Maternal Bisphenol A Programs
Offspring Metabolic Syndrome

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Metabolic Syndrome

• **Traits:**
  - Obesity
  - Hypertension
  - Type 2 diabetes mellitus
  - Dyslipidemia

• **Mortality:** Leading cause of death in the United States

• **Obesity:** U.S. adults 65% overweight, 31% obese,
  Childhood obesity 20%

• **Hypertension:** 29% of U.S. population

• **Diabetes:** 27% of U.S. population
Obesity Trends* Among U.S. Adults From 1990 to 2010

(BRFSS; *BMI ≥30, or about 30 lbs. overweight for 5’4” person)
Etiology of Obesity

- Food Availability
- High Fat Diets
- Reduced Energy Expenditure
- Propensity for Obesity
- Developmental Programming
Developmental Programming

Fetal Nutrition, Stress
? Environmental Toxins

Altered cell number
and differentiation

Modified gene expression
altered function
### Bisphenol A (BPA)

#### Percent of canned foods that exceed safety threshold

<table>
<thead>
<tr>
<th>Food Type</th>
<th>Within factor of 5</th>
<th>Within factor of 5-10</th>
<th>Within factor of 10-100</th>
</tr>
</thead>
<tbody>
<tr>
<td>All foods</td>
<td>11%</td>
<td>9%</td>
<td>26%</td>
</tr>
<tr>
<td>Infant formula</td>
<td>33%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasta</td>
<td>33%</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Vegetable</td>
<td>29%</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Soup</td>
<td>11%</td>
<td>42%</td>
<td>37%</td>
</tr>
<tr>
<td>Beans</td>
<td></td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Tuna</td>
<td></td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Fruit</td>
<td>24%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meal replace</td>
<td>20%</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>Soda</td>
<td>17%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk products</td>
<td></td>
<td></td>
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</tbody>
</table>

BPA in a single serving compared to toxic dose:
- Red: Within factor of 5
- Orange: Within factor of 5-10
- Pink: Within factor of 10-100

Percent of canned foods that exceed safety threshold.
BPA: Endocrine Disruptor

Diagram showing the mechanism of BPA interaction with various receptors and proteins.
NHANES: BPA Levels during Pregnancy

Maternal Serum: 1 - 2 ng/ml
Breast Milk: 1.1 ng/ml
Placenta: 1 – 105 ng/ml
Amniotic Fluid: 8.3 – 8.7 ng/ml
Fetal Serum: 0.2 – 9.2 ng/ml
Models of BPA-Induced Fetal Programming

• *In vivo* maternal BPA exposure
  – Offspring phenotype
  – *In utero* effects on fetal appetite and adipose development

• *In vitro* fetal BPA exposure
  – Effect on neural stem cells
  – Effect on adipose tissue development
Maternal Bisphenol A (BPA)

OFFSPRING
- **Litter size:** Culled to 4 males and 4 females at birth
- **Nursing:** All pups nursed by same dams until p21
- **Weaning:** At p21 to ad libitum food and BPA-free water
Maternal BPA: Offspring Body Weights

- 1 Day

Increased Body Weights in Males

6 Month
Maternal BPA: Body Fat of 6 Month Offspring

Increased Body Fat in Males
Major Contributors of Obesity

Food Intake

Fat
Appetite Regulation
ARC Nucleus Development

- ARC cells arise from Neural Stem Cells in periventricular region
- Appetite (NPY) and Satiety (POMC) neurons populate the ARC during fetal life and this continues to develop during postnatal life
Hypothesis of Enhanced Appetite

Fetal BPA exposure

Appetite (NPY) neurons

Satiety (POMC) neurons

Increased Food Intake
Hypothalamic Neurospheres

Undifferentiated

Early Differentiation
NSC Proliferative & Differentiation Factors

Neural Stem Cell (Nestin)

Self renewal

Oligodendrocyte  Neuron  Astrocyte

Proliferation
Neural Stem Cell

↑Hes1  Self renewal

↓Mash1

Neuron

Differentiation
Neural Stem Cell

↓Hes1

↑Mash1

Self renewal

Neuron
Methods: NSC
*In Utero* BPA Exposure

Hypothalamic NSCs from 1 day old newborns of Control and BPA treated dams.

- Culture media - complete medium

**Analysis**

- Immunostaining of 10 micron sections:
  - **Nestin** – NSC marker
  - **Hes1** - proliferative factor
NSC Immunostaining: 1 Day Newborn

Control

- DAPI (nuclear) + Nestin (NSC)

BPA

- DAPI (nuclear) + Hes1 (Prolif)

Cultured for 7 days in complete medium; 10 micron sections
Methods: *In Vitro* BPA Exposure

**BPA Treatment of Neural Stem Cells**

- Hypothalamic NSCs: 1 day old newborns
- BPA treatment:
  - Neurospheres Cultures: Self renewal or differentiated
  - Dose - 1, 10, 20 μM x 5 days

**Analysis**

- MTT assay: NSC proliferation
- Protein expression (Western Blot):
  - **Nestin** – NSC marker
  - **Hes1** - proliferative factor
  - **Tuj1** - neuronal marker
  - **GFAP** - astrocyte
  - **Mash1** - neurogenic factor
BPA: Neural Stem Cell Proliferation

Increased Proliferation

BPA 0μM

BPA 10μM

Proliferation Index MTT

BPA (μM)

0 1 10 20
BPA: Neural Stem Cell Proliferation Factors

Increased Proliferative Potential
BPA: Neural Stem Cell Differentiation

Increased Neuron to Astrocyte Ratio

Control Tuj1/GFAP Mash1
% of Control
0
40
80
120
160 * *

Ratio

Increased Neuron to Astrocyte Ratio
Programmed Adipogenesis

- Adipose Proliferation and Differentiation
- Lipogenesis
Regulation of Adipogenesis

Peroxisome proliferator-activated receptor gamma 2 (PPARγ2)
**In Utero BPA Exposure**

Control and BPA treated dams: 3 week old offspring

Retroperitoneal adipose tissue

**Analysis**

- **Protein Expression (Western Blot):**
  - $\text{PPAR}_{\gamma}$ – adipogenic transcription factor
  - $\text{C/EBP}_{\alpha}$ – adipogenic transcription factor
  - $\text{SREBP1}$ – lipogenic transcription factor
BPA: Adipocyte Transcription Factors

**Adipogenic**

- **C/EBPα**

- **PPARγ**

**Lipogenic**

- **SREBP1**

*Increased Adipocyte Differentiation and Lipogenesis*
Methods: *In Vitro* BPA Exposure

**Adipocytes**
- Subcutaneous adipose tissue: 1 day old newborns
- Cultures: pre-adipocytes or differentiated adipocytes
- BPA treatment:
  - Dose - 1, 10, 20 µM
  - Period of treatment - 5 days

**Analysis**
- MTT assay: Preadipocyte proliferation
- Oil O Red stain: Preadipocyte lipid storage
- Protein expression (Western Blot):
  - PPARγ – adipogenic transcription factor
  - C/EBPα – adipogenic transcription factor
  - SREBP1 – lipogenic transcription factor
BPA: Preadipocytes

Adipogenesis

Lipogenesis

Increased Proliferation and Lipid Storage
BPA: Differentiated Adipocytes Transcription Factors

**Adipogenic**

- C/EBPα
- PPARγ

**Lipogenic**

- SREBP1c

Increased Adipocyte Differentiation and Lipogenesis
Fetal Programming of Obesity: Effect of BPA

Fetal BPA Exposure

Appetite

Adipose mass

Fetal BPA Exposure to the fetus can lead to changes in appetite and increase adipose mass, contributing to the programming of obesity.
Conclusions

• *In utero* exposures altered nutrition and/or environmental agents may have marked effect on children and grandchildren

  • Need to refocus environmental agent effects beyond toxicity
  • Animal studies to explore mechanisms
  • Correlation of animal and human effects
  • Longer term human studies

• In the meantime, “all things in moderation”