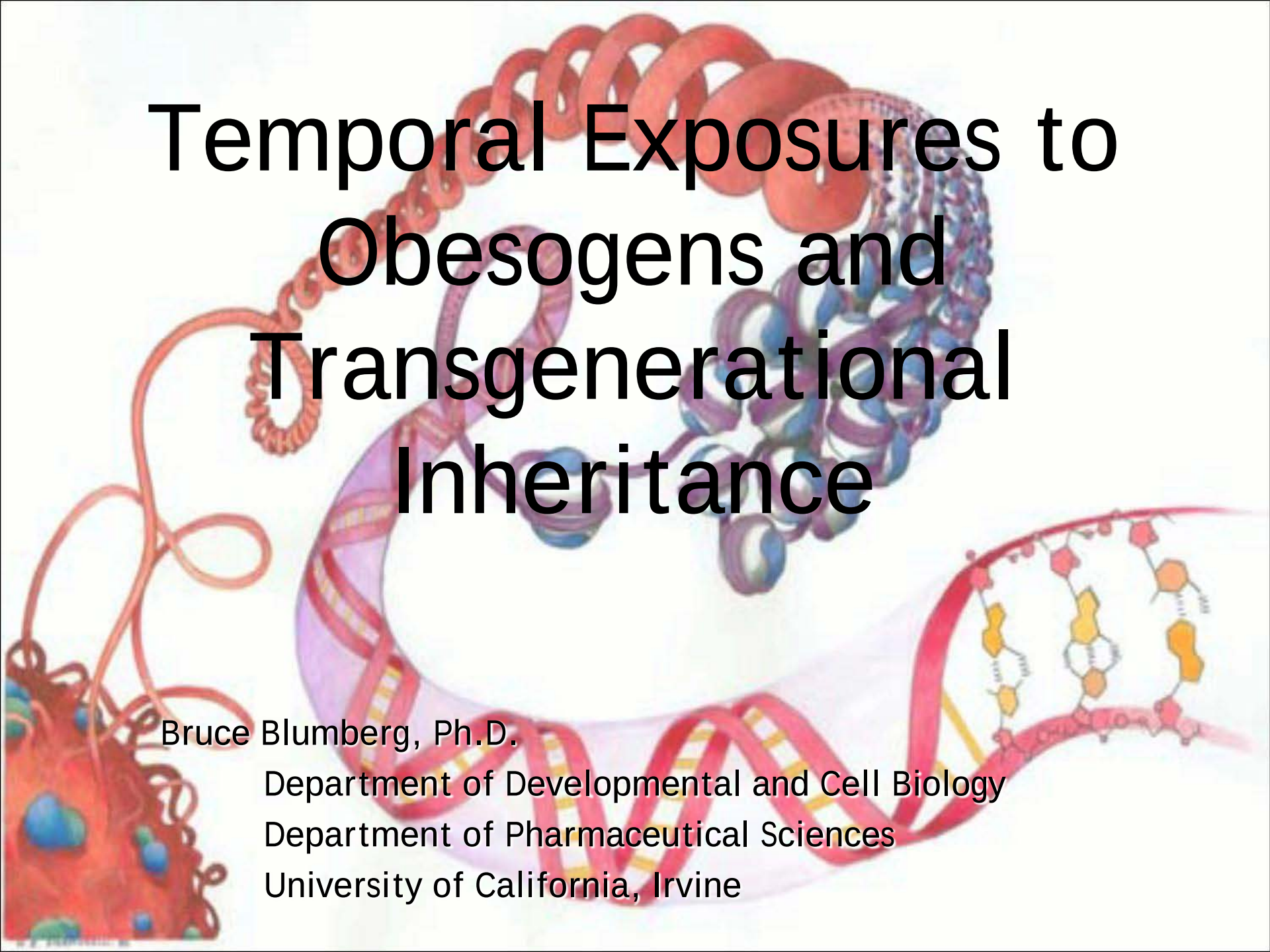
Temporal Exposures to Obesogens and Transgenerational Inheritance



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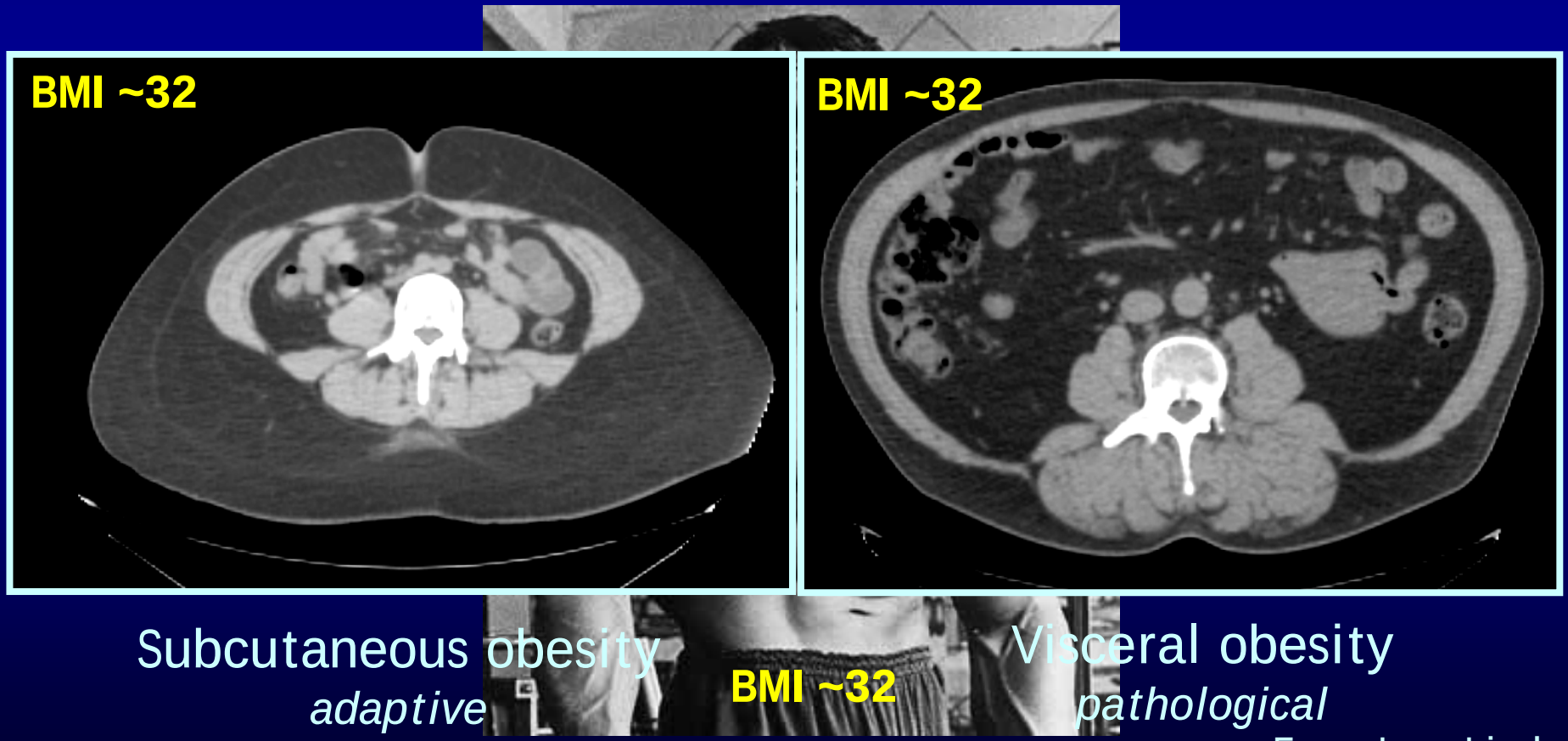
Main Points

- Obesogens exist and contribute to obesity epidemic
- Obesogen action may involve reprogramming of stem cells
- Obesogen exposure modifies response to diet
- Effects of obesogen exposure are heritable
- What do we know about temporal sensitivity windows?

F0 F1 F2 F3

The Worldwide Obesity Epidemic

- >35% 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

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
- 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

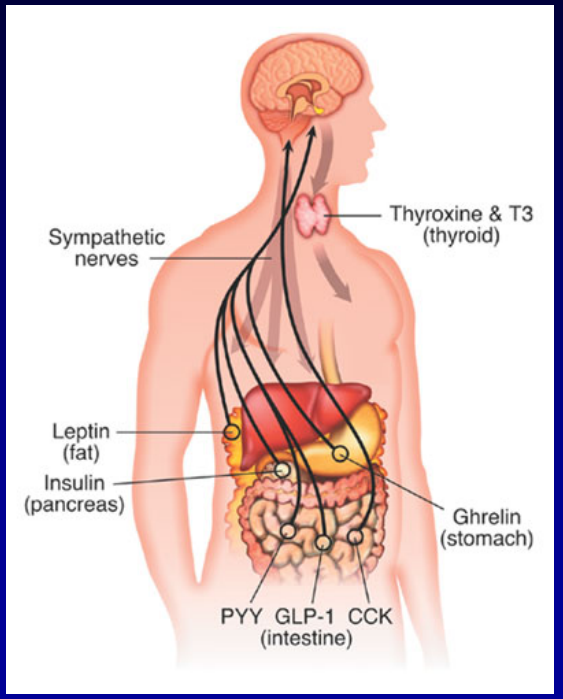
How does obesity occur ?

- 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 increased chemical use
  - Heindel (2003) "Endocrine Disruptors and the Obesity Epidemic"
- **Many chemicals have effects on the endocrine system**

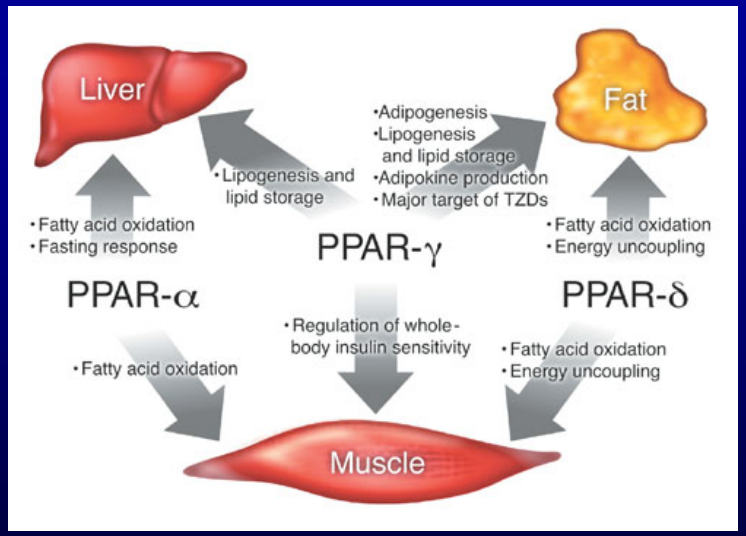
WALL-E, Disney/PIXAR, Inc

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

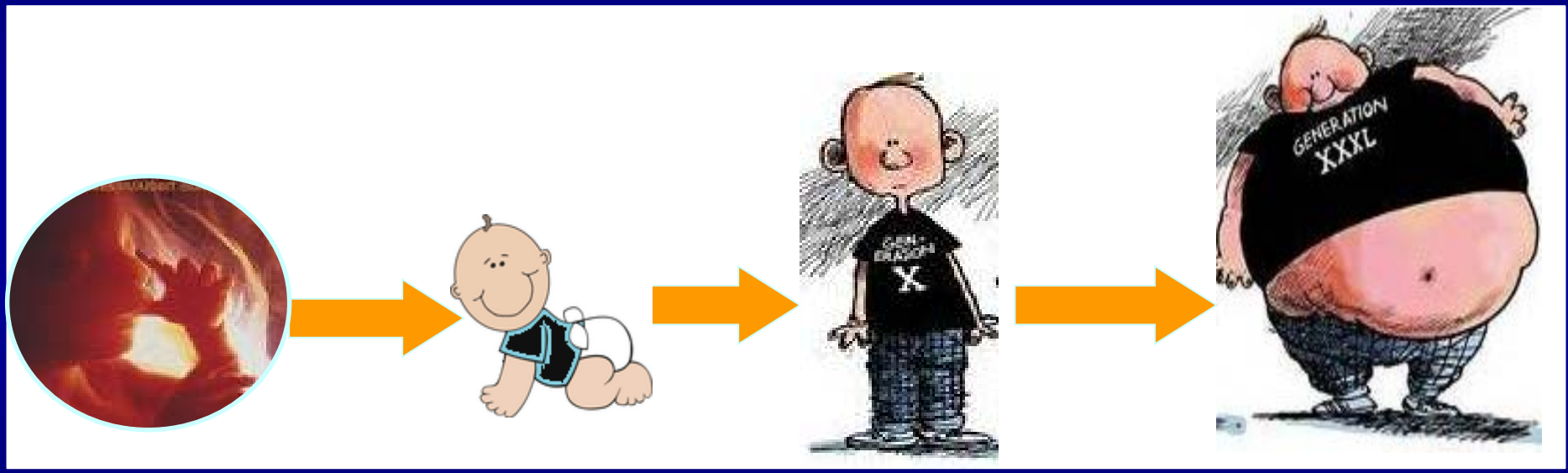


- Hormonal control of fat cell development and lipid balance
  - Regulated through nuclear hormone receptors RXR, PPARγ
  - PPARγ – master regulator of fat cell development
    - increased fat cell differentiation
    - Increased storage in existing cells
    - Increased insulin sensitivity



From Nature Medicine 10, 355 - 361 (2004)

Endocrine Disrupting Chemicals (EDCs)



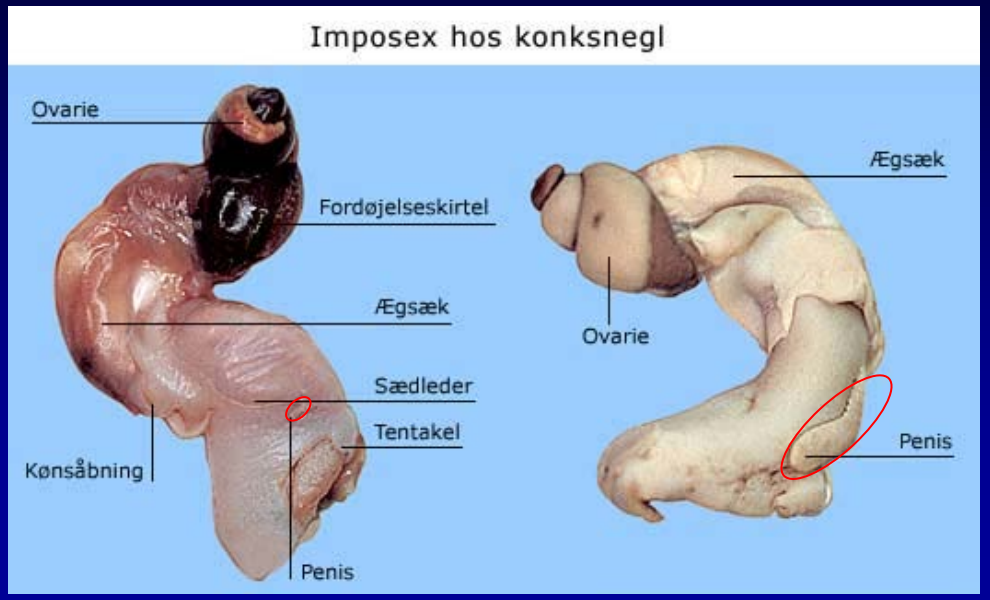
- Are EDC-mediated disturbances in endocrine signaling pathways involved in adipogenesis and obesity

EDCs and the obesogen hypothesis

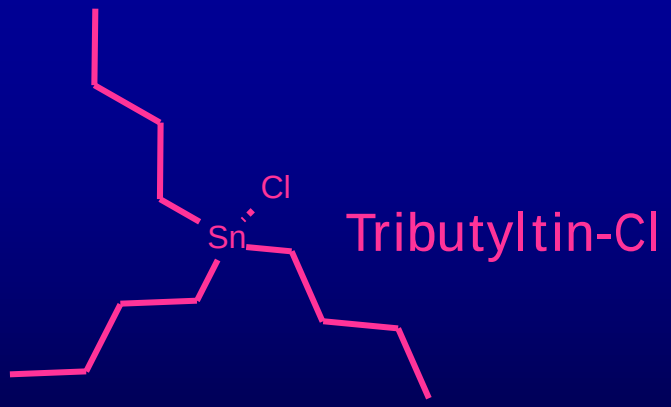
- ***Obesogens*** - chemicals that inappropriately stimulate adipogenesis and fat storage, disturb adipose tissue homeostasis, or alter control of appetite/satiety to lead to weight gain and obesity
- Pre- and postnatal exposure to EDCs such as environmental estrogens (ER) increases weight
  - DES, genistein, 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, aklylphenols, 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?



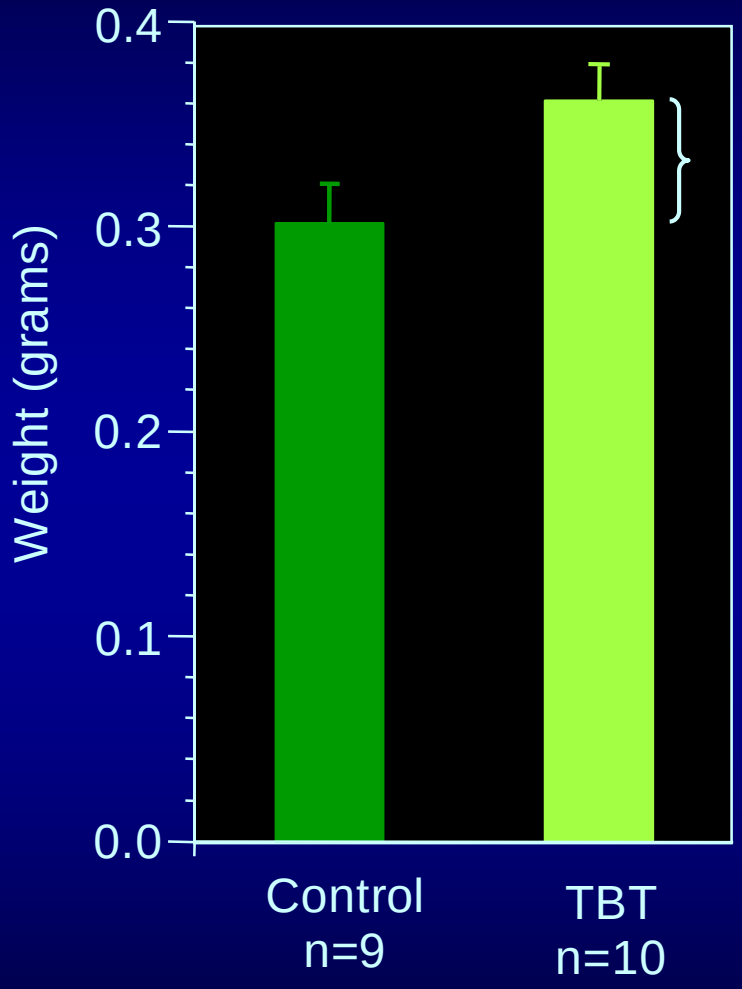
- We found that tributyltin (TBT)
  - Binds and activates at ppb (low nM) two nuclear receptors, RXR and PPARγ critical for adipogenesis
  - TBT induced adipogenesis in cell culture models (nM)
  - Prenatal TBT exposure led to weight gain in mice, in vivo



Grun et al., Molec Endocrinol, 2006

TBT increases testis fat pad weight

at 10 weeks



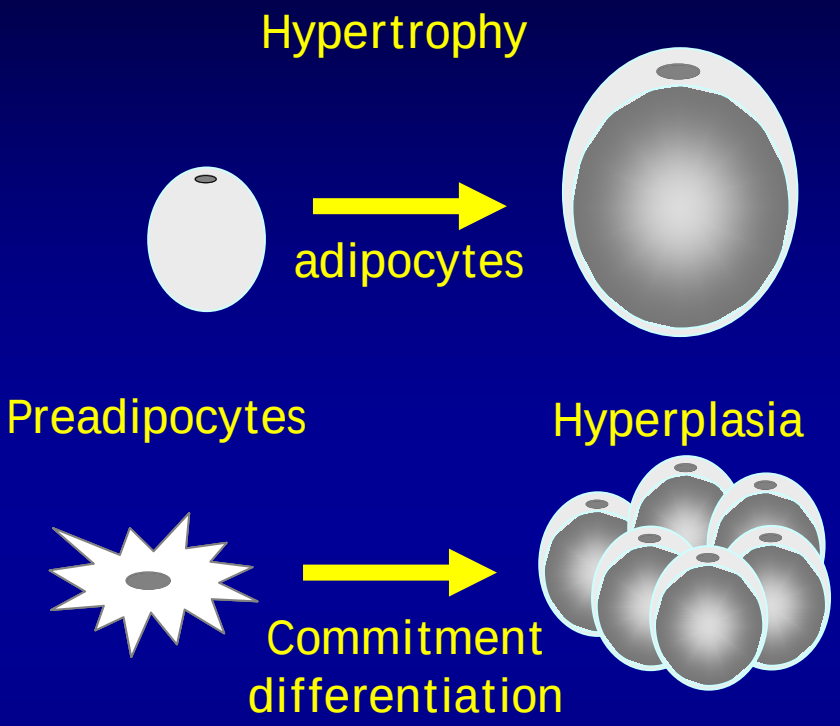
16% increase
p = 0.037

Fat depot size increases at the expense of overall body mass

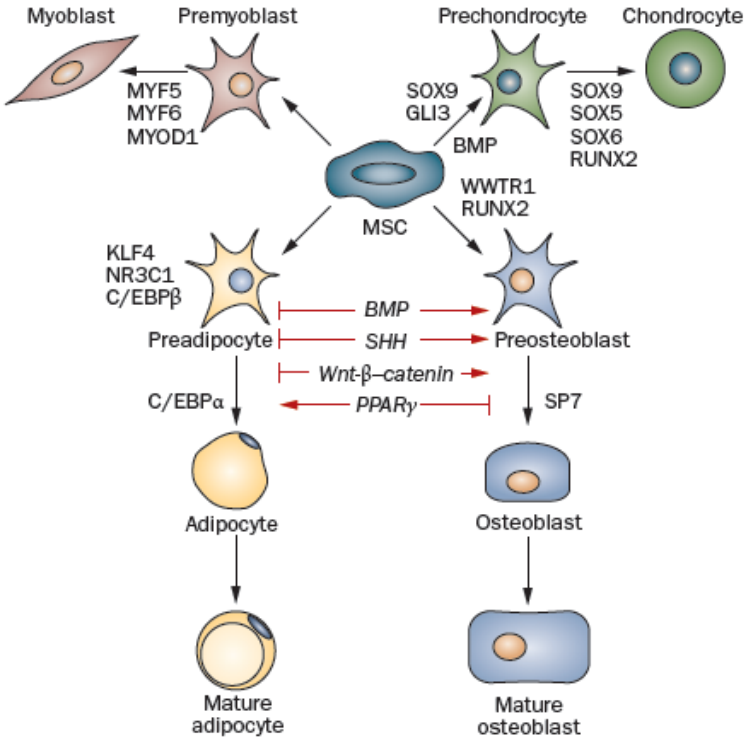
Grun et al., Molec Endocrinol, 2006

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?



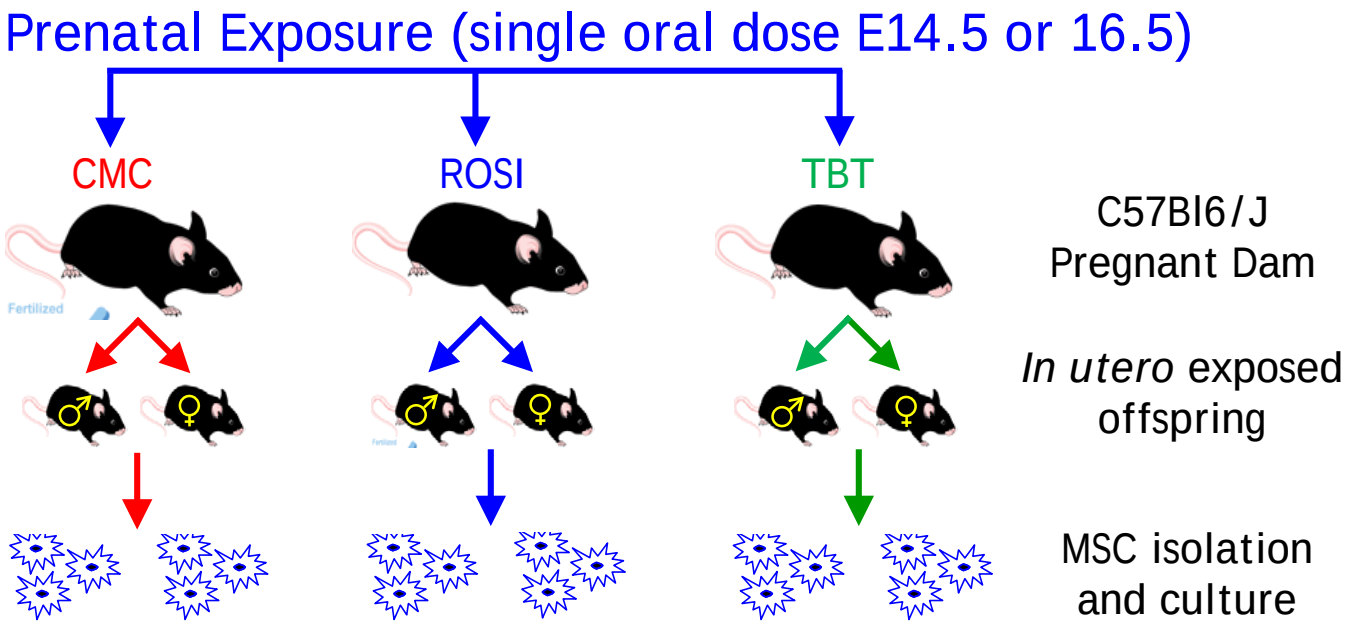
- Mesenchymal stem cells (MSCs) (a.k.a. multipotent stromal cells) precursors to many lineages including bone, cartilage, and adipose.
  - MSCs differentiate into adipocytes following rosiglitazone exposure
  - MSCs may (or may not) home to adipose depots after induction
- ***Hypothesis:*** TBT induces adipogenesis in MSCs



Takada et al., 2009 Nature Reviews Immunology 5, 442-447

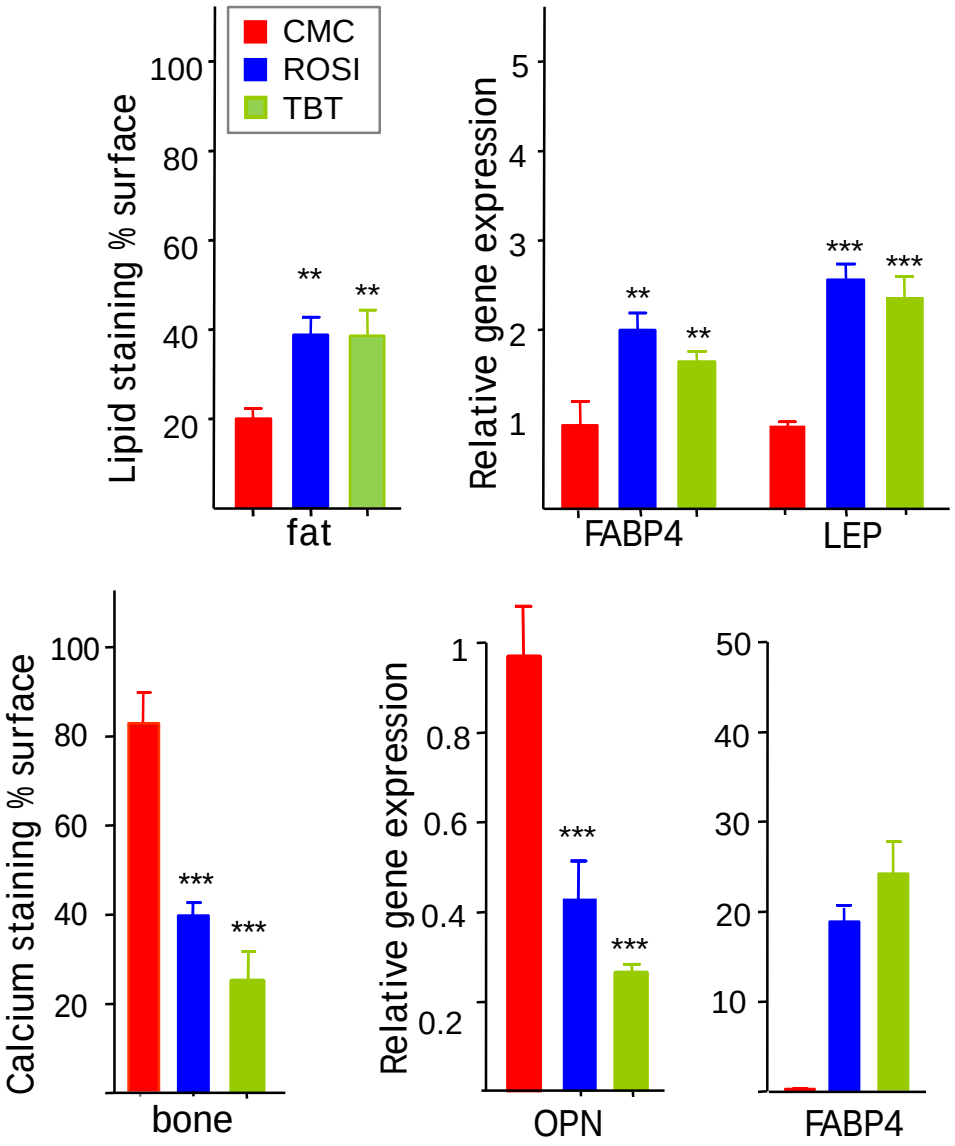
MSCs can give rise to many cell types in vivo

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



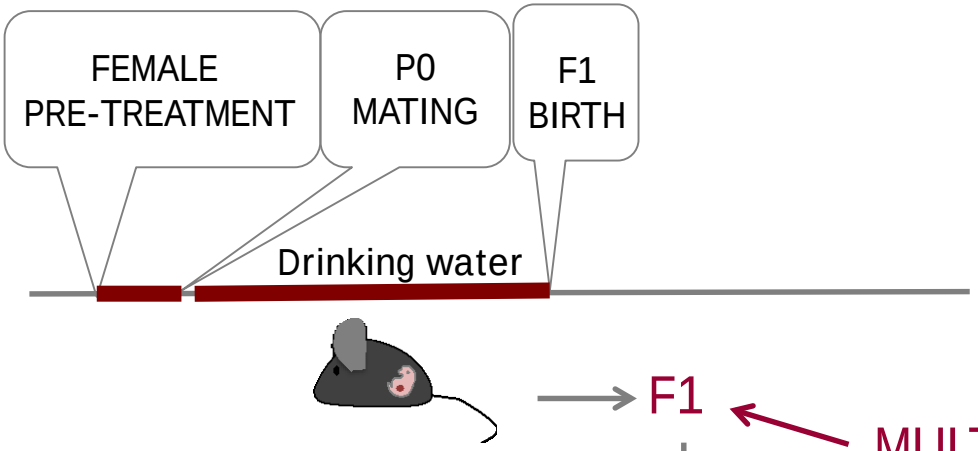
Kirchner et al, 2010 Molecular Endocrinology 24, 526-539

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 heritable ?



TREATMENTS

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

F1 ← MULTI-GENERATIONAL EFFECT

F1 → Sacrificed / Mating → F2

F2 → TRANS-GENERATIONAL EFFECT

F2 → Sacrificed / Mating → F3 → Sacrificed

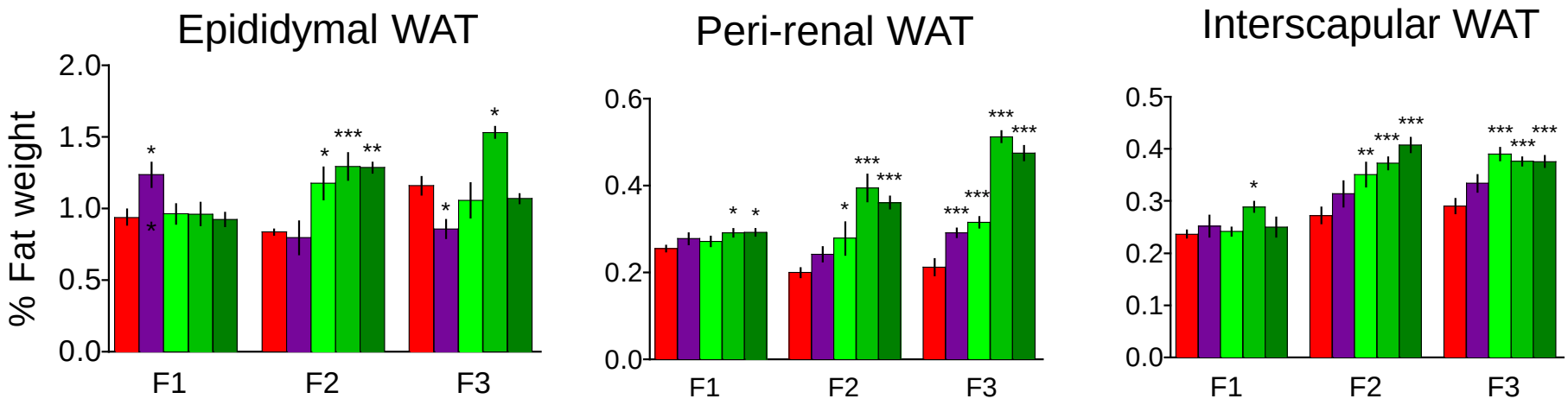
8 weeks old

- 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

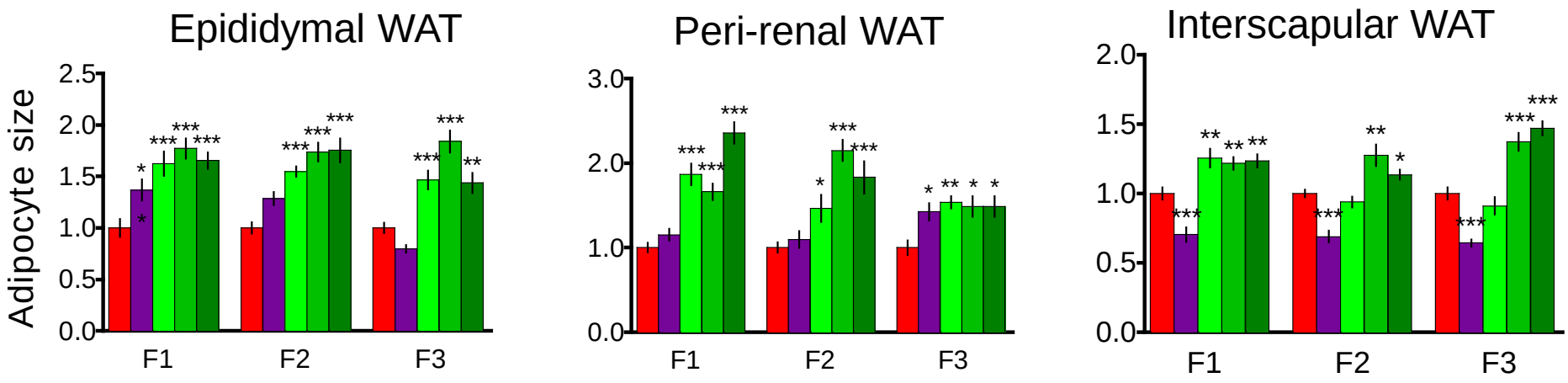


vehicle **ROSI** **TBT 5.4 nM** **TBT 54.2 nM** **TBT 542 nM**

Chamorro-Garcia et al., Environ Health Perspect, 2013

TBT exposure has transgenerational effects

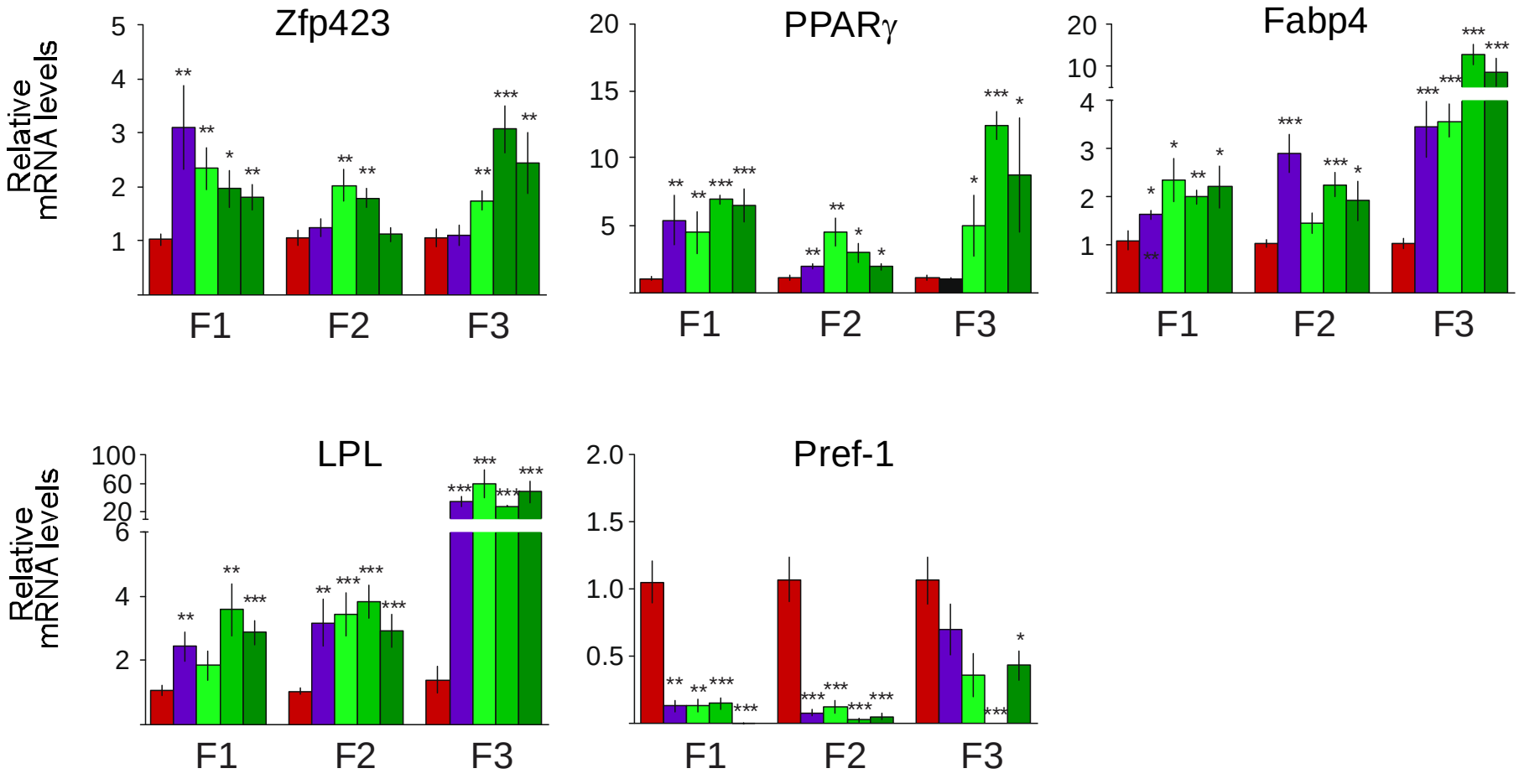
Larger fat cells



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

Obesogen exposure and development

- Organotins are exceptionally potent agonists of RXR and PPARγ at environmentally-relevant levels (ppb)
  - ~5 nM EC_{50}, 12.5 nM K_d on RXRα
  - ~20 nM EC_{50} and K_d on PPARγ

- 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

- TBT exposure induces a transgenerational "thrifty phenotype, altering 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 – potential AOP
  - PPARγ-RXR (differentiation)
  - Adipogenic commitment
  - Aromatase expression/function – estradiol levels
  - Glucocorticoid levels

Obesogens and temporal exposures

- TBT - prenatal exposures -> later life effects
  - Prenatal alone (B6)
    - Chronic dose – effects through F3, F4
  - Prenatal + nursing (B6)
    - Chronic dosing – effects through F4
- Other TBT exposures?
  - Adolescent exposure (KM) obesity, hepatic steatosis, islet cell apoptosis, altered glucose homeostasis
  - Adult exposure (B6 mice, Wistar rats) - increased adiposity, inflammation
- TZDs (Actos, Avandia – PPARγ activators) weight gain in adult humans
- Other chemicals with transgenerational effects on obesity?
  - DDT (prenatal) -> F3 obesity in 50% of animals (Skinner et al, 2013)
  - Plastics mixture -> F3 obesity (Manikkam et al, 2013)

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

- Are humans exposed to sufficient levels of TBT for concern?
  - PVC is up to 3% w/w (0.1 M) organotins
  - TPT used as fungicide on high value crops, used in water systems
  - Average blood level of 27 nM TBT in 32 random people tested
  - 84 ng/g liver of Japanese men ≈ 270 nM
  - Blood TPT levels from ~0.5–2 nM in Finnish fishermen
    - TBT not found in blood, but at ≈ 100 nM in placenta
    - Offspring with highest prenatal TBT exposure are fatter at 18 months

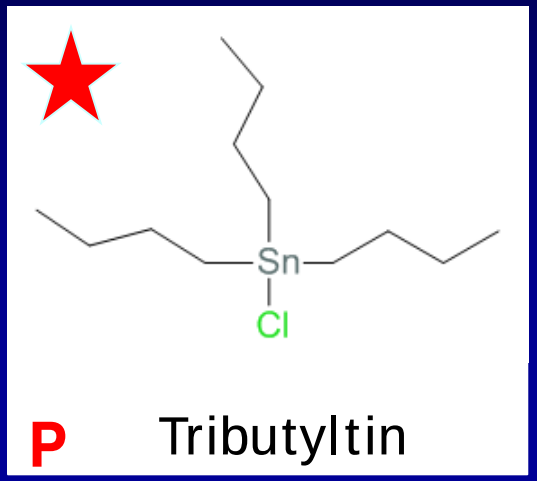
- Human exposure to organotins may reach levels sufficient to activate high affinity receptors
  - 1000 x lower dose than natural dietary RXR and PPARγ ligands

Is the environment making us fat?

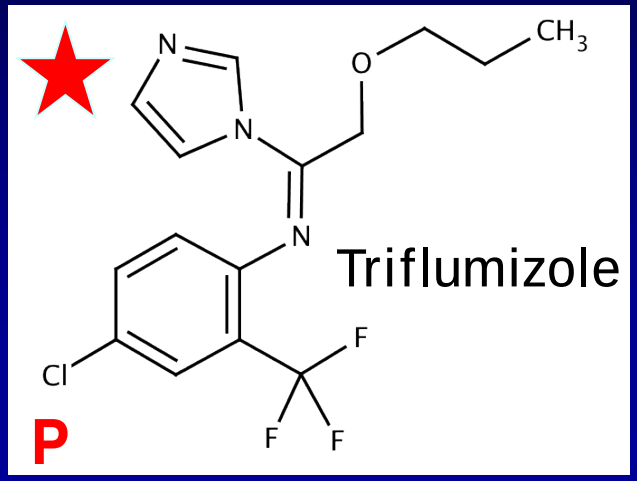
Obesogens - Just the Tip of the Iceberg ?

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

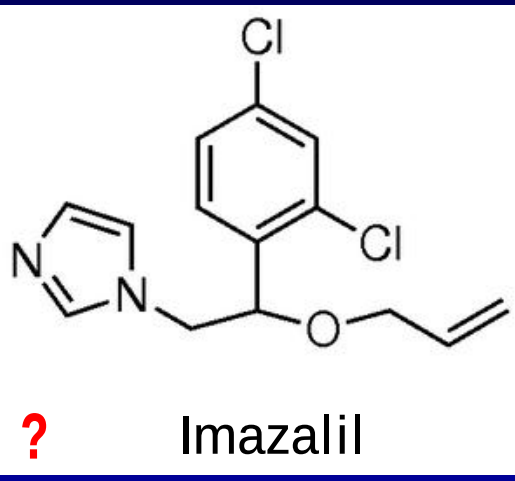
Surprisingly, Many Fungicides are Obesogens



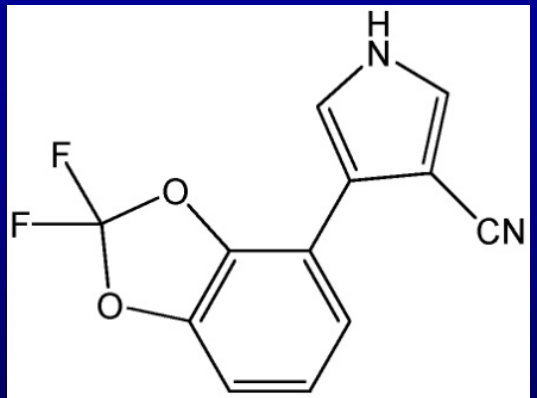
P Tributyltin



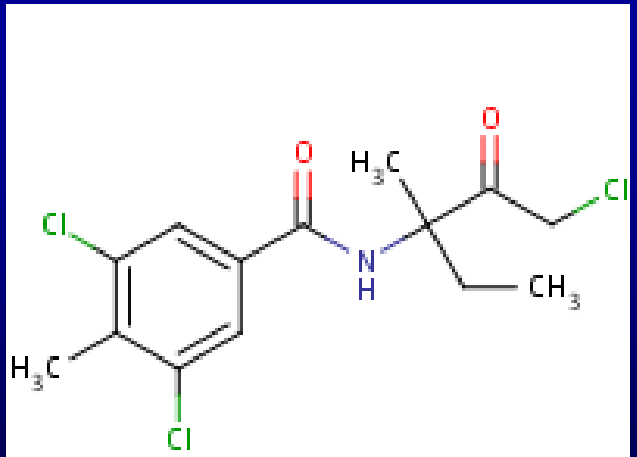
P Triflumizole



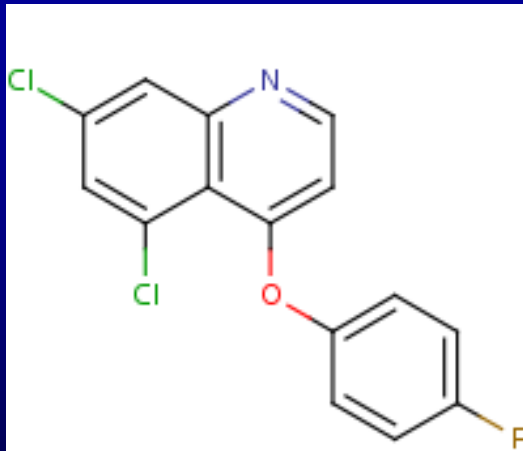
? Imazalil



R Fludioxonil



P Zoxamide



P Quinoxyfen

Janesick et al, 2016 *Environmental Health Perspectives*, *in press*

Obesogens - Just the Tip of the Iceberg ?

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

- 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, 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
- 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 to demonstrate substantial certainty of harm to humans before acting to reduce exposures ?
- How to integrate possibility of transgenerational effects into current risk assessment paradigms?

ehp ENVIRONMENTAL HEALTH PERSPECTIVES
http://www.ehponline.org

On the Utility of ToxCast™ and ToxPi as Methods for Identifying New Obesogens

Amanda Shaine Janesick, Giorgio Dimastrogiovanni, Lenka Vanek, Christy Boulos, Raquel Chamorro-García, Weiyi Tang, and Bruce Blumberg

http://dx.doi.org/10.1289/ehp.1510352

SUMMARY

- Screening for PPARγ and RXR transactivation appears to identify a good number of bona fide or potential obesogens
  - But not using ToxCast assays
  - Many obesogens activate neither receptor

- Combination of receptor activation and adipogenesis assays is a good predictor of activity in vivo (n=3)

- Toxcast assays for RXR and PPARγ activity are highly suspect
  - Attagene gives 5x more positives than other assays
  - Phase I ≠ Phase II results in the SAME ASSAYS
  - Why do HTS nuclear receptor assays work so poorly?

- A fair number of obesogens remain to be identified

- UCI - Blumberg Lab
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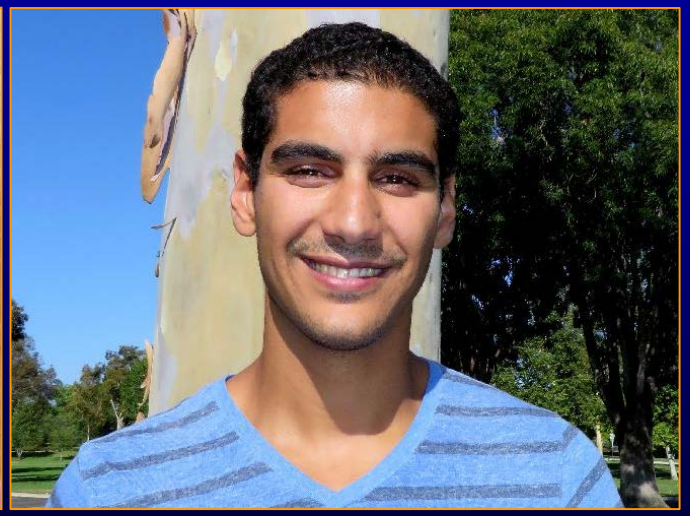

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