DEVELOPMENTAL FACTORS IN ADULT OBESITY: DIET, ENVIRONMENTAL CHEMICALS AND IMPORTANCE OF ANIMAL MODELS

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University of Missouri-Columbia, USA

Bisphenol A

Funded by research grants from the National Institutes of Health (NIH)
IUGR  
(Premature)  
(Maternal Type II diabetes)  
(Reduced placental blood flow)  

MACROSOMIA
Proposed factors influencing induction of IUGR
Babies that are overweight at birth are at risk for adult obesity and metabolic disease.

Some babies with intrauterine growth restriction (IUGR) are at risk for a rapid period of postnatal weight gain, adult obesity and metabolic disease.

**THRIFTY PHENOTYPE HYPOTHESIS**

<table>
<thead>
<tr>
<th>Birth</th>
<th>Body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrosomia</td>
<td>IUGR</td>
</tr>
<tr>
<td>Normal</td>
<td>IUGR</td>
</tr>
</tbody>
</table>
EXPERIMENTAL APPROACHES FOR ANIMAL RESEARCH ON THE FETAL BASIS OF METABOLIC SYNDROME

Treatment of Pregnant Females

- Caloric restriction
- Protein restriction
- High fat diet
- Exercise
- Dexamethasone administration
- Knockout mice (e.g. 11β-HSD)
- Environmental chemicals - PLASTIC
Even et al. 1994
J. Reprod. Fertil.
<table>
<thead>
<tr>
<th>Uterine Position</th>
<th>Placental Blood Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>O 1</td>
<td>31.5</td>
</tr>
<tr>
<td>O 2</td>
<td>33.1</td>
</tr>
<tr>
<td>O 3</td>
<td>20.6</td>
</tr>
<tr>
<td>M-2</td>
<td>20.8</td>
</tr>
<tr>
<td>M-1</td>
<td>9.3</td>
</tr>
<tr>
<td>M 0</td>
<td>18.8</td>
</tr>
<tr>
<td>M 1</td>
<td>27.3</td>
</tr>
<tr>
<td>M 2</td>
<td>29.7</td>
</tr>
<tr>
<td>C 3</td>
<td>31.3</td>
</tr>
<tr>
<td>C 2</td>
<td>43.5</td>
</tr>
<tr>
<td>C 1</td>
<td>366</td>
</tr>
</tbody>
</table>

RADIOLABELED MICROSPHERES

Kirkpatrick et al
Unpublished
PERCENT INCREASE IN BODY WEIGHT DURING THE 7 DAYS AFTER WEANING IN MALE MICE
N = 294 pups
PERCENT INCREASE IN BODY WEIGHT DURING THE 7 DAYS AFTER WEANING IN FEMALE MICE
FEMALE BODY WEIGHT

7 Weeks Old

4 Weeks Old

3 Weeks Old (Weaning)

Day of Birth

Grams

N = 287 pups
GLUCOSE TOLERANCE IN MALE MICE

Coe et al. unpublished
COULTER COUNTER ANALYSIS OF FAT CELL DIAMETER IN MALE MICE

Coe et al.
unpublished

* indicates difference between High and Low birth wt groups (P<0.05)
ADULT EPIDIDYMIDAL ADIPOCYTE GENE EXPRESSION: COMPARISON OF MALES CATEGORIZED WITH IUGR AND MACROSOMIA AT BIRTH

Taylor et al. unpublished
ADULT EPIDIDYMIDAL ADIPOCYTE GENE EXPRESSION: COMPARISON OF MALES CATEGORIZED WITH IUGR AND MACROSOMIA AT BIRTH

Taylor et al. unpublished
IUGR and Macrosomia Result in Differences in Adult Obesity

Hundreds of Genes in Epididymidal Adipocytes Differ in Activity

Affymetrix Mouse Genome 430 Array
Compensatory Ovulation Following Hemiovariectomy

- High risk - Heavy
- Optimum
- High risk - Light
- Optimum
- High risk - Heavy

Blood flow

- Descending aorta
- Ovariectomy
- Uterine loop artery
- Uterus
- Internal iliac artery
EXPERIMENTAL DESIGN

Randomly assign adult CD-1 mice to be fed different mouse feeds

1. **Purina 5008**: Pregnancy and lactation chow
   - **Purina 5001**: Post-weaning chow
     - soy based rodent feeds
     - high levels of phytoestrogens: genistein and daidzein

2. **Purina 5K96C**
   - casein based feed
   - undetectable phytoestrogens (MCF-7 cells)

Examine offspring at different ages
Serum Estradiol (pg/ml)

Maternal Female pup Male pup

Casein-based 5K96C Low Phytoestrogens Stimulates endogenous Estradiol

Ruhlen et al. Submitted
EFFECT OF FEED ON BODY WEIGHT IN FEMALE MICE

Ruhlen et al.
Submitted

5008/1
5K96C
SERUM LEPTIN IN ADULT MICE

Ruhlen et al.
Submitted
ESTROGENIC ACTIVITY IN DIFFERENT LOTS OF CASEIN-BASED PURINA 5K96C FEEDS
MCF-7 CELL BIOASSAY

Welshons and Taylor unpublished

CV = 84%

Purina 5008

HIGH

LOW

PPM Genistein Equivalents

Welshons and Taylor unpublished
2-MONTH OLD CD-1 FEMALE MICE

GONADAL FAT

Weight (mg)

NC 5K96C  Cont  5008/1

SERUM LEPTIN

Serum Leptin (ng/ml)

NC 5K96C  Cont  5008/1

* indicates statistical significance.
USES OF BISPHENOL A IN PRODUCTS
Production Capacity > 6.5 Billion Pounds / Year

Polycarbonate Bottles
- The premier bottle, #7 recycle code on the bottom is your guarantee of quality!
- Glass-like, non-porous material
- NO Plastic Leaching
- NO Dioxin Leaching
- Durable
- No Heat or Cold distortion

Manual Water Bottle Pump
- Fits most 2, 3 or 5 gal. bottles
- Dispenses water easily with just a light touch

America's Leading Water Products Wholesaler: 800.592.8371
www.newwaveenviro.com
BISPHENOL A LEACHES FROM NEW POLYCARBONATE BABY BOTTLES

Taylor, vom Saal, Welshons and Rottinghaus (unpublished)
NATURAL AND MANMADE ESTROGENS

POLYCARBONATE PLASTIC & RESIN
Bisphenol A

NATURAL HORMONE
Estradiol

ESTROGENIC DRUG
Diethylstilbestrol (DES)
INTRAUTERINE POSITION INFLUENCES SERUM ESTRADIOL AND TESTOSTERONE IN MALE MOUSE FETUSES

F. vom Saal

<table>
<thead>
<tr>
<th>Position</th>
<th>E_2 (pg/ml)</th>
<th>T (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2M</td>
<td>78</td>
<td>5.7</td>
</tr>
<tr>
<td>1M</td>
<td>94</td>
<td>4.6</td>
</tr>
<tr>
<td>2F</td>
<td>101</td>
<td>4.2</td>
</tr>
</tbody>
</table>

E_2 = 78 pg/ml
T = 5.7 ng/ml

E_2 = 94 pg/ml
T = 4.6 ng/ml

E_2 = 101 pg/ml
T = 4.2 ng/ml
PRENATAL BISPHENOL A EXPOSURE INCREASES BODY WEIGHT AT WEANING IN MICE WITH ELEVATED FETAL ESTROGEN LEVELS

Maternal BPA Oral Dose: 2 µg/kg/day, GD 11-17

High Medium Low Fetal Estradiol

Bisphenol A activates cAMP Responsive Element Binding Protein (CREB) at Human Exposure Levels (95% of Americans)

Calafat et al. 2005; Zhang et al. 2003; Quesada et al. 2003
Gene in differentiating fat cell

Promoter mRNA production site DNA sequence

P-CREB
EMBRYONIC EXPOSURE TO BISPHENOL A INCREASES POSTNATAL GROWTH RATE IN MICE

Wean Weight

Birth Weight

CONTROL

BISPHENOL A
1 nM (228 pg/ml)

~4 pups / litter

Takai et al., 2000
BISPHENOL A STIMULATES INSULIN SECRETION IN PANCREATIC β CELLS IN MICE

All Manmade Chemicals and Bisphenol A: Production Parallels the Global Obesity Epidemic

Modified from: Baillie-Hamilton, 2002
# BISPHENOL A  
(as of December 2007)  
EVIDENCE FOR BIAS DUE TO SOURCE OF FUNDING IN RELATION TO REPORTED OUTCOME IN RESEARCH WITH ANIMALS

<table>
<thead>
<tr>
<th>SOURCE OF FUNDING</th>
<th>STUDY OUTCOME</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HARM</td>
<td>NO HARM</td>
<td></td>
</tr>
<tr>
<td>Government</td>
<td>153 (92%)</td>
<td>14 (8%)</td>
<td>167</td>
</tr>
<tr>
<td>Chemical Corporations</td>
<td>0 (0%)</td>
<td>13 (100%)</td>
<td>13</td>
</tr>
</tbody>
</table>

vom Saal and Hughes, 2005  
Environ. Health Perspect.  
Also > 180 studies of molecular mechanisms
BIASED OUTCOME DUE TO SOURCE OF FUNDING IN LOW-DOSE BISPHENOL A RESEARCH (as of December 2005)

STUDIES THAT USED THE ESTROGEN-INSENSITIVE CHARLES RIVER SPRAGUE-DAWLEY (CD-SD) RAT

<table>
<thead>
<tr>
<th>SOURCE OF FUNDING</th>
<th>REPORTED STUDY OUTCOME</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HARM</td>
<td>NO HARM</td>
</tr>
<tr>
<td>Government</td>
<td>0 (0%)</td>
<td>7 (100%)</td>
</tr>
<tr>
<td>Chemical Corporations</td>
<td>0 (0%)</td>
<td>3 (100%)</td>
</tr>
<tr>
<td></td>
<td>0 (0%)</td>
<td>10 (100%)</td>
</tr>
</tbody>
</table>

vom Saal and Hughes, 2005 Environ. Health Perspect.
Reported low-dose effects have not been replicated in repeat studies conducted in independent laboratories. The vast majority of available data shows no low dose effect whatsoever.
A scientific consulting firm says that it aids companies in trouble, but critics say that it manufactures uncertainty and undermines science.

“We will harness, focus and involve the scientific and intellectual capital of our company with one goal in mind — creating the outcome our client desires.”
The FDA sees no reason to ban bisphenol A.

The FDA approved bisphenol A for use in food and beverage containers in 1963. The standard was that bisphenol A was Generally Regarded As Safe (GRAS), since it had been in use for a few years and there was no evidence of harm.

George Pauli, Ph.D.
Associate Director for Science and Policy
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
PUBLISHED STUDIES SINCE 1997 REPORTING LOW-DOSES EFFECTS OF BISPHENOL A IN MICE AND RATS DURING DEVELOPMENT

- 17 = Altered brain structure & chemistry
- 21 = Altered behavior
- 5 = Increased prostate size and cancer
- 9 = Lowered sperm production
- 6 = Altered adult hormone levels
- 7 = Abnormal mammary glands
- 3 = Early puberty in females
- 7 = Increased subsequent body weight
- 2 = Altered immune system
HUNDREDS OF PUBLISHED SCIENTIFIC STUDIES SHOW:

1) High exposure is occurring: fetuses - adults.
2) Developing fetuses and babies are more sensitive to BPA than adults.
3) BPA is an endocrine disruptor that causes a wide range of abnormalities in animals.
4) **Alternative plastics that are much more stable and safer than BPA are available.**

SHOULD BISPHENOL A (BPA) BE BANNED IN BABY PRODUCTS AND FOOD AND BEVERAGE CONTAINERS?
# STUDENTS AND COLLABORATORS

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