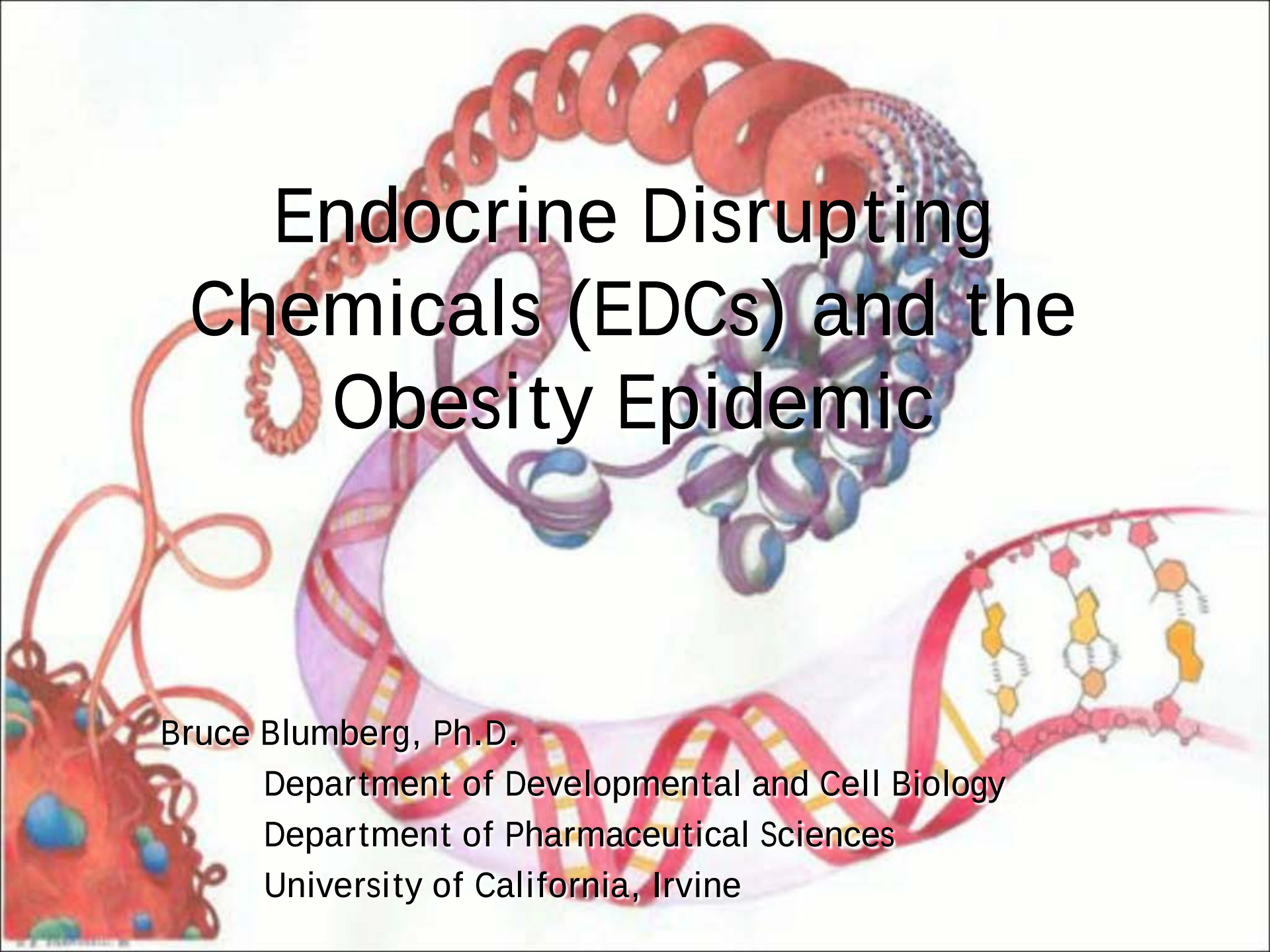


Endocrine Disrupting Chemicals (EDCs) and the Obesity Epidemic

Bruce Blumberg, Ph.D.
Department of Developmental and Cell Biology
Department of Pharmaceutical Sciences
University of California, Irvine

Main Points

- Obesogens exist and contribute to obesity epidemic
- Obesogen action may involve reprogramming of stem cells
- Effects of obesogen exposure are heritable

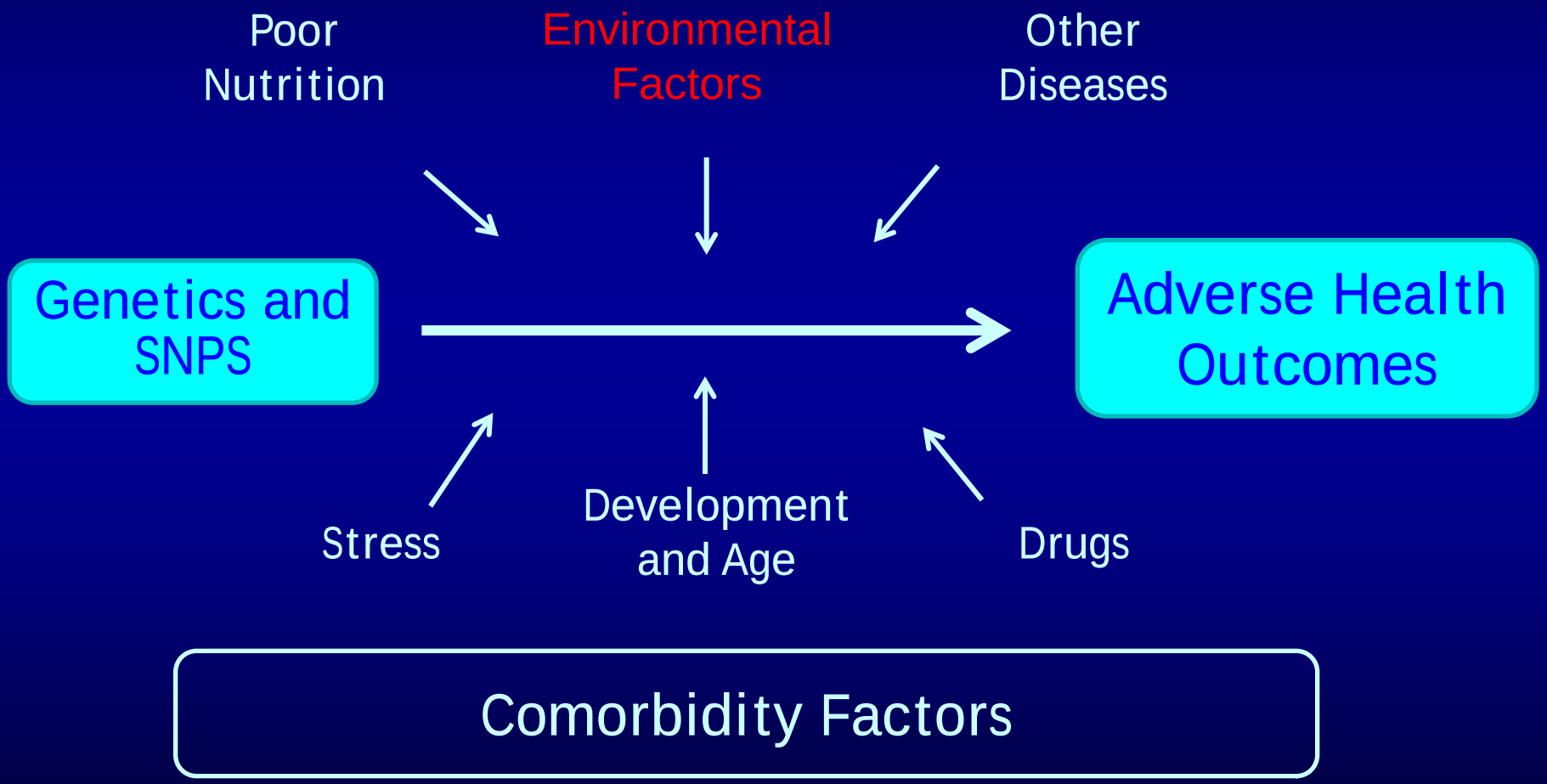
Non-communicable Diseases On the Rise

- **Leukemia, brain cancer:** over 20% increase since 1975
- **Asthma:** doubled between 1980 and 1995, stays elevated
- **Difficulty in conceiving and maintaining pregnancy:** 40% more women in 2002 than in 1982 (doubled in women aged 18-25)
- **Autism diagnosis:** increased 1000% over the past 3 decades
- **Obesity:** Increased 3 fold in US over past 40 years doubled worldwide last 20 years

- Account for 35 million deaths each year
- 60% of all deaths globally (80% occur in developing countries)

- **Genetic changes cannot explain increases in these diseases!**

Why do Certain People Develop Disease While Others Remain Healthy?



- **Role of environmental factors is important and under explored**

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 ?

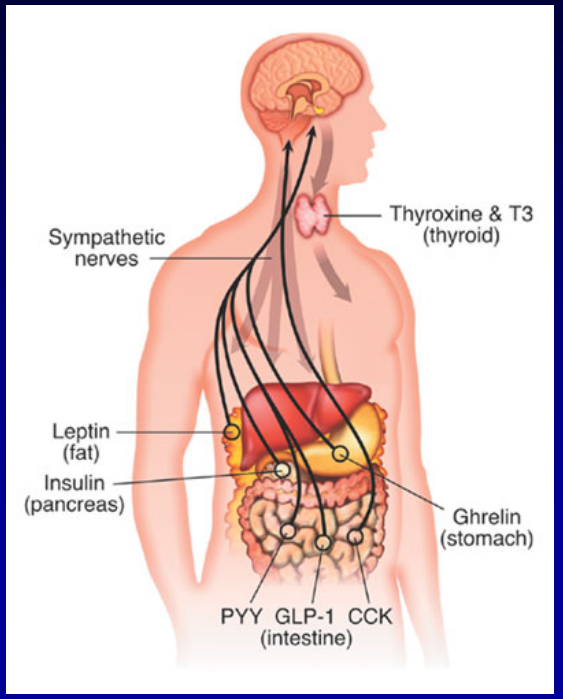
- Prevailing wisdom – "couch potato syndrome"
  - Positive energy balance, i.e., too much food, too little exercise

How does obesity occur ?

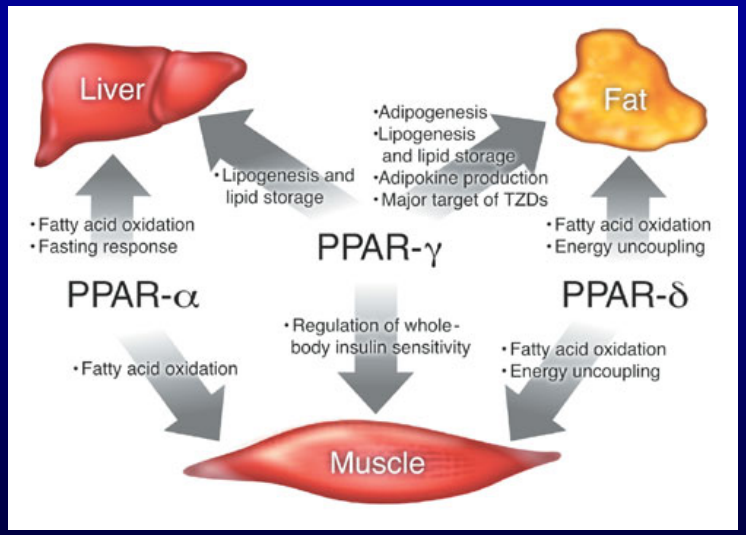
- Prevailing wisdom – "couch potato syndrome"
  - Positive energy balance, i.e., too much food, too little exercise
- Are there other factors in obesity ?
  - Stress (elevated glucocorticoids)
  - Inadequate sleep (stress?)
  - "Thrifty" genes which evolved to make the most of scarce calories
  - Viruses, 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**

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



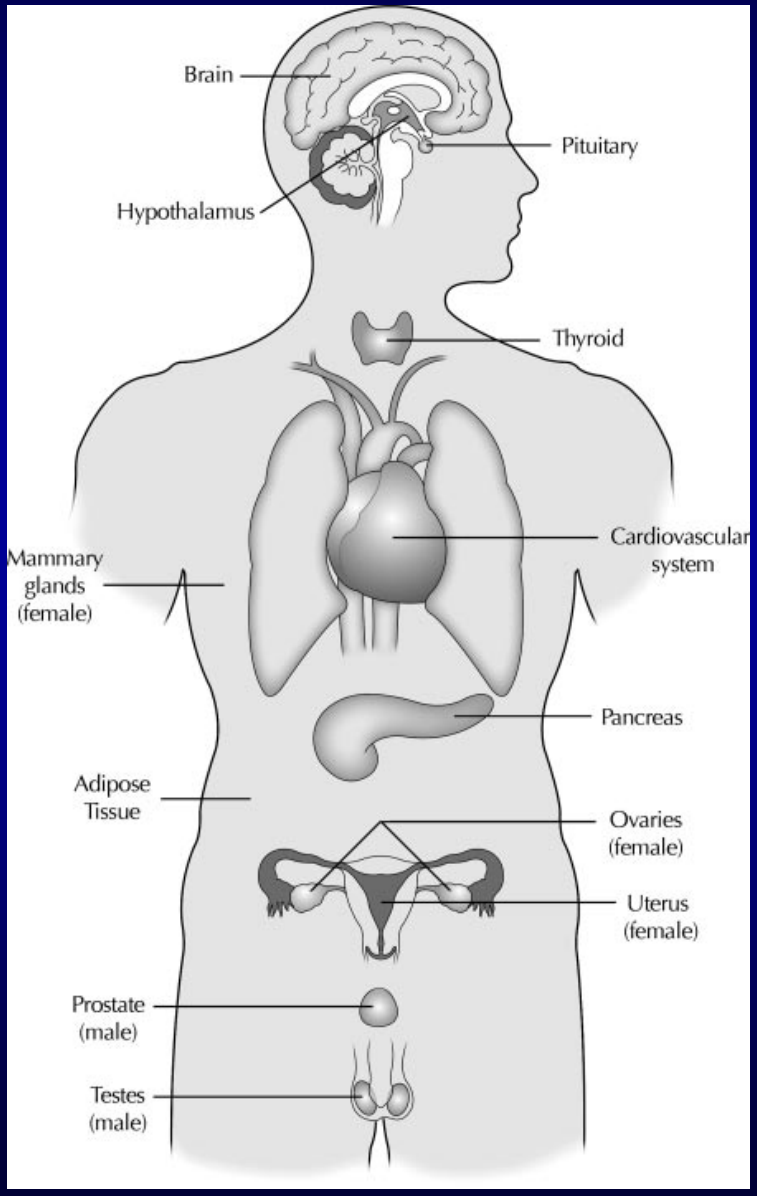
- Hormonal control of fat cell development and lipid balance
  - Regulated through nuclear hormone receptors RXR, PPARγ
  - PPARγ – master regulator of fat cell development
    - increased fat cell differentiation
    - Increased storage in existing cells
    - Increased insulin sensitivity



From Nature Medicine 10, 355 - 361 (2004)

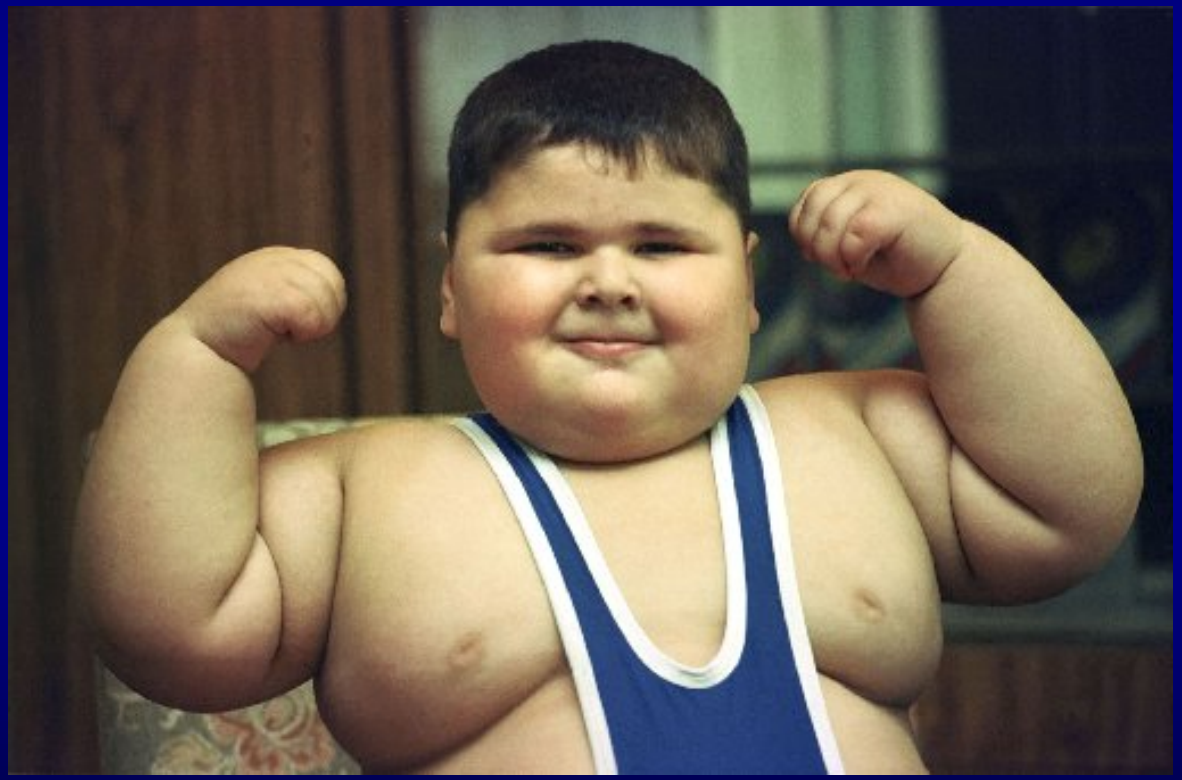
Endocrine Disrupting Chemicals (EDCs)

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 (POPs)
  - dietary components (pesticides)
  - personal care products
  - cleaning materials
  - food packaging
- Everyone is exposed

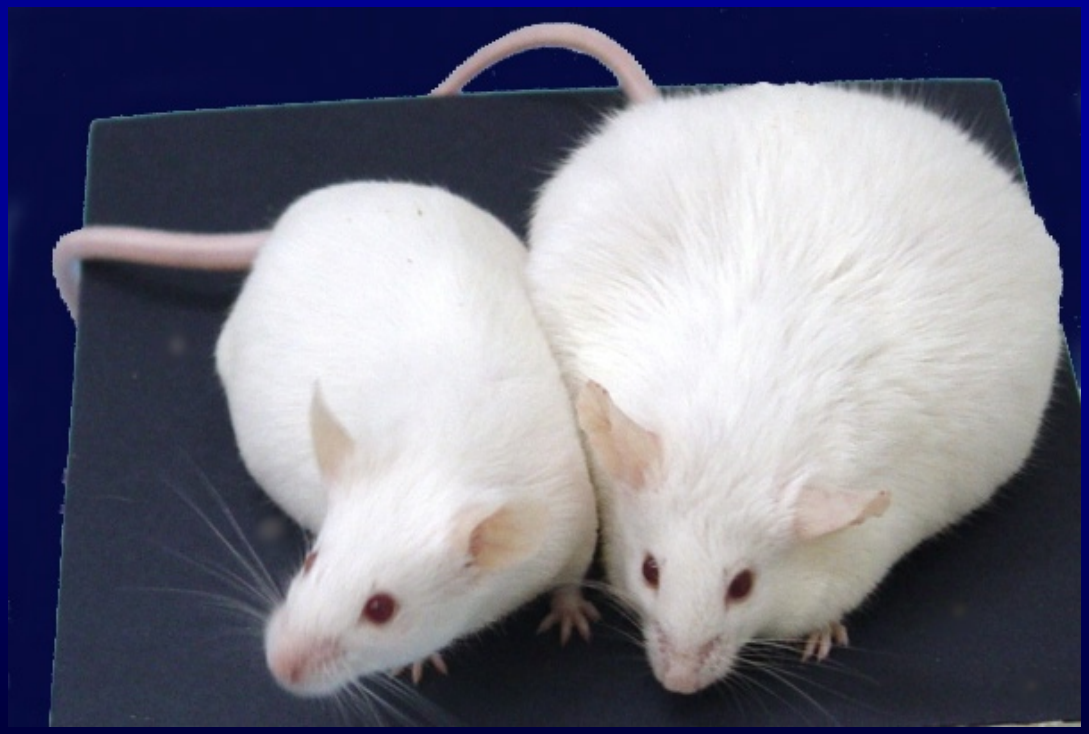
Do EDC-mediated disturbances in endocrine signaling pathways play a role in obesity?



Do EDC-mediated disturbances in endocrine signaling pathways play a role in 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



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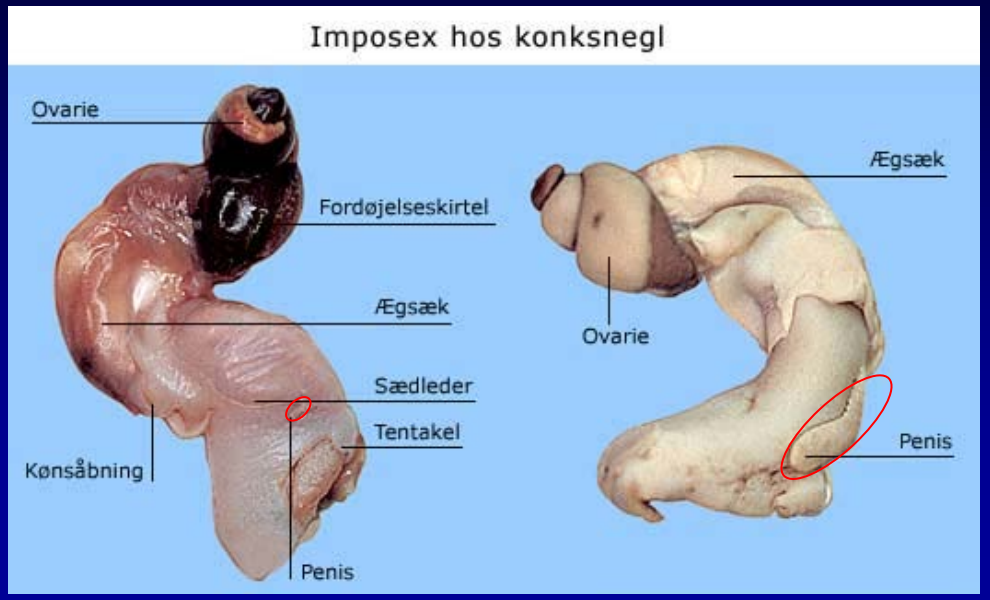
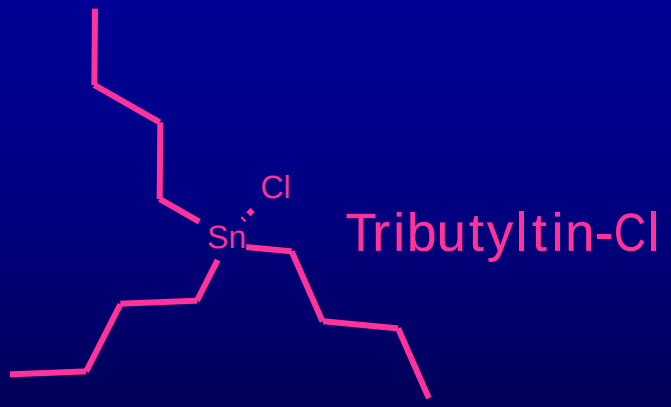
- Pre- and postnatal exposure to EDCs such as environmental estrogens (ER) increases weight
  - DES, genistein, bisphenol A
- Thiazolidinedione anti-diabetic drugs (PPARγ)
  - Increase fat storage and fat cell number at all ages in humans
- Urinary phthalates correlate with waist diameter and insulin 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?



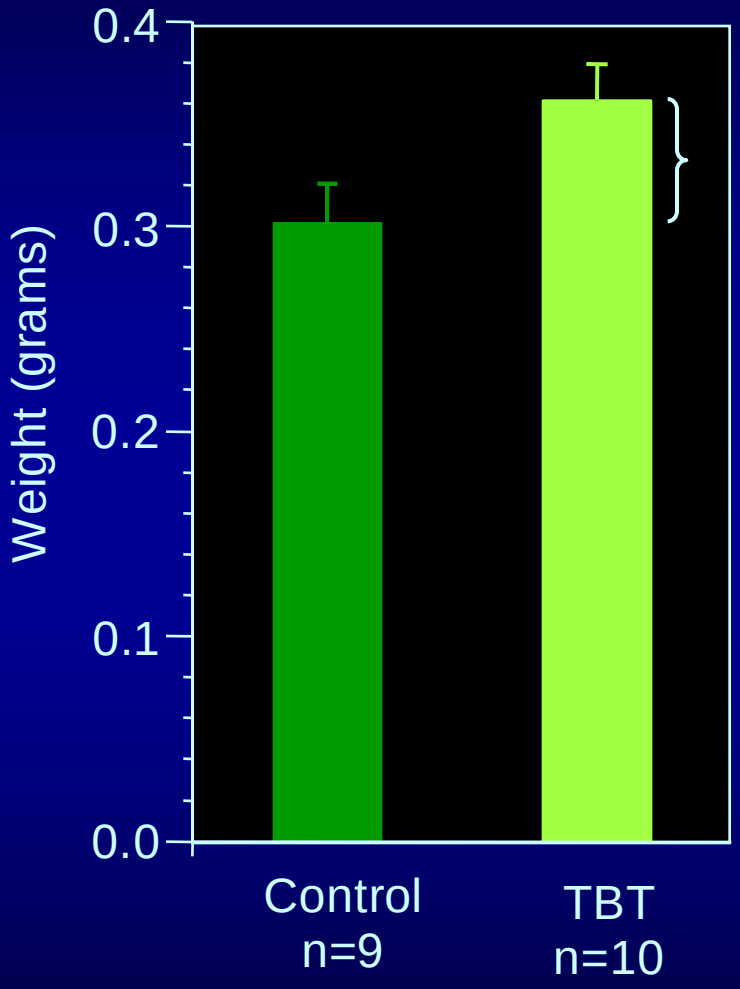
- We found that tributyltin (TBT)
  - Binds and activates at ppb (low nM) two nuclear receptors, RXR and PPARγ critical for adipogenesis
  - TBT induced adipogenesis in cell culture models (nM)
  - Prenatal TBT exposure led to weight gain in mice, in vivo



Grun et al., Molec Endocrinol, 2006

TBT increases testis fat pad weight

at 10 weeks



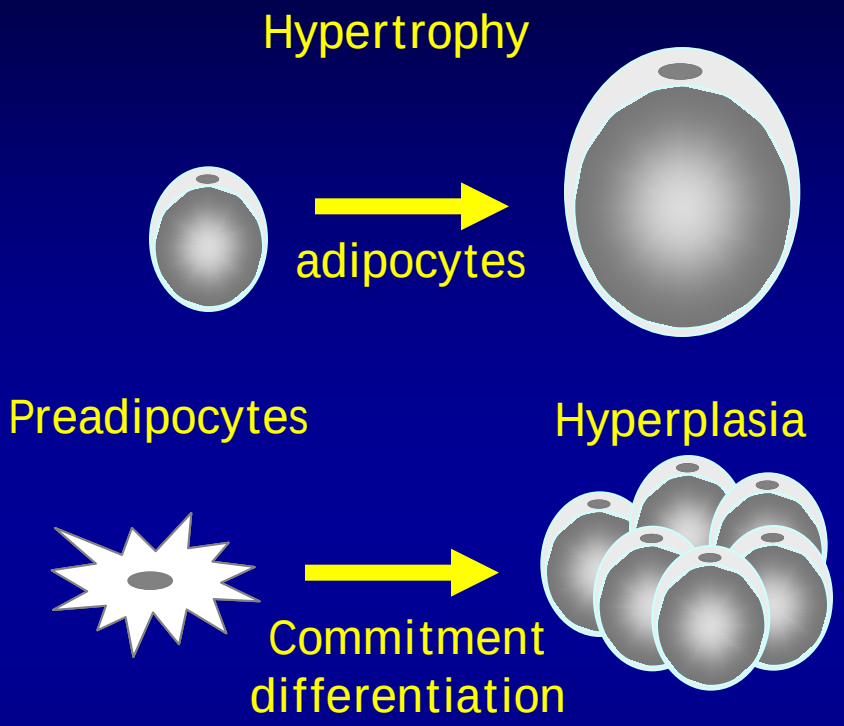
16% increase
p = 0.037

Fat depot size increases at the expense of overall body mass

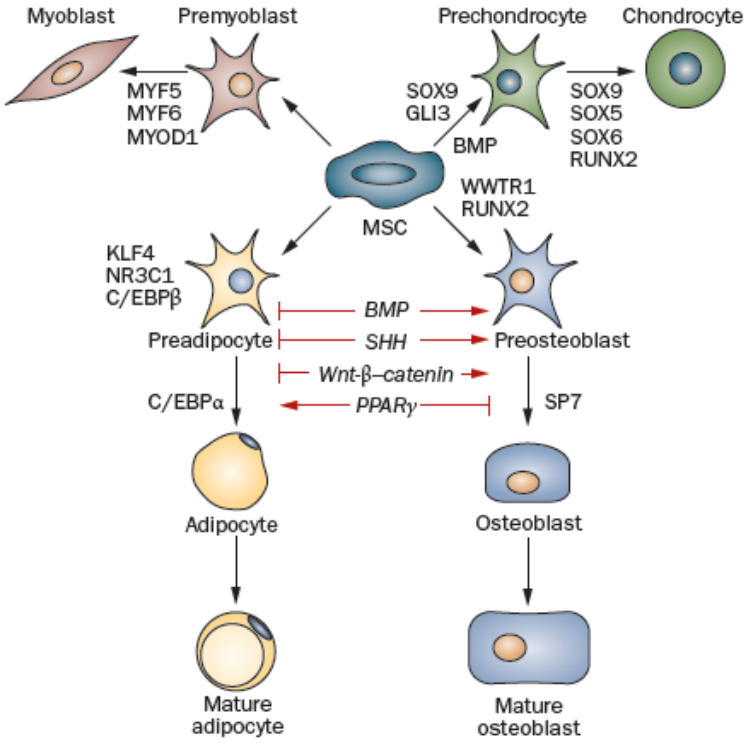
Grun et al., Molec Endocrinol, 2006

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?



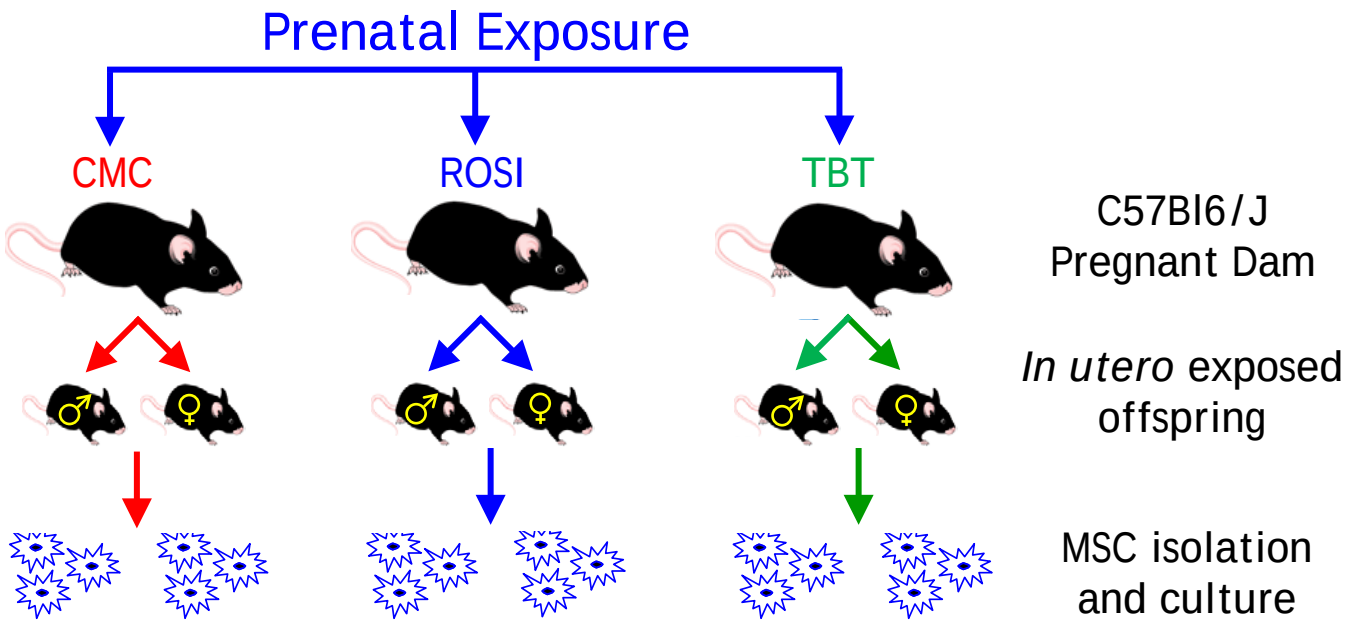
- Mesenchymal stem cells (MSCs) (a.k.a. multipotent stromal cells) precursors to many lineages including bone, cartilage, and adipose.
  - MSCs differentiate into adipocytes following rosiglitazone exposure
  - MSCs may (or may not) home to adipose depots after induction
- ***Hypothesis:*** TBT induces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



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

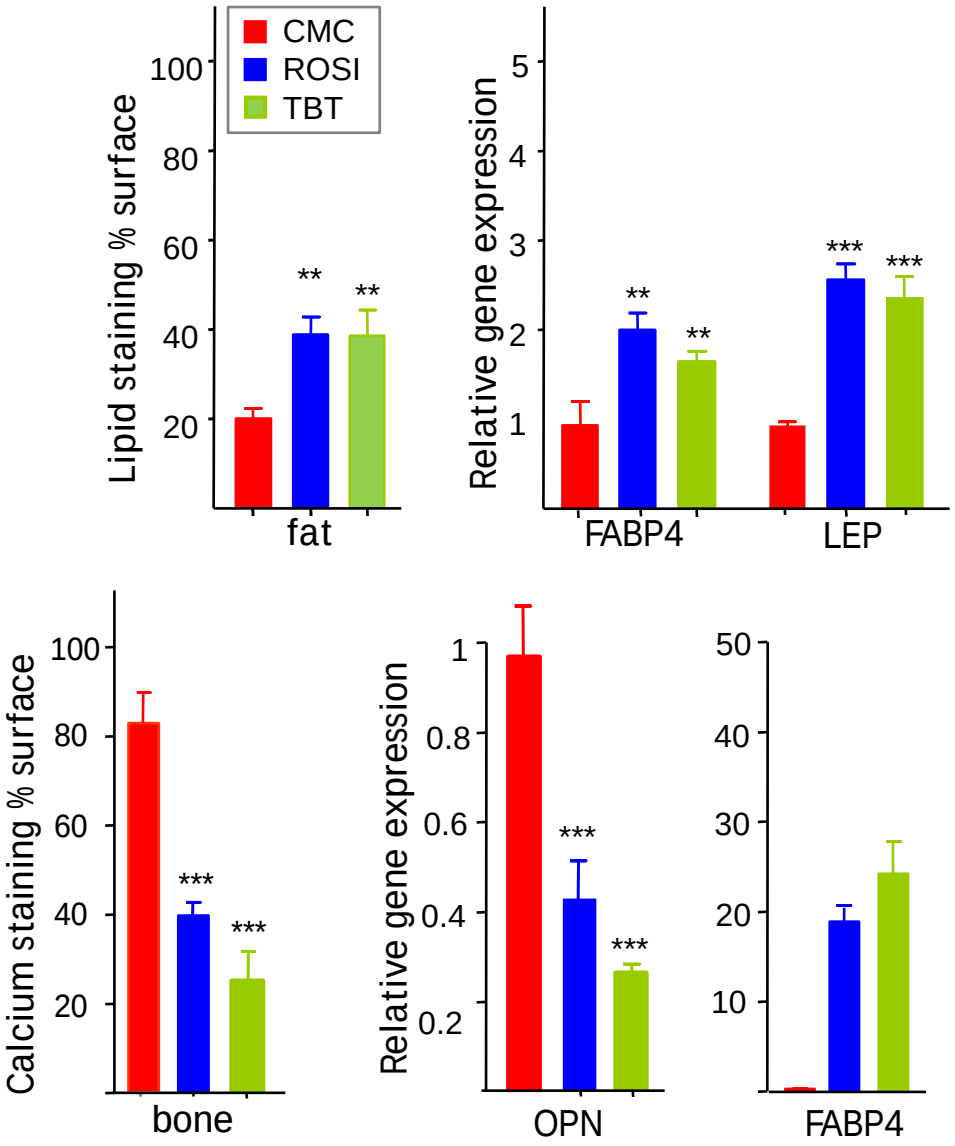
MSCs can give rise to many cell types in vivo

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



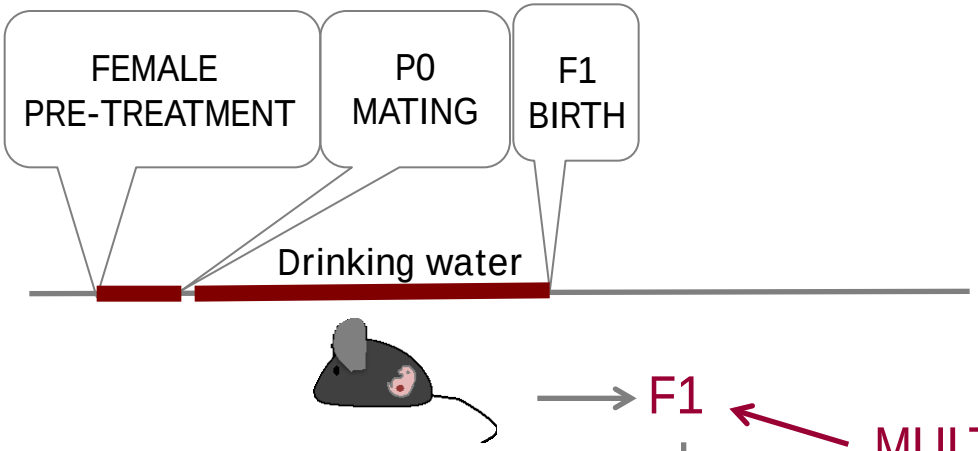
Kirchner et al, 2010 Molecular Endocrinology 24, 526-539

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 → F3 → Sacrificed

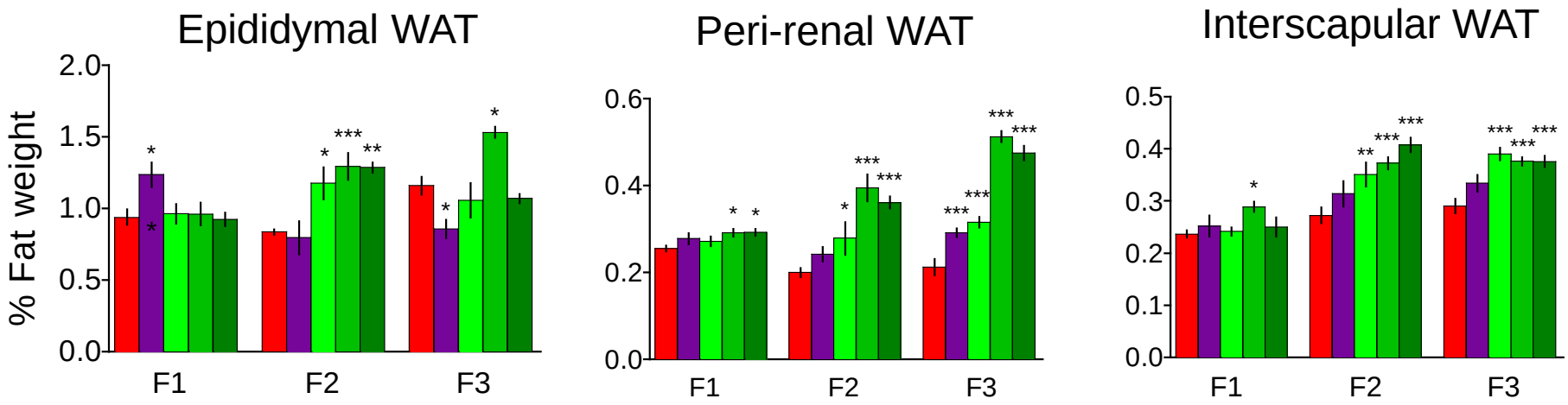
8 weeks old

- 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

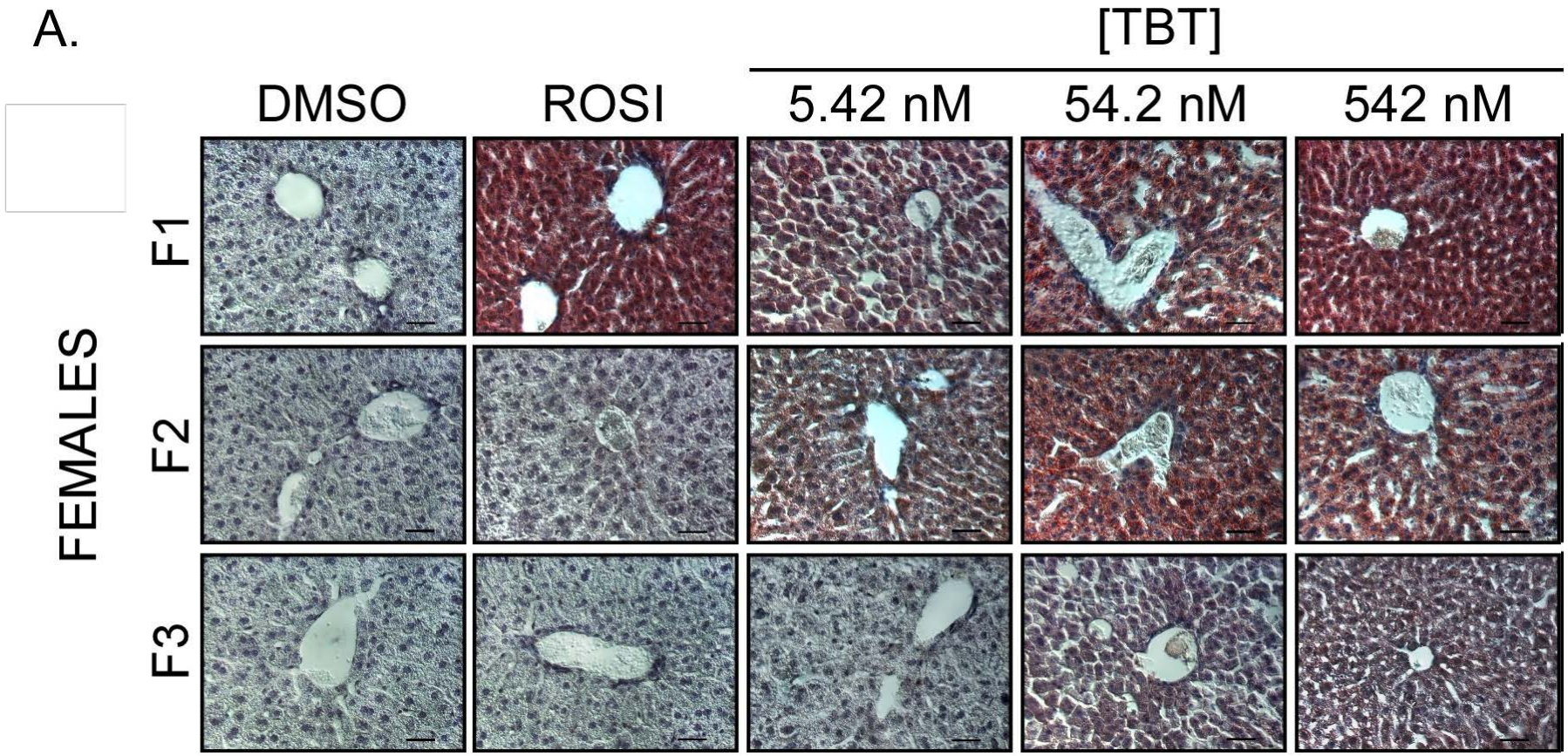


vehicle **ROSI** **TBT 5.4 nM** **TBT 54.2 nM** **TBT 542 nM**

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

TBT exposure has transgenerational effects

Non-alcoholic fatty liver disease



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)
- TBT drives adipocyte differentiation in cell culture
- TBT exposure during development induces adipogenesis in many vertebrates
  - Mouse, rats, frogs, fish
  - Inhibits bone formation in culture and in females
- Maternal TBT exposure causes transgenerational effects
- TBT detected in people at levels that cause effects in animals

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

- 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

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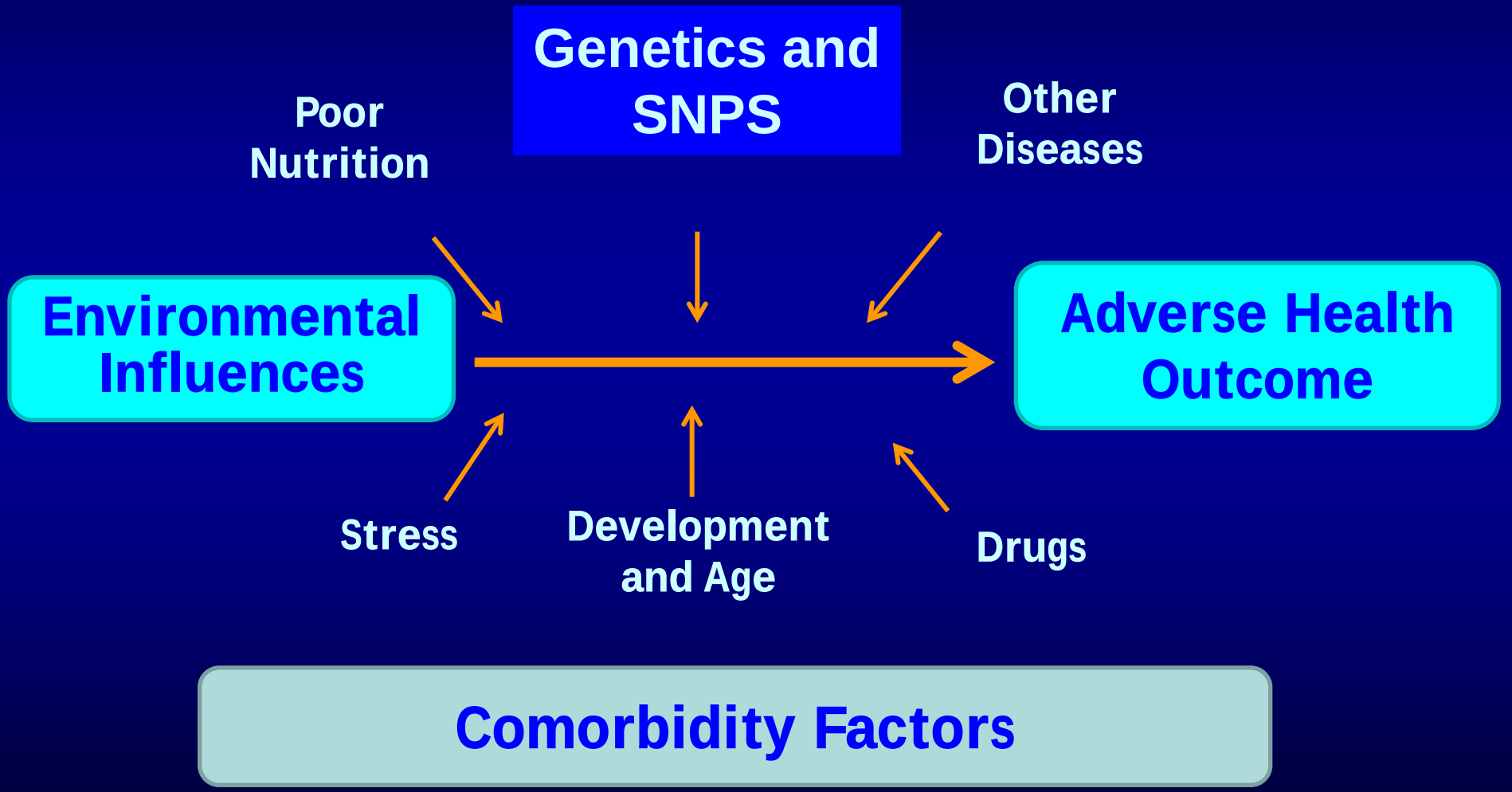
- Prenatal obesogen exposure reprograms exposed animals to be fat
  - Epigenetic changes alter fate of stem cell compartment -> more preadipocytes and more adipocyte progenitors
- Obesogens shift paradigm from treatment to prevention during pregnancy, childhood and puberty
  - Reduced exposure to obesogens, optimized nutrition

An better paradigm for chronic diseases ?



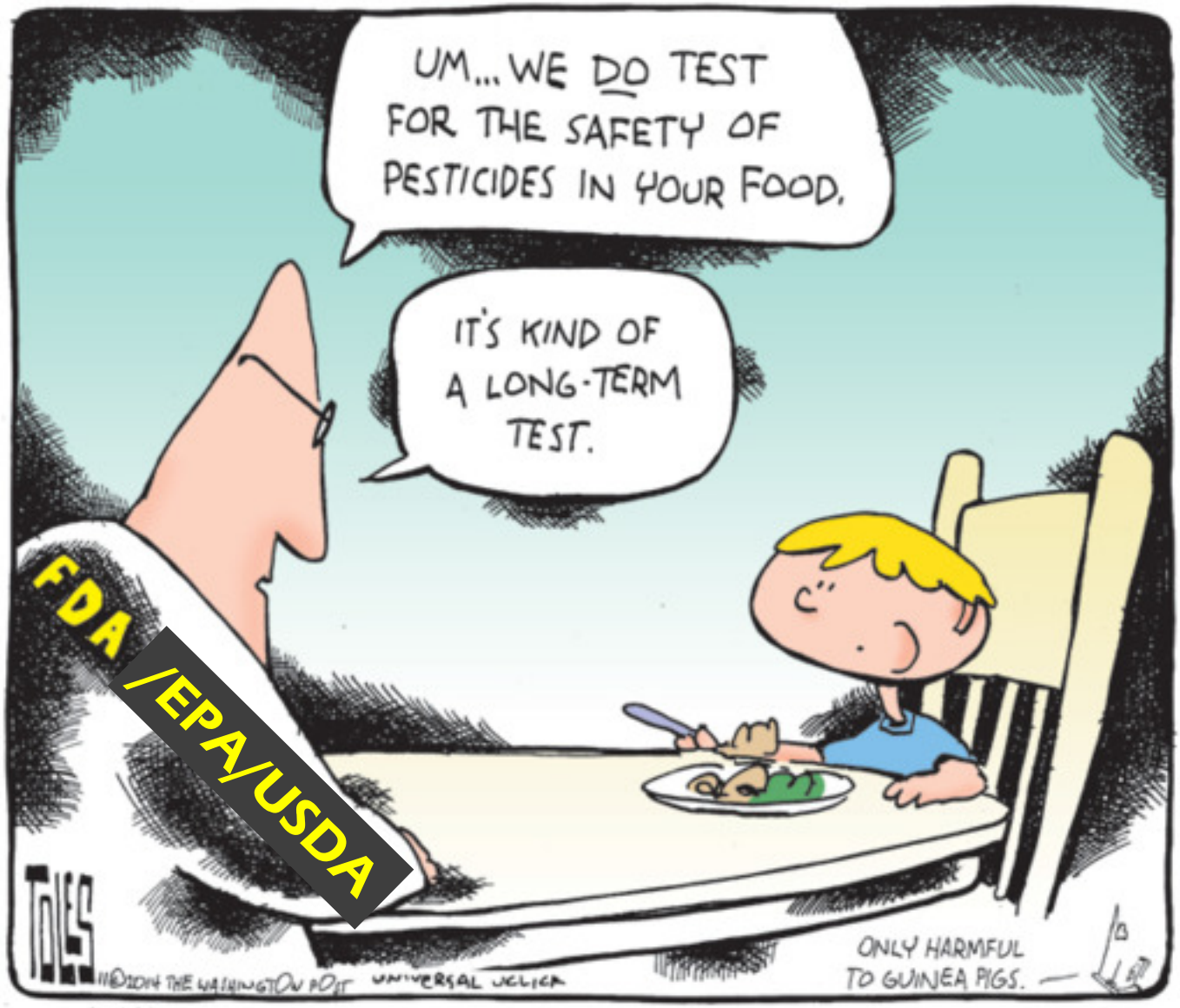
Chemicals with Transgenerational Effects

- 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 ?

Testing for effects of chemical exposure



- UCI - Blumberg Lab
  - Kotaro Azuma
  - Kayla Barekat
  - Raquel Chamorro-García
  - Riann Egusquiza
  - Catrine Ibrahim
  - Amanda Janesick
  - Takashige Kawakami
  - Ron Leavitt
  - Eric Martinez
  - Mary Sargious
  - Bassem Shoucri
  - Weiyi Tang
  - Griselda Valdez
- MGH/Harvard Medical School
  - Toshi Shioda
- Former lab members
  - Christy Boulos
  - Giorgio Dimastrogiovanni
  - Felix Grun
  - Elina Karimullina
  - Séverine Kirchner
  - Heidi Käch
  - Jasmine Li
  - Lenka Vanek
- NINS – Okazaki, Japan
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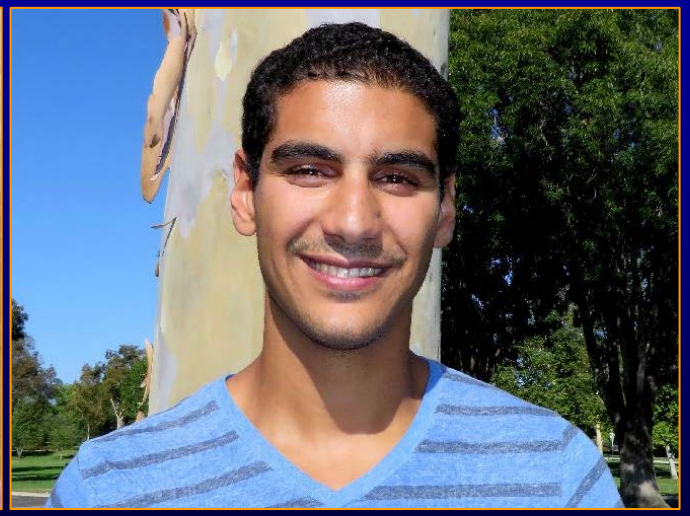
- NIHS - Tokyo, Japan
  - Jun Kanno
- Uppsala University
  - Monica & Lars Lind



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