illicit drugs, smoking
- Environmental exposure (PCBs)
- Placental problems
- Chronic health problems in the mother
- Inadequate maternal weight gain
- Socioeconomic factors
- Poor nutrition
- Age: teen mothers or women over 40
Low birth weight

Percent of live births, 2007

Source: Centers for Disease Control
National Vital Statistics System
Obesity—BMI ≥ 30
Diabetes

Source: Centers for Disease Control and Prevention
Stroke

Stroke Death Rates, 2000-2006
Adults Ages 35+, by County

Source: Centers for Disease Control
National Vital Statistics System
Hypertension

Percentage of Adults Aged 20 Years and Older Who Have Been Told They Have High Blood Pressure, 2007

Source: Centers for Disease Control National Vital Statistics System
Western diet fed-dam data

Samuelsson et al., Hypertension 2008
Offspring data

• Body weight differences
• Increased fat mass
• Hypertensive
• Impaired glucose tolerance

Samuelsson et al., Hypertension 2008
Paternal influence

Ng et al., Nature 2010
Developmental origins

• Barker hypothesis
  – Maternal malnutrition leads to obesity, diabetes, and heart disease in offspring.
  – Fetal/developmental programming
  – Developmental origins of health and disease (DOHaD)

– High Initial Damage Load Hypothesis
  • Early development produces an exceptionally high load of initial damage, which is comparable with the amount of subsequent aging-related deterioration accumulating during the rest of the entire adult life.
  • Predicts that even small progress in optimizing the early-developmental processes can potentially result in a remarkable prevention of many diseases in later life.
“You live in two worlds; the world of your mother and the world into which you are born.”

Slide credit: Barbara Alexander
Environment Special:
The oceans—why 70% of our planet is in danger

The Facebook Movie:
The secret history of social networking

How the first nine months shape the rest of your life

The new science of fetal origins
BY ANNIE MURPHY PAUL
But how is this occurring?

Epigenetic Mechanisms
DNA to protein

DNA

Template strand of DNA

Synthesis of RNA (transcription)

mRNA

Synthesis of polypeptide (translation)

Polypeptide

NH₂ - COOH
Chromatin

• Made up of
  – DNA and proteins
    • Histones.
    • Non-histone chromatin proteins.

• Fundamental packaging of DNA.
  – Need to highly condense DNA to fit in cell.
  – Yet – must be accessible when needed.
Chromatin Structure – Successive Coiling

http://www.cbs.dtu.dk/staff/dave/roanoke/genetics980218.html
Chromatin is Dynamic

- Structure of chromatin is remodeled
  - ATP-dependent process
  - Important to allow
    - Replication
    - Transcription
    - Repair
    - Packaging
  - Histone code informs chromatin structure
    - Histone modifications serve to recruit other proteins by specific recognition of the modified histone
Epigenetics

• ‘Epi’ means over or above
  – ‘epi’genetics
• Conrad Waddington coined the term in his description of cellular fate.
  – Epigenetic landscape
Epigenetics

“Epigenetics is the study of heritable changes in phenotype (appearance) or gene expression caused by mechanisms other than changes in the underlying DNA sequence.”

- DNA methylation
- Histone modification
  - Acetylation, phosphorylation, and ubiquitination
- Non-coding RNA
  - Short interfering RNA, microRNA, etc.
- Genomic imprinting

Epigenetic regulation is critical for mammalian development and cellular differentiation.
Epigenetic overview
Block transcription

Figure 2. Mechanisms of DNA methylation-mediated repression. (a) DNA methylation in the cognate DNA binding sequences of some transcription factors (TF) can result in inhibition of DNA binding. By blocking activators from binding targets sites, DNA methylation directly inhibits transcriptional activation. (b) Methyl-CpG-binding proteins (MBPs) directly recognize methylated DNA and recruit co-repressor molecules to silence transcription and to modify surrounding chromatin. (c) In addition to their DNA methyltransferase activities, DNMT enzymes are also physically linked to histone deacetylase (HDAC) and histone methyltransferase (HMT) activities. In this case, the addition of methyl groups to DNA is coupled to transcriptional repression and chromatin modification. (d) DNA methylation within the body of genes can also have a dampening effect on transcriptional elongation. MBPs might be involved in inhibiting elongation, either directly or by their effects on the surrounding chromatin structure.
Histones

- Highly conserved
- Basic proteins with a globular structure
- ‘Poor’ tail
  - Up to 30% of mass of histones
  - Relatively unstructured and flexible
  - Not part of globular domain
  - Quite important in epigenetics

Marks et al. Nature Reviews Cancer 2001
Histone modifications

- Small covalent modifications

- Methylation
  \[ R \text{--CH}_3 \]

- Acetylation
  \[ H_3C \text{--CO} \]

- Phosphorylation
  \[ R\text{--O--P--OH} \]
Lots to study—histone code
Example: Acetylation

- Reduces affinity for histone to DNA (DNA is negatively charged)
- Leads to more open chromatin conformation
- Recruits complexes to allow for transcription
Acetylation
Multiple methyl groups

- Arginine
  - Mono-methyl arginine
  - Symmetrical di-methyl arginine
  - Asymmetrical di-methyl arginine

- Lysine
  - Mono-methyl lysine
  - Di-methyl lysine
  - Tri-methyl lysine

Histone modifying enzymes
Epigenome

Figure 1. Cytosine and Histone Methylation

Cytosine methylation is the only known covalent modification of DNA in mammals. In contrast, histones are subject to hundreds of modifications, including acetylation, methylation, phosphorylation, and ubiquitination. (A) illustrates the structures and effects of cytosine methylation (repressive/red) and two histone marks: H3K27 methylation (repressive/red) and H3K4 methylation (activating/green). (B) illustrates the diversity of histone H3 modifications.
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• Maternal obesity and high fat diet consumption

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Lab overview

Developmental origins of health and disease

Maternal obesity
PCB exposure

Maternal exercise

OFFSPRING
Diabetes
Obesity
Cancer

Increase

Decrease
Aging intervention—Short-term
High health return on early investments

Mice
Pregnancy ~3 weeks
Lifespan ~150 weeks

Humans
Pregnancy ~40 weeks
Lifespan ~4160 weeks
Maternal exercise
Exercise during pregnancy

• Many known maternal benefits
  – Cardiovascular health
  – Body composition and weight maintenance
  – Muscle discomfort and cramps
  – Glucose regulation
  – Labor complications
Offspring effects

- Maternal exercise improves learning and memory in the offspring.
- Unclear metabolic effects.
Hypothesis
Voluntary exercise during pregnancy and nursing improves health in offspring.
ICR (CD1) mouse

- Outbred strain
- Good breeders
- Maternal care
- ~21 day gestation period
- Litter size is 11-12
Maternal intervention

Carter et al, AJP 2012
Maternal running data

![Graph showing maternal running data with time in days on the x-axis and running distance in km/day on the y-axis. The graph includes a peak in running distance around day 20, with a decline and stabilization after day 30. There are annotations indicating 'pups born' at specific time points.](image)
No negative effects

Body weight was completely normal throughout life.
Female glucose disposal

![Graph showing glucose disposal over time for sedentary and exercise groups.](image)

Sedentary; n = 20
Exercise; n = 18

**AUC (g/dl * min)**

![Bar graph showing AUC comparison between sedentary and exercise groups.](image)

**P < 0.01 compared to Sedentary**
Male glucose disposal

- **Sedentary; n = 19**
- **Exercise; n = 18**

* P < 0.05 compared to Sedentary
Body composition

- No change in female offspring born to sedentary or exercised dams.
- Male offspring born to exercised dams
  - Increased lean mass
  - Decreased fat mass
- Sex specific differences.
Is the effect species specific?

[Graph showing running distance (km day$^{-1}$) over time (day) with data points indicating peaks and troughs, and an arrow pointing to the point labeled "Pups born."]

Carter et al, MSSE 2013
Future directions for project

- Test controlled exercise rather than voluntary
- Timing of exercise important?
- Mechanism
  - Epigenetics
- High fat diet consumption in offspring
- Gestational diabetes
  - Offspring outcomes
Lab overview

Developmental origins of health and disease

Maternal obesity
PCB exposure

Maternal exercise

OFFSPRING
Obesity
Diabetes
Cardiovascular disease

Increase
Decrease
Goal: To identify a maternal diet in ICR mice that negatively influences offspring body composition and glucose tolerance.

Easy!!!
Maternal obesity

Lard-based diet
- 10% fat
- 60% fat
- 45% fat

Butter-based diet
- 11% fat
- 60% fat
- 32% fat
Normal offspring

• Offspring were completely normal even if they are born to obese moms consuming extremely high fat diets.
  – Body weight
  – Body composition
  – Glucose tolerance
  – Heart function

• Does this mean that these mice cannot be negatively programmed?

NO!!!!
Developmental origins of health and disease

- Maternal obesity
- PCB exposure
- Maternal exercise

OFFSPRING
- Obesity
- Diabetes
- Cardiovascular disease
Superfund Research Program

• Joint program sponsored by the National Institute of Environmental Health Sciences and the Environmental Protection Agency.

• Network of university grants that are designed to seek solutions to the complex health and environmental issues associated with the nation's hazardous waste sites.

• 3 biomedical projects
• 2 nonbiomedical projects
Polychlorinated Biphenyls (PCBs)

• Chemicals widely used as coolants.
• Banned in 1977
• More than one million capacitors and 14,000 transformers containing PCBs are still in use in the U.S.
• Primary sources of PCB exposure
  • Ground water or soil contamination
  • Food contamination from food storage in silos with PCB-coated interiors
  • Consumption of fish from contaminated waterways
• Additional sources of PCB exposure are still being identified…
PCBs: origins and environmental fate

Humans are harmed by intake through food, water, and air. Babies are harmed from mothers' blood and breast milk.

Slide credit: Nicki Baker
PCB 126—Coplanar PCB

- 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126)

- Dioxin-like
  - Characteristic dioxin toxicity

- Dioxin (TCDD)—contaminant in Agent Orange
  - Manufacturing by-product, extremely toxic
  - FDA: “Almost every living creature has been exposed to dioxins”

- Toxic Equivalency Factor (TEF)
  - A potency index/health risk value where other compounds are compared to TCDD
Concerns

• Persistent organic pollutants
  – Fat soluble, prone to accumulation
  – World-wide ban

• Health
  – Cancer
  – Cognitive impairment
  – Inflammation
  – Obesity/diabetes
  – Reproductive failure
    • Fertility
    • Miscarriage
    • Low birth weight
### Maternal exposure

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1 µmole/kg PCB 126</th>
<th>5 µmole/kg PCB 126</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prior to mating</strong></td>
<td>![Mouse]</td>
<td>![Mouse]</td>
<td>![Mouse]</td>
</tr>
<tr>
<td><strong>Pregnancy</strong></td>
<td>![Mouse]</td>
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</tr>
<tr>
<td><strong>Nursing</strong></td>
<td>![Mouse]</td>
<td>![Mouse]</td>
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</tr>
</tbody>
</table>

No PCB exposure

**Pup survival, body weight, inflammation**
Pregnancy weight gain is decreased
Maternal PCB and LBW

![Graph showing offspring body weight (g) at different ages (P7, P14, P21). The graph compares Control and PCB groups.](image)

![Image of a mouse labeled 'Control' and another labeled 'PCB'.](image)
Less Frequent Dosing

Maternal body weight (g)

-20 -10 0 10 20

Offspring body weight (g)

P3 P7 P14 P21

Age (days)

Control PCB126

Mating gestation

Day -28 -21 -14 -7 0 7 14 21

Wean
Hypothesis

Maternal Inflammation

Glucose Intolerance

Fetal Programming

Obesity and Glucose Intolerance
**In utero or milk**

- **Vehicle**
  - In Utero Environment
  - No Exposure
  - Nursing P0-P21
  - After Weaning 7 Weeks Old
  - Normal Glucose Tolerance
  - ????

- **PCB126**
  - In Utero Exposure
  - Postnatal Exposure
  - In Utero + Postnatal Exposure
  - Impaired Glucose Tolerance
  - ????
Funded Aims

Perinatal PCB Exposure
  ↓
  AhR
  ↓
  Nrf2
  ↓
Inflammation
  ↓
Offspring Programming
  ↓
Adult Disease
  ↓
Diabetes
  ↓
Obesity

Aim 1

Aim 2

Aim 3
Maternal Exercise
Outline

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Aging intervention—Short-term
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Humans
Pregnancy ~40 weeks
Lifespan ~4160 weeks
Neonatal foreskin – Can we link gene expression, protein levels, or markers of oxidative stress with maternal or fetal characteristics?

Maternal and birth weight

Smoking

Alcohol use

Exercise

Environmental contaminants
Foreskin as a model for other disease states

- Great tissue model because there are multiple cell types within the tissue
- Readily available where circumcisions are performed, and the tissue is routinely discarded
NanoString nCounter

- Isolated RNA
- Design a codeset for genes of interest
  - Stress response
  - Transporters/receptors
  - Adipokines
  - Cytokines
Smoking during pregnancy

- 30% of women delivering at UK Hospital
Neonatal Biomarkers of In Utero Tobacco Exposure

- **Aim 1**
  - Neonatal Foreskin
  - Developing Embryo, Fetus, Neonate
  - Smoking in Pregnancy

- **Aim 2**
  - Biomarkers
  - RNA
  - Epigenetics
  - Oxidative Stress
  - Functional Analyses
  - Stress Response
  - Glucose Transport
  - Adipogenesis

• Collaborators
  - Kristin Ashford, PhD
  - John O’Brien, MD
  - Rich Charnigo, PhD
  - Carmen Marsit, PhD—Dartmouth
Where do we go from here?

• Obviously very preliminary.
• We are looking to see if protein levels match RNA data.
• New tissue for programming studies.
  – How does it compare to placenta, cord blood?
• Risk assessment for environmental exposures???
Summary

• Maternal exercise protects improves offspring health.

• Are maternal “exposures” more important than maternal obesity?
  – Our data in a mouse model suggest PCBs are worse.

• Much, much, much more work needs to be done.
Our goal is to figure out if there are epigenetic changes in the offspring that are induced by maternal behavior/environment.
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