Obesogens, Stem Cells and the Maternal Programming of Obesity

Bruce Blumberg, Ph.D.

Department of Developmental and Cell Biology
Department of Pharmaceutical Sciences
Developmental Biology Center
University of California, Irvine
The Worldwide Obesity Epidemic

- 34% of the US population are clinically obese (BMI > 30)
  - Double worldwide average (Flegal et al. JAMA 2010;303:235-241)

- 68% are overweight (BMI > 25)
Obesity Trends* Among U.S. Adults
(*BMI ≥30, or about 30 lbs. overweight for 5’4” person)
Obesity In European Adults

With the limited data available, prevalences are not age standardised & data are not always directly comparable. The illustrations above are to give an impression of the changes that have taken place over the last 20 years. Self reported surveys (illustrated with dots) may underestimate true prevalence. Sources and references are available from obesity@iaso.org. © International Association of Obesity, London May 2009.
The Worldwide Obesity Epidemic

- 34% of the US population are clinically obese (BMI > 30)
  - Double worldwide average (Flegal et al. JAMA 2010;303:235-241)
- 68% are overweight (BMI > 25)
How does obesity occur?

- Prevailing wisdom – “couch potato syndrome” – Positive energy balance, i.e., too much food, too little exercise

- How does obesity occur?
  - Are there other factors in obesity?
    - Stress (elevated glucocorticoids)
    - Inadequate sleep (stress?)
    - “Thrifty” genes which evolved to make the most of scarce calories
    - Viruses, gut microbes, SNPs

- What about role of prenatal nutrition or in utero experience?
  - Southampton studies
    - Maternal smoking decreases birth weight and increases obesity

- What about the role of industrial chemicals in rise of obesity?
  - Baillie-Hamilton (2002) postulated a role for chemical toxins
  - Obesity epidemic roughly correlates with a marked increase in the use of chemicals (plastics, pesticides, etc.)
  - Many chemicals have effects on the endocrine system

![Graph showing the production of synthetic organic chemicals and the percentage of overweight adults in the United States during the twentieth century.](image)
Hormonal control of weight

- Hormonal control of appetite and metabolism
  - Leptin, adiponectin, ghrelin are key players
  - Leptin, adiponectin - adipocytes
  - Grehlin - stomach
  - Thyroid hormone/receptor
    - Sets basal metabolic rate

- Hormonal control of fat cell development and lipid balance
  - Regulated through nuclear hormone receptors RXR, PPARγ
  - PPARγ - master regulator of fat cell development
    - increased fat cell differentiation
    - Increased fat storage in existing cells
    - Increased insulin sensitivity

Endocrine Disrupting Chemicals (EDCs)

- *Endocrine disrupter* - a compound that mimics or blocks the action of endocrine hormones, either directly or indirectly
  - Often persistent pollutants or dietary components that disturb development, physiology and homeostasis

- Details scientific support for existence and effects of EDCs
  - Endorsed by American Medical Association
  - Led to H.R. 4190 - Endocrine Disruption Prevention Act of 2009

- Are EDC-mediated disturbances in endocrine signaling pathways involved in adipogenesis and obesity
EDCs and the obesogen hypothesis

- **Obesogens** - chemicals that inappropriately stimulate adipogenesis and fat storage, disturb adipose tissue homeostasis, or alter control of appetite/satiety to lead to weight gain and obesity

- Pre- and postnatal exposure to EDCs such as environmental estrogens (ER) increases weight
  - DES, genistein, bisphenol A

- Thiazolidinedione anti-diabetic drugs (PPARγ)
  - Increase fat storage and fat cell number at all ages in humans

- Urinary phthalates correlate with waist diameter and insulin resistance in humans

- several compounds cause adipocyte differentiation in vitro (PPARγ)
  - phthalates, BPA, akylyphenols, PFOA, organotins

- Existence of obesogens is plausible
Endocrine disruption by organotins

- Organotins -> imposex in mollusks
- Sex reverses genetically female flounder and zebrafish -> males
- Which hormone receptors might be organotin targets?

- We found that tributyltin (TBT)
  - Binds and activates at ppb (low nM) two nuclear receptors, RXR and PPARγ critical for adipogenesis
  - TBT induced adipogenesis in cell culture models (nM)
  - Prenatal TBT exposure led to weight gain in mice, in vivo

\[ \text{Tributyltin-Cl} \]

\[ \text{Sn}^+ \text{Cl} \]
### Nuclear receptor activation by organotins

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Nuclear Receptor LBD EC&lt;sub&gt;50&lt;/sub&gt; nM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hRXRα</td>
</tr>
<tr>
<td>LG268</td>
<td>2-5</td>
</tr>
<tr>
<td>AGN203</td>
<td>0.5-2</td>
</tr>
<tr>
<td>9-&lt;i&gt; cis&lt;/i&gt; RA</td>
<td>na</td>
</tr>
<tr>
<td>all-&lt;i&gt;trans&lt;/i&gt; RA</td>
<td>na</td>
</tr>
<tr>
<td>Butyltin chloride</td>
<td>na</td>
</tr>
<tr>
<td>Dibutyltin chloride</td>
<td>3000</td>
</tr>
<tr>
<td>Tributyltin chloride</td>
<td>3-8</td>
</tr>
<tr>
<td>Tetrabutyltin chloride</td>
<td>150</td>
</tr>
<tr>
<td>Di(triphenyltin) oxide</td>
<td>2-10</td>
</tr>
<tr>
<td>Butyltin-tris (2-ethylhexanoate)</td>
<td>na</td>
</tr>
<tr>
<td>Troglitazone</td>
<td>na</td>
</tr>
</tbody>
</table>
Prenatal TBT exposure causes permanent physiological changes resulting in predisposition to weight, despite normal diet and exercise.

Grun et al., unpublished
How does TBT exposure cause weight gain?

- Changes in the hormonal control of appetite and satiety?
- Altered ability of adipocytes to process and store lipids?
- Increased number of adipocytes or pre-adipocytes?
- Mesenchymal stem cells (MSCs) (now called multipotent stromal cells) precursors to many lineages including bone, cartilage, and adipose.
  - MSCs differentiate into adipocytes following rosiglitazone exposure
  - MSCs may (or may not) home to adipose depots after induction

**Hypothesis:** TBT induces adipogenesis in MSCs
Multi-lineage analysis of MSC potential

<table>
<thead>
<tr>
<th>Undifferentiated mADSCs gene expression profile</th>
<th>Positive cellular markers and genes</th>
<th>Negative cellular markers and genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSCs</td>
<td>CD90, CD105, Sca1</td>
<td>CD19, CD31, CD79a, c-kit</td>
</tr>
<tr>
<td>Hematopoietic lineage</td>
<td>CD34, CD68</td>
<td></td>
</tr>
<tr>
<td>Adipogenic lineage</td>
<td>Pref-1, aP2, PPARγ (+/-)</td>
<td>LEP, ADIPOQ</td>
</tr>
<tr>
<td>Osteogenic lineage</td>
<td>ALP (+/-), OPN</td>
<td>CST</td>
</tr>
<tr>
<td>Chondrogenic lineage</td>
<td>COL II, COL X (+/-)</td>
<td>ACAN</td>
</tr>
</tbody>
</table>

+ adipogenic media (14D) | + osteogenic media (21D) | + chondrogenic media (21D)
TBT induces adipogenic differentiation in MSCs

Kirchner et al., 2010 Molec Endocrinol, 24, 526-539
TBT induces adipogenic genes in MSCs

Kirchner et al., 2010 Molec Endocrinol, 24, 526-539
Adipogenic effects of TBT and ROSI require PPARγ

Kirchner et al., 2010 Molec Endocrinol, 24, 526-539
Osteogenic capacity of hADSCs

TBT overrides the effects of the bone-inducing cocktail, instead causing the cells to become adipocytes.

Kirchner et al., 2010 Molec Endocrinol, 24, 526-539
Effects of TBT on cultured MSCs

- TBT increases the amount of adipocyte differentiation in ADSCs
  - Increased number of cells with lipid
  - Increased amount of lipid stored in cells
  - Decreased expression of adipogenesis inhibitor Pref-1
  - Increased expression of pre- and adipocyte markers

- Adipogenic effects of TBT and ROSI require PPARγ
  - TBT and ROSI rescue effects of PPARγ antagonist
  - TBT acts through PPARγ

- TBT inhibits ability of osteogenic cocktail to induce ADSCs to become adipocytes

- What is the effect of prenatal exposure on ability of ADSCs to differentiate into adipocytes or other lineages?
In vivo assays to assess stem cell commitment

E16 – chemical exposure by gavage

CMC, TBT, ROSI

C57BLK6 - Pregnant dam
CD-1 unexposed surrogate

in utero exposed offspring

BM, WAT

mBMSCs, mADSCs

+DMSO, TBT or ROSI

Adipocyte differentiation conditions
Bone differentiation conditions
Cartilage differentiation conditions
Prenatal TBT exposure increases MSC differentiation into adipocytes

Kirchner et al., 2010 Molec Endocrinol, 24, 526-539
In utero TBT exposure inhibits osteogenesis

- Prenatal TBT exposure predisposes MSCs to become adipocytes at the expense of their ability to form osteocytes
- Prenatal TBT exposure inhibits calcium, and enhances lipid deposition
- What is the mechanism?

Kirchner et al., 2010 Molec Endocrinol, 24, 526-539
Effects of prenatal TBT on MSC pool

- TBT exposure biases the MSC compartment toward adipocytes
  - 7-15% more pre-adipocytes in TBT-treated than control animals
- Increased expression of adipocyte markers reflects increased number of pre-adipocytes
  - Decreased potential to form osteoblasts

Kirchner et al., 2010 Molec Endocrinol, 24, 526-539
How does prenatal TBT exposure promote adipocyte differentiation?

**Effects of in utero TBT exposure on adipogenic pathway genes**

<table>
<thead>
<tr>
<th>uninduced</th>
<th>+ TBT 14D</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARγ2+/−</td>
<td>PPARγ2+</td>
</tr>
<tr>
<td>Fabp4+</td>
<td>Fabp4+</td>
</tr>
<tr>
<td>LEP+</td>
<td>LEP+</td>
</tr>
<tr>
<td>Pref1−</td>
<td>Pref1−</td>
</tr>
<tr>
<td>GyK+</td>
<td>GyK+</td>
</tr>
<tr>
<td>PEPCK+</td>
<td>/</td>
</tr>
<tr>
<td>ADIPOQ</td>
<td>LPL+</td>
</tr>
<tr>
<td>Resistin</td>
<td>ADIPOQ+</td>
</tr>
<tr>
<td>IRS-2</td>
<td>/</td>
</tr>
</tbody>
</table>

---

Epigenetic effects of prenatal TBT exposure on promoter methylation of PPARγ target genes

Kirchner et al., 2010 Molec Endocrinol, 24, 526-539
How does TBT affect PPARγ regulators?

- Zfp423 regulates PPARγ expression (Gupta et al. 2010)
- BMP4 activates C/EBPβ (Bowers et al. 2007)
- Ezh2 represses Wnt during adipogenesis by methylating H3K27 at its promoter (Wang et al. 2010)
- Wnt10b represses adipogenesis, repressed by Ezh2 (Wang et al. 2010)
- Wnt5b promotes adipogenesis by inhibiting Wnt/β-catenin pathway (Christodoulides et al. 2008)
- Sox9 represses C/EBPβ/δ activity (Wang et al. 2009)
Conclusions - organotins and nuclear receptors

- Organotins are exceptionally potent agonists of RXR and PPAR\(_\gamma\)
  - ~5 nM EC\(_{50}\), 12.5 nM K\(_d\) on RXR\(_\alpha\)
  - ~20 nM EC\(_{50}\) and K\(_d\) on PPAR\(_\gamma\)

- TBT drives adipocyte differentiation in mouse and human cell cultures

- TBT exposure during development induces adipogenesis in two vertebrate species: mouse and *Xenopus*
  - TBT induces expression of expected RXR/PPAR\(_\gamma\) target genes involved in adipogenesis, *in vivo*.

- Induction of adipogenesis is novel and unexpected endocrine disrupting effect of TBT

- Multiple potential modes of action
  - PPAR\(_\gamma\)-RXR
  - Aromatase expression/function - estradiol levels
  - Glucocorticoid levels
  - Other stressors?
Conclusions - organotins and obesity

- Is organotin exposure a contributing factor for obesity?
  - Adult exposure rapidly induces adipogenic genes
    - Drugs that activate PPARγ increase obesity
  - Prenatal TBT exposure permanently alters adult phenotype
  - Prenatal TBT exposure recruits MSCs to adipocyte lineage and diverts them from bone lineage

- Are humans exposed to sufficient levels of TBT for concern?
  - PVC is up to 3% w/w (0.1 M) organotins
  - Prevalent contaminants in dietary sources
  - Fungicide on high value crops, used in water systems
  - Average blood level of 27 nM in 32 random people tested
  - TPT levels from ~0.5-2 nM in Finnish fishermen

- Human exposure to organotins may reach levels sufficient to activate high affinity receptors
  - 1000 x lower dose than natural dietary RXR and PPARγ ligands

Is the environment making us fat?
Obesogens - Just the Tip of the Iceberg?

- TBT/TPT
- Phthalates
- PFOA
- DES
- Bisphenol A
- Genistein
- Nicotine
- Air pollution
- BaP
- fructose
- COX2 inhibitors
- PCBs ?, PBDEs ?
- Organophosphate pesticides

What don’t we know yet?

- How many obesogens are out there?
- Body burdens in population
- Molecular targets of action beyond RXR
- Critical windows of exposure
- How does prenatal exposure alter adult phenotype?
- Is the prenatal reprogramming epigenetic?

fructose

BaP
Implications For Human Health

- Diet and exercise are insufficient to explain obesity epidemic particularly in the very young

- Obesogens inappropriately stimulate adipogenesis and fat storage
  - Prescription drugs
    - Thiazolidinedione anti-diabetic drugs (Actos, Avandia)
    - Atypical antipsychotics, anti-depressants
  - Environmental contaminants
    - organotins, environmental estrogens (BPA, DEHP), PFOA

- Prenatal obesogen exposure reprograms exposed animals to be fat
  - Epigenetic changes alter fate of stem cell compartment -> more preadipocytes and more cells committed to adipocyte lineage

- Obesogens shift paradigm from treatment to prevention during pregnancy, childhood and puberty
  - Reduced exposure to obesogens, optimized nutrition
  - Obesity is intractable once established
• UCI - Blumberg Lab
  Rachelle Abbey
  Stephanie Casey
  Raquel Chamorro-Garcia
  Amanda Freise
  Amanda Janesick
  Séverine Kirchner
  Jhyme Laude
  Jasmine Li
  Sophia Liu
  Nhieu Pham

• UCI - labs
  David Fruman
  Matt Janes
  Edward Nelson
  Eric Potma

• NINS - Okazaki, Japan
  Taisen Iguchi

• NIHS - Tokyo, Japan
  Jun Kanno

• University of Tokyo
  Satoshi Inoue
  Kotaro Azuma

Funding from NIEHS, US-EPA, UC-TSR&TP