Effects of Prenatal Obesogen Exposure Echo Down the Generations

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Main Points

- Obesogens exist and contribute to obesity epidemic
- Obesogen action can reprogram stem cell fate
- Effects of obesogen exposure are heritable
- Obesogen exposure modifies response to diet and fasting
- Prenatal TBT treatment leads to heritable epigenetic changes that alter susceptibility to obesity.
The Worldwide Obesity Epidemic

- 39.6% of the US population are clinically obese (BMI > 30)
  - Hales al, NCHS Data Brief 288, 2017 (4% increase since 2015)
  - 37.9% male vs 41.1% female
  - Disproportionately affects minority groups
    - 46.8% African American, 47% Hispanic, only 12.7% Asian

- 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
  - cancers
How does obesity occur?

- Prevailing wisdom - “couch potato syndrome”
  - Positive energy balance, i.e., too much food, too little exercise
Any history of diet or exercise in your family?
Canaries in the coal mine: a cross-species analysis of the plurality of obesity epidemics

Yann C. Klimentidis¹, T. Mark Beasley¹, Hui-Yi Lin⁴, Giulianna Murati⁵, Gregory E. Glass⁶, Marcus Guyton¹, Wendy Newton⁷, Matthew Jorgensen⁸, Steven B. Heymsfield⁹, Joseph Kemnitz⁷, Lynn Fairbanks¹⁰ and David B. Allison¹,²,3,*

A dramatic rise in obesity has occurred among humans within the last several decades. Little is known about whether similar increases in obesity have occurred in animals inhabiting human-influenced environments. We examined samples collectively consisting of over 20,000 animals from 24 populations (12 divided separately into males and females) of animals representing eight species living with or around humans in industrialized societies. In all populations, the estimated coefficient for the trend of body weight over time was positive (i.e. increasing). The probability of all trends being in the same direction by chance is 1.2 × 10⁻⁷. Surprisingly, we find that over the past several decades, average mid-life body weights have risen among primates and rodents living in research colonies, as well as among feral rodents and domestic dogs and cats. The consistency of these findings among animals living in varying environments, suggests the intriguing possibility that the aetiology of increasing body weight may involve several as-of-yet unidentified and/or poorly understood factors (e.g. viral pathogens, epigenetic factors). This finding may eventually enhance the discovery and fuller elucidation of other factors that have contributed to the recent rise in obesity rates.
Our pet cats and dogs are getting fat, but so are feral rats in cities and, crucially, 4 species of research animals living in controlled environments.

Something about living in proximity to humans is making animals fat.
Secular differences in the association between caloric intake, macronutrient intake, and physical activity with obesity

Ruth E. Brown, Arya M. Sharma, Chris I. Ardern, Pedi Mirdamadi, Paul Mirdamadi, Jennifer L. Kuk

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Summary

Background: To determine whether the relationship between caloric intake, macronutrient intake, and physical activity with obesity has changed over time.

Methods: Dietary data from 36,377 U.S. adults from the National Health and Nutrition Survey (NHANES) between 1971 and 2008 was used. Physical activity frequency data was only available in 14,419 adults between 1988 and 2006. Generalised linear models were used to examine if the association between total caloric intake, percent dietary macronutrient intake and physical activity with body mass index (BMI) was different over time.

Results: Between 1971 and 2008, BMI, total caloric intake and carbohydrate intake increased 10–14%, and fat and protein intake decreased 5–9%. Between 1988 and 2006, frequency of leisure time physical activity increased 47–120%. However, for a given amount of caloric intake, macronutrient intake or leisure time physical activity, the predicted BMI was up to 2.3 kg/m² higher in 2006 that in 1988 in the mutually adjusted model ($P < 0.05$).
Physical activity is INCREASING, not DECREASING

Energy balance model is insufficient to explain rise in BMI between 1988 and 2006
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 - Barker “thrifty phenotype hypothesis”
  - Dutch “Hunger Winter” 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

- 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
  - Ghrelin - 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

*From Nature Medicine 10, 355 - 361 (2004)*
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
  - Wrong signal, loss of signal, wrong place at wrong time
  - Hormones work at low concentrations and so do EDCs

• How are we exposed to EDCs?
  - persistent pollutants (food, water)
  - dietary components (pesticides)
  - food packaging
  - personal care products
  - cleaning materials
## Endocrine Disrupting Chemicals (EDCs)

<table>
<thead>
<tr>
<th>HERBICIDES</th>
<th>INSECTICIDES</th>
<th>INDUSTRIAL CHEMICALS</th>
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<tr>
<td>2,4,-D</td>
<td>Aldicarb</td>
<td>Bisphenol - A</td>
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<td>2,4,5,-T</td>
<td>beta-HCH</td>
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<td>Alachlor</td>
<td>Carbaryl</td>
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<td>Amitro</td>
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<td>Cadmium</td>
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<td>Chlordecone</td>
<td>Chloro- &amp; Bromo-diphenyl</td>
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<td>DBCP</td>
<td>Dioxins</td>
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<td>Dieldrin</td>
<td>Lead</td>
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<tr>
<td>Trifluralin</td>
<td>DDT and metabolites</td>
<td>Manganese</td>
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<td>FUNGICIDES</td>
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<td>Benomyl</td>
<td>Heptachlor / H-epoxide</td>
<td>Nonylphenol</td>
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<td>Lindane (gamma-HCH)</td>
<td>Octylphenol</td>
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<td>Malathion</td>
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<td>Hexachlorobenzene</td>
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<td>PCBs</td>
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<td>Oxychlordane</td>
<td>PFOA</td>
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<tr>
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<td>Parathion</td>
<td>p-tert-Pentylphenol</td>
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<td>Tributyltin</td>
<td>Synthetic pyrethroids</td>
<td>Phthalates</td>
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<tr>
<td>Vinclozolin</td>
<td>Transnonachlor</td>
<td>Styrene</td>
</tr>
<tr>
<td>Zineb</td>
<td>Toxaphene</td>
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**TESTOSTERONE SYNTHESIS INHIBITOR**

**ESTROGEN RECEPTOR AGONIST**

**THYROID HORMONE DISRUPTOR**

**ANDROGEN RECEPTOR ANTAGONIST**
Endocrine Disrupting Chemicals (EDCs)

**Pesticides**
- Alachlor
- Amitrole
- Atrazine
- Benomyl
- Carbaryl
- Chlordane
- Chlordecone
- Chlordane
- Dieldrin
- DDT and metabolites
- Dieldrin
- Endosulfan
- Ethylene thiourea
- Fenarimol
- Fluridone
- Hexachlorobenzene
- Maneb
- Methomyl
- Methoxychlor
- Parathion
- Tributyltin
- Vinclozolin
- Zineb
- Testosterone synthesis inhibitor
- Thyroid hormone disruptor
- Estrogen receptor agonist
- Androgen receptor antagonist

**Herbicides**
- 2,4-D
- 2,4,5-T
- Alachlor
- Atrazine
- Amitrole
- Linuron
- Metribuzin
- Nitrofen
- Trifluralin
- Over 1,000 EDCs

**Fungicides**
- Benomyl
- Ethylene thiourea
- Fenarimol
- Hexachlorobenzene
- Mancozeb
- Maneb
- Metiram - complex
- Tributyltin
- Vinclozolin
- Zineb

**Metal**
- Cadmium
- Chromium
- Copper
- Lead
- Manganese
- Zinc

**Fuels**
- Benzene
- Diethyl phthalate
- Dibutyl phthalate
- Ethylene glycol
- Glycol ethers
- Heptachlor / H-epoxide
- Hexachlorobenzene
- Lindane (gamma-HCH)
- Lindane (gamma-HCH)
- Methanol
- Methoxychlor
- Methoxychlor
- Methoxychlor
- Oxychlordane
- Parathion
- Synthetic pyrethroids
- Transnonachlor
- Toxaphene
- Testosterone synthesis inhibitor
- Thyroid hormone disruptor
- Estrogen receptor agonist
- Androgen receptor antagonist

**Industrial byproducts**
- Butylhydroxyanisole
- Cadmium
- Chloro- & Bromo-diphenyl
- Dioxins
- Furans
- Lead
- Manganese
- Methyl mercury

**Plastics**
- Butylhydroxyanisole
- Cadmium
- Chloro- & Bromo-diphenyl
- Lead
- Manganese
- Methyl mercury

**Plasticizers**
- Butylhydroxyanisole
- Cadmium
- Chloro- & Bromo-diphenyl
- Lead
- Manganese
- Methyl mercury

**Metals**
- Cadmium
- Chromium
- Copper
- Lead
- Manganese
- Zinc

**INDUSTRY**
- Butylhydroxyanisole
- Cadmium
- Chloro- & Bromo-diphenyl
- Lead
- Manganese
- Methyl mercury

**Cosmetics**
- Butylhydroxyanisole
- Cadmium
- Chloro- & Bromo-diphenyl
- Lead
- Manganese
- Methyl mercury

**Industrial byproducts**
- Butylhydroxyanisole
- Cadmium
- Chloro- & Bromo-diphenyl
- Lead
- Manganese
- Methyl mercury

**Plastics**
- Butylhydroxyanisole
- Cadmium
- Chloro- & Bromo-diphenyl
- Lead
- Manganese
- Methyl mercury
Endocrine Disrupting Chemicals (EDCs)

- Are EDC-mediated disturbances in endocrine signaling pathways involved in adipogenesis and obesity
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Endocrine Disrupting Chemicals (EDCs)

- 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
  - 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 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
  - Prenatal exposure epigenetically reprograms MSC fate toward fat lineage

Grun et al., Molec Endocrinol, 2006
Kirchner et al, Molec Endocrinol 2010
Results

- No increase in body weight
- Increased WAT weight
- Increased adipocyte size
- Increased WAT number
- Whitened BAT
- Adipogenic bias in MSCs
- Fatty liver

Chamorro-Garcia et al., Environ Health Perspect, 2013
How does TBT exposure elicit transgenerational effects?

TREATMENTS
DMSO
TBT 5 nM (~50x < NOAEL)
TBT 50 nM (~5x < NOAEL)

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

Chamorro-Garcia et al., Nature Communications, 2017
Effects of diet on F4 animals

Chamorro-Garcia et al., Nature Communications, 2017
F4 TBT males are resistant to fasting-induced fat loss

TBT animals do not mobilize fat comparably to controls

Chamorro-Garcia et al., Nature Communications, 2017
Body weight is not changed by TBT exposure

Chamorro-Garcia et al., Nature Communications, 2017
Body weight is not changed by TBT exposure

Body weight is not an acceptable surrogate for obesity!

Chamorro-Garcia et al., Nature Communications, 2017
Higher fat diet causes obesity in **F4** males

CALORIES PROVIDED BY FAT

<table>
<thead>
<tr>
<th></th>
<th>13.2%</th>
<th>21.6%</th>
<th>13.2%</th>
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</tr>
</tbody>
</table>

% to body weight

Mouse age (weeks)

Chamorro-Garcia et al., Nature Communications, 2017
Smaller effect of diet on F4 females

Chamorro-Garcia et al., Nature Communications, 2017
Observations

Biological
- Fat accumulation (8 weeks)
- Response to diet (19 weeks)
- Failure to mobilize fat in fasting conditions

Molecular
- DNA methylation analyses
- Gene expression analyses

MSCs (8 week old)
Liver (33 week old)
Fat tissue
Differentially Methylated Regions in F4 Male WAT

How do we get DMRs in F4 generation when germline reprogramming erases existing methylation every generation?
Methylation and Differential Gene Expression

Chamorro-Garcia et al., Nature Communications, 2017

![Diagram showing methylation and differential gene expression. B. Subset I: Gene x. Subset II: Gene y, Gene z. Subset III: Multiple genes with hypermethylated and hypomethylated DMW regions.]

Hypermethylated DMW  Hypomethylated DMW
isoDMBs are abundant in the genome
• TBT alters methylation of genes involved in a variety of cellular processes, including many lipid and fatty acid metabolism genes

Chamorro-Garcia et al., Nature Communications, 2017
Leptin is Within a Hypo-methylated isoDMB

A.

IsoDMB #1626 (475.9 Kb)
Chr6:28,971,601-29,446,900

Isochores

Ref Seq

Lep

Isochores

L1 L2 H1 H2 H3

Gene Expression
Not changed Overexpressed

B.

Normalized counts

DMR 1

* DMSO TBT

3,000 bp

DMR 2

* 3,000 bp

Chamorro-Garcia et al., Nature Communications, 2017
F4 Over-expression of Leptin mRNA and Protein (only in males)

Obesity + elevated leptin levels = leptin resistance
TBT Exposure Alters Chromatin Structure Favoring Expression of Obesity-promoting Genes

- isoDMBs map to regions of increased GC content, which are known to correspond to TADs and loops - chromatin structure

- We observe isoDMBs rather than specific DMRs in genes

Chamorro-Garcia et al., Nature Communications, 2017
ATAC-seq of F3/F4 sperm reveals TBT-altered local chromatin accessibility

• Hypomethylated isoDMBs associated with inaccessible sperm chromatin and vice versa
• Is accessibility in sperm related to methylation status in somatic cells?

Chamorro-Garcia et al., Nature Communications, 2017
Inaccessible regions in F3/F4 sperm that are Hypomethylated in WAT contain key metabolic genes

![Graph A: Significant DAIs shared by F3 and F4](image)

- Predicted [minimum and maximum]
- Predicted [5th and 95th percentile]
- Observed

![Graph B: Venn Diagram](image)

- F3 Acc.
- F4 Acc.
- F3 Inacc.
- F4 Inacc.

<table>
<thead>
<tr>
<th>Inacc. in F3</th>
<th>GO term</th>
<th>Inacc. in F4</th>
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<tr>
<td># genes</td>
<td>p value</td>
<td>p value</td>
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<td>818</td>
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<td>496</td>
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<td>883</td>
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<td>483</td>
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</tbody>
</table>
Chromatin Accessibility is Already altered in F1 PGCs

- Pool gonads by litter and sex
- Isolate PGCs at e13.5 by FACS, tagment and deep sequence
Chromatin Accessibility is Already altered in F1 PGCs

- F0 TBT exposure leads to changes in chromatin accessibility
  - Male-specific
  - Occurs by E13.5

- F1 PGCs are, in fact the F2 generation

- TBT effects may be carried by altered chromatin structure/accessibility in the male germline

- Chromatin structure is inherited, leading to secondary regional changes in DNA methylation and gene expression
Obesogen exposure and development

- Organotins are exceptionally potent agonists of RXR and PPAR\(\gamma\) at environmentally-relevant levels (ppb)
  - \(~5\ \text{nM } \text{EC}_{50}\), \(12.5\ \text{nM } K_d\) on RXR\(\alpha\), \(~20\ \text{nM } \text{EC}_{50}\) and \(K_d\) on PPAR\(\gamma\)

- TBT drives adipocyte differentiation in cell culture models, and in 2 vertebrate species: mouse and *Xenopus*

- The effects of maternal TBT exposure are transgenerational
  - Fat depot size, adipocyte size, MSC gene expression, hepatic fat
  - Altered DNA methylation in F3 and F4 animals
  - Probable heritable, large scale changes in chromatin structure

- TBT exposure induces a transgenerational, leptin-resistant “thrifty phenotype, altering response to diet composition and fasting
  - Increased fat accumulation vs. control
  - TBT makes animals resistant to weight loss from fasting
  - TBT animals over-express leptin and are likely leptin-resistant

- Multiple potential modes of action
  - PPAR\(\gamma\)-RXR (differentiation)
  - Adipogenic commitment (probably RXR-dependant)
  - Aromatase expression/function - estradiol levels
Implications For Human Health

- Diet and exercise alone are insufficient to explain obesity epidemic
- Obesogens inappropriately stimulate adipogenesis and fat storage
  - Prescription drugs
    - Thiazolidinedione anti-diabetic drugs (Actos, Avandia)
    - Atypical antipsychotics, tricyclic 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
  - Altered chromatin structure and accessibility leads to regional changes in DNA methylation and gene expression favoring obesity
- Obesogens shift paradigm from treatment to prevention during pregnancy, childhood and puberty
  - Reduced exposure to obesogens, optimized nutrition
Implications For Human Health

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Testing for effects of chemical exposure

UM... WE DO TEST FOR THE SAFETY OF PESTICIDES IN YOUR FOOD.

IT'S KIND OF A LONG-TERM TEST.

ONLY HARMFUL TO GUINEA PIGS.
• UCI
  Raquel Chamorro-García
  Carlos Diaz-Castillo
  Riann Egusquiza
  Linzi Hosohama
  Victor Hung
  Kevin Nee
  Bassem Shoucri
  Lauren Urban
  Gin Wang
  Sigal Willner

  Grant MacGregor

• Former lab members
  Tim Abreo
  Christy Boulos
  Giorgio Dimastrogiovanni
  Felix Grun
  Amanda Janesick
  Séverine Kirchner
  Eric Martinez
  Zdenka Moosova
  Lenka Vanek

• NINS - Okazaki, Japan
  Taisen Iguchi

• NIHS - Tokyo, Japan
  Jun Kanno

• Io Therapeutics
  Rosh Chandraratna

Funding from NIEHS
Why We Eat Less and Exercise More but Still Struggle to Lose Weight

THE OBESOGEN EFFECT

BRUCE BLUMBERG, PHD
with Kristin Loberg

https://www.theobesogeneffect.com/