Xenobiotic Obesogens: Organophosphate pesticides and the Obesity Epidemic.

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Xenobiotics and environmental exposures – a role in obesity?

- Obesity and energy balance
- Fetal programming
- Xenobiotics as obesogens?
  - Organophosphate pesticides
  - Bisphenol A
  - phthalates
  - organotins
  - Perfluoroalkyl acids

Picture from: Geoff Price “Understanding Capitalism Part IV: Capitalism, Culture and Society” Feb 4, 2005 @ rationalrevolution.net
\[ E \text{ intake} \quad \text{E expenditure} \]

- \( E_{\text{in}} \approx E_{\text{exp}}. \)
  - Weight neutral
- \( E_{\text{in}} > E_{\text{exp}}. \)
  - Obesity
- \( E_{\text{in}} \approx E_{\text{exp}}. \)
  - Obesity

Environmental/genetic influences
Morbid Obesity due to Leptin Signaling Defects

Ob⁻/Ob⁻ leptin deficient

LRb T1138S leptin receptor defect
Leptin Deficits
Farooqi and O’Rahilly

- Leptin deficiency
  - Early onset morbid obesity
  - High fat mass
  - Infertility
  - T-cell defects recurrent infections
  - Rare: autosomal recessive
  - Readily diagnosed
  - Rx with recombinant leptin

- Leptin receptor deficiency
  - Milder phenotype
  - No specific Rx
Defects in MC4R Receptor

Farooqi and O’Rahilly

- Loss of α-MSH mediated anorexia

Phenotype
- Hyperphagia
- Accelerated linear growth
- Increased bone density
- ↑ adipose and lean tissue mass

Multiple alleles
- Codominant
- Homozygous > heterozygous

Prevalence
- ≈ 6% in severe childhood onset obesity
- 1/2000 general population

Dx/Rx
- DNA sequence from WBC
- Athena Diagnostics - $810
- No specific treatment

Figure 3 Clinical phenotype of MC4R deficiency. MC4R mutations result in a dominantly inherited obesity syndrome. Left: 9-year-old with MC4R mutation. Right: 16-year-old sibling with normal MC4R.
E \textit{intake} \quad \text{E \textit{expenditure}}

- \( E \text{ in} \approx E \text{ exp.} \)
  - Weight neutral
- \( E \text{ in} > E \text{ exp.} \)
  - Obesity
- \( E \text{ in} \approx E \text{ exp.} \)
  - Obesity

Environmental/genetic influences
Higher risk adult diseases with lower birth size

- Standard mortality ratio for deaths from CVD
- Prevalence IGT or DM
- % insulin resistant
- LDL (mmol/l)
Maternal nutrition imbalance and programming of the metabolic syndrome

- Thrifty phenotype hypothesis
  - Nutritional imbalances in utero foreshadow postnatal deprivation
  - Fetal phenotype is “programmed” for efficiency
    - AKA: adaptive response
  - Mismatch between pre/post natal environment results in disease

- ↓ birth weight and catch up growth associated with ↑ risk of central adiposity and the metabolic syndrome
  - Dutch famine vs. siege of Leningrad in WWII
    - Present in all ethnicities examined
  - Animal models
    - Placental insufficiency and hypoxia
    - Caloric restriction
    - Macro or micronutrient restriction
    - Macronutrient imbalances
### In utero programming and post-natal susceptibility to obesogenic diets.

<table>
<thead>
<tr>
<th>Prenatal exposure (maternal diet)</th>
<th>Postnatal exposure</th>
<th>“cafeteria diet”</th>
</tr>
</thead>
<tbody>
<tr>
<td>chow</td>
<td>chow</td>
<td>normal</td>
</tr>
<tr>
<td>low protein</td>
<td>↓ wt, fat mass</td>
<td>↑ wt, fat mass</td>
</tr>
<tr>
<td>chow</td>
<td>chow</td>
<td>high fat</td>
</tr>
<tr>
<td>low calorie</td>
<td>+/- ↓ wt, fat mass</td>
<td>↑ wt, fat mass</td>
</tr>
<tr>
<td>chow</td>
<td>chow</td>
<td>high fat</td>
</tr>
<tr>
<td>high fat</td>
<td>↑ wt, fat mass</td>
<td>↑↑ wt, fat mass</td>
</tr>
</tbody>
</table>
Maternal Obesity @ Conception Programs
Obesity in the Offspring
Shankar K et al, Am J Physiol Regul Interg Comp Physiol 2008;294:R528-38
Maternal Obesity @ Conception Programs

Obesity in the Offspring

Shankar K et al, Am J Physiol Regul Interg Comp Physiol 2008;294:R528-38
Excessive Gestational Weight Gain and Child’s Weight Status

<table>
<thead>
<tr>
<th>Birth Wt. Status</th>
<th>Inadequate</th>
<th>Adequate</th>
<th>Excessive</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGA &lt;10&lt;sup&gt;th&lt;/sup&gt;</td>
<td>22%</td>
<td>50%</td>
<td>28%</td>
</tr>
<tr>
<td>AGA 10-90&lt;sup&gt;th&lt;/sup&gt;</td>
<td>14%</td>
<td>36%</td>
<td>50%</td>
</tr>
<tr>
<td>LGA &gt; 90&lt;sup&gt;th&lt;/sup&gt;</td>
<td>7%</td>
<td>26%</td>
<td>66%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Child BMI @ age 3</th>
<th>85-94th</th>
<th>≥ 95th</th>
</tr>
</thead>
<tbody>
<tr>
<td>85-94th</td>
<td>1.0</td>
<td>3.8</td>
</tr>
<tr>
<td>≥ 95th</td>
<td>2.09</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Adult fat mass is determined by both adipocyte size and number


![Graphs showing the relationship between body fat mass and fat cell volume for subcutaneous and visceral fat in females and males.](image)
Number of adipocytes is determined during childhood/adolescence and is stable in adulthood even with significant weight loss.
Adipocyte hyperplasia

- Adipocyte hyperplasia (number expansion) begins earlier in obese subjects
  - 5.7 vs. 2.1 years of age
- Degree of hyperplasia higher in obese subjects
- Number, but not volume, of adipocytes fixed by early adulthood
- Adipocyte differentiation/recruitment potential mode of action for obesogens?
  - Thiazolidinediones as proof of concept
    - Weight gain (subcutaneous fat) associated with pioglitazone and rosiglitazone stimulation of PPARγ and adipocyte differentiation
Effect of maternal smoking during pregnancy on child overweight (n= 84,563, @ 3-33 years of age)

![Graph showing odds ratios for maternal smoking and child overweight by age and sex]
Maternal Genistein Consumption Promotes Hyper-methylation and in utero programming. 

Dolinoy et al, *EHP* 2006;1141:567
Ecological Systems Theory Model
Davison KK, Birch LL *Obes Rev* 2001;2:159-71

**Energy Intake**
- Essential calories
- Discretionary calories

**Energy Expenditure**
- Basal metabolism
- Processing food
- Maintenance/repair
- Growth
- Physical activity

**Genetic factors**
- Epigenetic/Metabolic factors

**Food and Beverage Intake**

**Physical Activity**

**Patient**

**Family/Home**

**School/Peers**

**Community**

**Media/Govt./Industry**

**Culture/Society**

**Ecological Systems Theory Model**
Synthetic chemical production and overweight in the US


Chemical production and % Adults overweight
Synthetic compounds reported to promote weight gain


- Pesticides
  - Organophosphates
  - Organochlorines
  - Carbamates
- Polychlorinated/brominated biphenyls
- Perfluoralkyl acids
- Components of plastics
  - Bisphenol-A
  - Phthalates
- Metals
  - Cadmium, lead, organotins
- Solvents
Effect of chronic chlorpyrifos exposure on growth of female Long-Evans rats (5 mg/kg day\(^{-1}\), \(\approx 5\% \text{ LD50}\))

Meggs, Brewer and Collier
Effect of chronic chlorpyriphos exposure on organ weights in rats

![Bar chart showing the comparison of organ weights between control and OP-exposed groups.](image-url)
TNF-alpha, leptin and insulin levels in rats after 2 weeks of continuous exposure to chlorpyriphos
Effect of chlorpyrifos on pre-adipocyte differentiation in vitro

MDI (stimulates differentiation)

MDI + DMSO (vehicle)

MDI + DMSO + chlorpyrifos @ 0.004 mg/ml
Chlorpyriphos exposure up-regulates PPAR\(_{\gamma}\) expression in adipose tissue

*Figure 1*

**PPAR\(_{\gamma}\)**

**\(52 \text{kDa}\)**

**\(60 \text{kDa}\)**

**Control**

**OP Exposed**

1 4 5 15 16 17

**\(\beta\)-Tubulin**

52 kDa

60 kDa

*Figure 1*
Perinatal Exposure to Chlorpyrifos (1 mg/kg) Results in Dyslipidemia in Adult Off-Spring
Slotkin, Brown, Seidler EHP 2005;113:1291-94
Perinatal Exposure to Chlorpyrifos (1 mg/kg) Results in Insulin Resistance in Adult Off-Spring
Slotkin, Brown, Seidler EHP 2005;113:1291-94
Is the Endocannabinoid System Responsible for Organophosphate Mediated Obesity?

Arcuate Nucleus

NPY
AgRP

POMC
α-MSH

Paraventricular Nucleus

orixeginic

MCH

Orexins

anorixeginic

Endocannabinoids

Fatty acid amide hydrolase (FAAH)

X

organophosphates

anandamide

2-arachidonylglycerol

Monoacyl glycerol Lipase (MAGL)
Acknowledgements

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  - Kori Brewer PhD
  - Bill Meggs MD, PhD
  - Phil Pekala PhD
  - David Collier MD, PhD
  - Garrett Franklin BS
  - Brian Whitfield BS
Bisphenol A
vom Saal and Hughes EHP 2005;113:926-33

- Building block of polycarbonate
  - Bottles, can liners, dental resins
  - 6.4 billion pounds/year
- Readily leaches
  - Extremes of pH, temperature and age, detergents
- Potent “endocrine disruptor”
  - Gestational exposure (maternal consumption of ppb):
    - permanent androgen mediated prostate hypertrophy in rodents
    - Decreased sperm count
    - aneuploidy
    - Intraductal hyperplasia and carcinoma in situ in rats
- Other effects
  - Cognitive-behavioral
    - Excessive aggression in males
    - Hyperactive/inattentive
Bisphenol A
vom Saal and Hughes EHP 2005;113:926-33

- **Common in humans**
  - 95% of urine samples +
  - Maternal/fetal plasma, placenta, breast milk
  - Amniotic fluid levels 5X maternal plasma
  - Biologically relevant concentrations
    - Median BPA levels in humans exceed levels required to cause adverse effects in mice
    - 94/115 published in vivo studies demonstrate biological effects at low levels (below the LOAEL of 50 mg/kg/day)
    - 40/115 show effects below reference value (50 ug/kg/d)
LOAEL vs. Reference Dose for BPA (mg/kg day $^{-1}$)

Reference Dose (≈ safe)        Lowest Observed Adverse Effect Level (LOAEL)
Toxic and endocrine disruption dose response curves for BPA are distinct.

Reference Dose (≈ safe)  Lowest Observable Adverse Effect (LOAE)
BPA accelerates the differentiation of 3T3 cells into adipocytes

Maternal exposure to BPA

- Oral exposure (50mg/kg) in dams
- Shifts coat color of offspring to yellow
- Shifts offspring towards obese
- Associated with hypo-methylation of target DNA
- Shows that in-utero exposure to xenobiotic influences chemical modification and expression of DNA

Dolinoy, Huang, Jirtle PNAS 2007
Effect of in utero exposure to bisphenol-A on weight gain in offspring (rats)

Rubin et al. 2001 Env Health Perspect 109;675-80
“Chronic” exposure to bisphenol-A induces insulin resistance and glucose intolerance (Alonso-Magdalena P, Env Health Pers 2006;114:106-12)

- 4 day oral intake
- 100 μg/kg/day
- Only 2X U.S. EPA’s reference dose and 1/500th LOAEL
Rates of BPA leaching from off the shelf baby bottles (24 hrs @ 80\degree C)

Figure 4: Bisphenol A Extracted from Polycarbonate Baby Bottles
Phthalates

- Commonly used (diesters of phthalic acid)
  - Plasticizers that are non covalently bound to substrates
  - Plastics, cosmetics, shampoos, toys, pacifiers, meds, blood bags
  - Common in people
    - 75% US adults ≥ 5 urinary metabolites
    - Present in breast milk, formula
    - NICU infants

- Known endocrine disruptors
  - Anti-androgeneric in fetus
  - Levels in cord blood and breast milk positively associated with:
    - cryptorchidism and hypospadius
    - reduced sperm count and testosterone levels are correlated

- Low testosterone in men associated with:
  - Obesity
  - Insulin resistance
  - Diabetes
The normal testis, left, comes from a healthy male rat. The right tissue comes from a rat exposed to DEHP, a type of phthalate, while its reproductive tract was maturing. It is a small testis filled with fluid. The corresponding tissue from the other side of the same animal exhibits no visible testis or sperm-storing epididymis.
Waist circumference, insulin resistance (HOMA) and urinary levels of phthalate metabolites

Stahlhut et al 2007 (poster)

Data from NHANES
1292 adult ♂
Similar relationship for 5 different metabolites
[Phthalate metabolite] positively associated with obesity and insulin resistance
Figure 1. Concentration of six phthalate monoesters (μg/L) in human breast milk samples from Denmark \((n = 65)\) and Finland \((n = 65)\), 1997–2001, collected between 1 and 3 months postnatally as additive aliquots. Data are given as percentile distribution. (A) mMP, (B) mEP, (C) mBP, (D) mBzP, (E) mEHP, (F) miNP.
Organotins

- Organic derivatives of tin (e.g. tributyltin)
- Antifungals, wood preservatives, PVC products
- Potent activators (1-10 nM) of:
  - PPARγ receptor (target of thiazolidinediones)
  - Retinoid X receptor
  - Adipocyte differentiation
- In utero exposure in animals:
  - Increased ectopic fat deposition in offspring
  - Hepatic steatosis
- Levels in human samples
  - Range 3-100 nM
  - Mean 27 nM
  - Low transfer to breast milk
Lead (in utero)

10 days old

Effect noted in:
Male rats only
Greater at low dose

1 year old

Why?

- Marked decrease in spontaneous locomotor activity for male rats.

Perfluoroalkyl Acids (PFAAs)

Environmental Health Perspectives  May 2007 115:A251-256

1. PFAAs
   - Surfactants/components in > 200 applications
     - Water/stain repellents

2. Perfluorooctanoic acid (PFOA)
   - Teflon etc

3. Perfluorooctanoyl sulfonate (PFOS)
   - Scotchguard, Stainmaster
   - Long t½ in humans (4-7 yrs!)

Sources
- Food
  - Wrappers – eg. Microwave popcorn!
- Cook-ware
- Soil/dust
  - DuPont sites in NC
- Water
- Breast milk
Gestational Exposure to PFOA (Teflon etc.) and Body Composition at 80 Weeks

Environmental Health Perspectives  May 2007  115:A251-256
A Role for Perfluorinated Chemicals (PCAs) in Human Obesity and Metabolic Disease?

- **Ubiquitous**
  - > 90% adults have PCAs
    - PFOS @ 30.4 ppb
    - PFOA @ 5.2 ppb

- **Cord blood**
  - ≈ 10% maternal serum levels
  - 100% with PFOS
  - 99% with PFOA

- **Breast milk**
  - ≈ 1% maternal serum levels
  - 200 ngm/day to infant

- **Correlations**
  - “Dose dependent” decrease in birth weight and cord blood levels
    - - 69 gms for PFOS
    - - 104 gms for PFOA

Apelberg et al., *EHP* 2007 115;1670-1676
Neutral/negative
- E in ≈ E exp.
- Weight neutral

Positive
- E in > E exp.
- Obesity
- Obesity due to programming
A perfect storm?
Recommendations to minimize risk

- Avoid polycarbonate food packaging (bisphenolA)
  - Hard, clear - labeled as “PC” or #7,
  - # 1, 2, 5 aren’t sources of bisphenolA
  - If polycarbonate is used:
    - Avoid washing at high temperatures
    - Avoid harsh detergents
    - Avoid scratching
    - Discard if cloudy or crazed

- Avoid polycarbonate baby bottles
  - Leaching accelerates after 50-100 washes
  - Select glass or polypropylene (softer more opaque)
Recommendations to minimize risk

- Never heat food in plastic containers
  - Microwave in ceramic or glass containers
  - Don’t let cling wrap touch food – use paper towel
- Avoid micro waving in grease or water resistant paper packaging (perfluoroalkyl acids)
  - Microwave popcorn, pot pies etc.
- Avoid food in plastic wrap
  - Pliable cling wrap contains phthalates as plasticizer
  - Especially avoid meats and cheeses in cling wrap
    - Buy @ deli and have wrapped in paper (PFAAs??)
  - Cut away portion in contact with wrap
Recommendations to minimize risk

- Avoid canned foods (BPA from lining)
  - Single soda per day ↑ odds of obesity
  - Glass, frozen or fresh
- Avoid ready to feed and concentrated formula in cans (BPA)
- Choose organic food options (OP pesticides)
- Avoid smoking
  - Nicotine replacement contraindicated in pregnancy
- Use filtered tap water
  - Activated carbon filter
  - Don’t reuse bottled water bottles
Recommendations to minimize risk

- Look for PVC-free products (organotins, phthalates)
  - Consider high quality wooden toys
    - Plan Toys, Turner Toys, Haba, Selecta, Holztiger
- Limit infant care products and unless medically necessary avoid lotions and powders
  - Look for “phthalate free” products
- Avoid scented products and cosmetics
  - Cologne, lotion, personal care products
- Avoid scented household products (phthalates)
  - Glade plug ins etc. ??
- Minimize inhalation and hand-mouth ingestion
  - Vacuuming
  - Routine filter maintenance
Energy Expenditure

Energy Intake

Food and Beverage Intake

Physical Activity

Genetic factors
Epigenetic/Metabolic factors

Energy Expenditure
Basal metabolism
Processing food
Maintenance/repair
Growth
Physical activity

Essential calories
Discretionary calories

Culture/Society

Media/Govt./Industry

Community

School/Peers

Family/Home

Patient

Davison KK, Birch LL Obes Rev 2001;2:159-71
Regulation of Gene Expression through Epigenetic Processes