Transgenerational Inheritance of Prenatal Obesogen Exposure

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Main Points

- Obesogens exist and contribute to obesity epidemic
- Obesogen action may involve reprogramming of stem cells
- The effects of obesogens are permanent and heritable
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) - 86% estimated by 2020

Subcutaneous obesity: adaptive

Visceral obesity: pathological

From Lars Lind
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Obesity Trends* Among U.S. Adults
(*BMI $\geq 30$, or about 30 lbs. overweight for 5’4” person)

1990
- No Data
- <10%
- 10%–14%
- 15%–19%
- 20%–24%
- 25%–29%
- ≥30%

1999
- No Data
- <10%
- 10%–14%
- 15%–19%
- 20%–24%
- 25%–29%
- ≥30%

2008
- No Data
- <10%
- 10%–14%
- 15%–19%
- 20%–24%
- 25%–29%
- ≥30%

~17,000
30,961
22,401

Sources: CDC (map), U.S. Census bureau (numbers)
The Worldwide Obesity Epidemic

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• Obesity accounts for a huge fraction of healthcare costs
  - $85.7 billion annually in US (2005), $147 billion (2009)
  - New model (J. Health Economics, 2012) - $209.7 billion in 2008 $
    • 20.6% of US healthcare costs.

• Obesity is associated with increases in
  • Metabolic syndrome -> type 2 diabetes
  • cardiovascular disease
  • hypertension
  • stroke
How does obesity occur?

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

- 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

- Is there a role for 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
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 storage in existing cells
    - Increased insulin sensitivity

**Endocrine Disrupting Chemicals (EDCs)**

*affect many organ systems*

- “*Endocrine Disruptor* - an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action.” - The Endocrine Society, 2012
  - Hormones work at low concentrations and so do EDCs

- How are we exposed to EDCs?
  - persistent pollutants (POPs), pesticides, food packaging, personal care products, etc

- Developmental exposures can have permanent effects - DOHaD
  - Are effects heritable?
Endocrine Disrupting Chemicals (EDCs)

• Are EDC-mediated disturbances in endocrine signaling pathways involved in adipogenesis and obesity
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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
  - Many chemicals linked with obesity in epidemiological studies

- Several compounds cause adipocyte differentiation in vitro (PPARγ)
  - Phthalates, BPA, alkylphenols, 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 PPAR\(\gamma\) critical for adipogenesis
  - TBT induced adipogenesis in cell culture models (nM)
  - Prenatal TBT exposure led to weight gain in mice, in vivo

Grun et al., Molec Endocrinol, 2006
TBT increases testis fat pad weight at 10 weeks

Fat depot size increases at the expense of overall body mass

Grun et al., Molec Endocrinol, 2006
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
MSCs can give rise to many cell types in vivo

- PPARγ controls choice between fat and bone pathways
- Expression and activation of PPARγ favors the fat and inhibits bone formation.

Kirchner et al., 2010 Molecular Endocrinology 24, 526-539

CMS

CMC

ROS1

TBT

C57Bl6/J Pregnant Dam

In utero exposed offspring

MSC isolation and culture

Takada et al., 2009 Nature Reviews Immunology 5, 442-447
Prenatal TBT exposure reprograms MSCs to become fat cells instead of bone cells

Kirchner et al, 2010 Molecular Endocrinology 24, 526-539
Are effects of TBT exposure transgenerational? (i.e., permanent)

**TREATMENTS**
- DMSO
- ROSI 0.5 uM
- TBT 5.42 nM (50x < NOAEL)
- TBT 54.2 nM (5x < NOAEL)
- TBT 542 nM

**MULTI-GENERATIONAL EFFECT**

- FEMALE PRE-TREATMENT
- P0 MATING
- F1 BIRTH

Drinking water

8 weeks old
- Body weight
- Adipose tissue weight
- Adipocyte size
- MSC gene expression profile
- Liver adiposity
- Hepatic gene expression

Chamorro-Garcia et al., Environ Health Perspect, 2013
TBT exposure has transgenerational effects

Heavier fat depots

Chamorro-Garcia et al., Environ Health Perspect, 2013
TBT exposure has transgenerational effects

*Increased expression of fat-specific genes in MSCs*

Chamorro-Garcia et al., Environ Health Perspect, 2013
Epigenomic analysis of MSC fate

- Genomic DNA of MSCs from F3 male and females (TBT, vehicle)

- Methylminer deep-sequencing analysis (2 x 10^8 reads) (Toshi Shioda, MGH)

- 440,000 DNA methylation peaks identified for the entire MSC genome
  - ~450 reads/peak

- Computational statistical screenings (p < 0.001)
  - 900 peaks stronger in the vehicle vs TBT exposed MSCs
    - Methylation suppressed by TBT
  - 6215 peaks stronger in the TBT vs. vehicle-exposed MSCs.
    - Methylation increased by TBT

- Preliminary data suggest relevant epigenetic changes can be found in DNA of MSCs in F3 animals descended from F0 in utero treatment.
Epigenomic analysis of MSC fate

- Full genome analysis of MSCs and sperm from F1 -> F4 animals prenatally exposed to TBT or vehicle.
  - RNA-seq
    - mRNA-seq
    - lncRNA-seq
    - miRNA panel
  - Whole genome methylation and hydroxymethylation
    - MeDIP-seq, OH-MeDIP-seq
    - Bisulfite seq
  - Methyl histone ChIP-Seq
    - H3K4me3 - active chromatin
    - H3K9me3 - repressive mark
    - H3K27me3 - repressive mark

- Link transcription with changes in DNA and histone methylation to identify “epimutations” responsible for transgenerational inheritance
Transgenerational effects of TBT exposure

**TREATMENTS**
- DMSO
- TBT 5 nM
- TBT 50 nM

**ENDPOINTS**
- 8 weeks old - MSCs
  - Transcriptomics
  - Methylomics
  - Lineage commitment
  - Body weight
  - Body composition
  - Serum analysis
  - Epididymal sperm

**MULTI-GENERATIONAL EFFECT**
- Sacrificed
- Mating
- F2

**TRANS-_GENERATIONAL EFFECT**
- Sacrificed
- Mating
- F3

Diet Test
Effects of diet on F4 animals

- **F4 BIRTH**
- **13.2% FAT DIET**
- **DIET SWITCH (WEEK 19)**
  - **21.6% FAT DIET**
- **DIET SWITCH (WEEK 25)**
  - **13.2% FAT DIET**
- **4H FASTING MRI (WEEK 32)**
- **O/N FASTING MRI**
- **EUTHANASIA (WEEK 33)**
What early pathways is TBT uniquely acting through?

**Standard adipogenesis assay**

- **Plating**
- **Confluency**
  - +DMSO, +ROSI 500 nM, +TBT 5/50/100 nM
- **Induction cocktail (MDI)**
- **Stop assay**

**Day 0**
- **Day 3**
- **Day 7**
- **Day 14**

**RNA-Seq**

*Search for genes altered by TBT, but not ROSI after 3 days*
Transcripts upregulated in MSCs at 3 days

- ROSI 500 nM (544)
- TBT 5 nM (559)
- TBT 100 nM (253)
- TBT 50 nM (561)
TBT-induced genes and transgenerational effects

• Approach
  – Select genes induced by TBT (but not ROSI) at 3 days
  – Subtract genes induced by TBT (but not ROSI) at 7 days
  – Stringent DAVID analysis for gene representation

• 11 clusters identified
  – Lipid, fatty acid, carboxylic, organic acid biosynthesis
  – Lipid, sterol, cholesterol biosynthesis and metabolism
  – Lipid transport, localization and binding
  – Lipid, cholesterol, sterol metabolism
  – Metal binding
  – Zn finger proteins (types 2-12)
  – Transcription factors (including TRβ, LXRα, Nurr1, various Hox genes)
  – Oxidoreductase, NAD, NADP, short chain dehydrogenases
  – Endoplasmic reticulum and nuclear envelope
  – Matrix metalloproteases
  – Protein tyrosine phosphatase
TBT and MSC commitment

• *Undifferentiated* MSCs collected from prenatally exposed mice have an adipogenic gene expression profile

• Mice treated with TBT, *but not* Rosiglitazone, show a strong transgenerational phenotype

• Standard *in vitro* assays do not identify obesogens that can commit MSCs to the adipose fate, since treatment is in conjunction with adipogenic cocktail (MDI)
Obesogen exposure and development

• Organotins are exceptionally potent agonists of RXR and PPAR\(\gamma\) at environmentally-relevant levels (ppb)
  - ~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 cell culture models

• TBT exposure during development induces adipogenesis in two vertebrate species: mouse and *Xenopus*

• The effects of maternal TBT exposure are transgenerational
  - Fat depot size, adipocyte size, MSC gene expression, hepatic fat

• TBT exposure alters response to diet composition and fasting
  - Increased fat accumulation vs. control
  - TBT makes animals resistant to weight loss from fasting

• 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
- COX2 inhibitors
- PCBs ?, PBDEs ?
- fructose
- many fungicides
- Organophosphate pesticides

- What don’t we know yet?
  - How many obesogens are out there
  - Body burdens in population
  - Molecular targets of action beyond RXR-PPARγ
  - Critical windows of exposure
  - How does prenatal exposure alter adult phenotype?
  - Is the prenatal reprogramming epigenetic?
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, estrogens (BPA, DEHP), PFOA/S, DDE, POPs
    - Many fungicides, organophosphates, parabens

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

- Obesogens shift paradigm from treatment to prevention during pregnancy, childhood and puberty
  - Reduced exposure to obesogens, optimized nutrition
Chemicals with Transgenerational Effects

- Tributyl tin (RXR, PPARγ) plastic, industrial use, water pipes - increased fat mass, reprogram stem cells to produce more fat cells over time, fatty liver disease (Chamorro-Garcia et al, 2013)
- Vinclozolin (anti-androgen) - fungicide, impairs male reproductive function (Anway and Skinner, 2005)
- Plastics mixture, BPA, DEHP, DBP, (estrogen, anti-androgen) obesity, reproductive diseases, sperm epimutations (Manikkam et al, 2013)
- Hydrocarbons, JP-8 jet fuel (?) obesity, reproductive diseases, sperm epimutations (Tracey et al, 2013)
- BPA, estrogen (plastics, thermal paper, recycled paper, food packaging), altered social interactions, modified gene expression (Wolstenholme et al, 2012)
- DDT, estrogen (pesticide) - 50% of F3 males and female rats develop obesity (Skinner et al, 2013)
Chemicals with Transgenerational Effects

- Existence of transgenerational effects raises the stakes in the argument about whether and what chemicals to regulate.

- What will be the cost of waiting for conclusive evidence of harm in humans before acting?
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