

Identification of environmental obesogens

or

Is the environment making us fat?

Bruce Blumberg, Ph.D.

Department of Developmental and Cell Biology

Department of Pharmaceutical Sciences

Institute for Genomics and Bioinformatics

University of California, Irvine

The Worldwide Obesity Epidemic

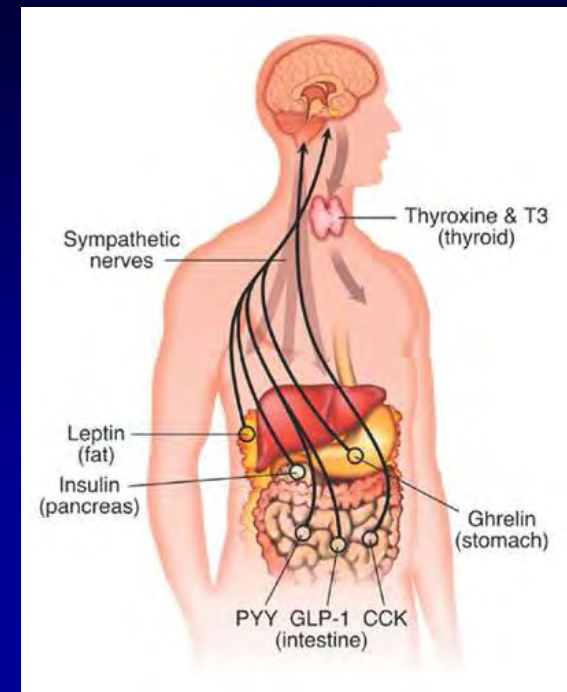
- 60 million people in the US are clinically obese
 - >30% above “ideal” body weight
- Obesity accounts for 8% of healthcare costs in Western countries
 - \$75 billion annually in US (2005)
- Obesity is associated with “metabolic syndrome” -> type 2 diabetes and cardiovascular disease
 - Central (abdominal obesity)
 - Atherogenic dyslipidemia (high triglycerides, high LDL, low HDL)
 - Hypertension
 - Insulin resistance
 - Prothrombotic state
 - Pro-inflammatory state (elevated CRP)

How does obesity occur ?

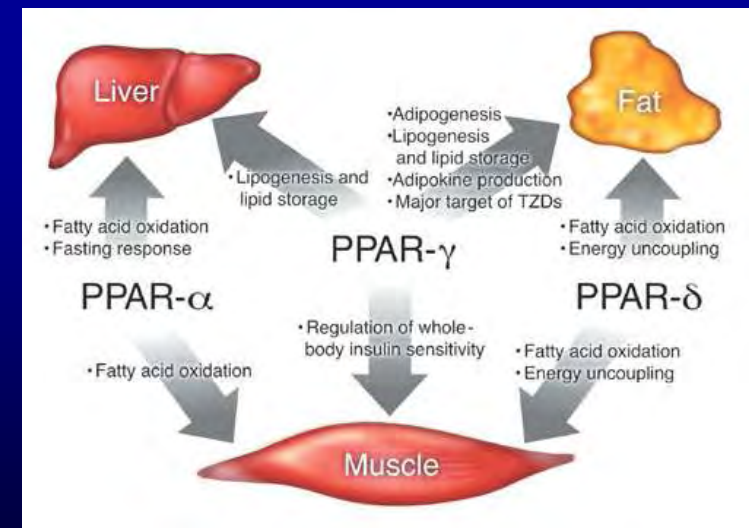
- Prevailing wisdom - “couch potato syndrome”
 - Positive energy balance, i.e., too much food, too little exercise
- Other factors ?
 - Stress (elevated glucocorticoids)
 - Inadequate sleep (stress?)
 - “Thrifty” genes which evolved to make the most of scarce calories
 - Viruses, SNPs
- What about role of prenatal nutrition or in utero experience?
 - 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

Hormonal control of weight

- Hormonal control of appetite and metabolism
 - Leptin, resistin adiponectin ghrelin are key players
 - Leptin, adiponectin, resistin - adipocytes
 - Ghrelin - stomach
 - Thyroid hormone/receptor



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

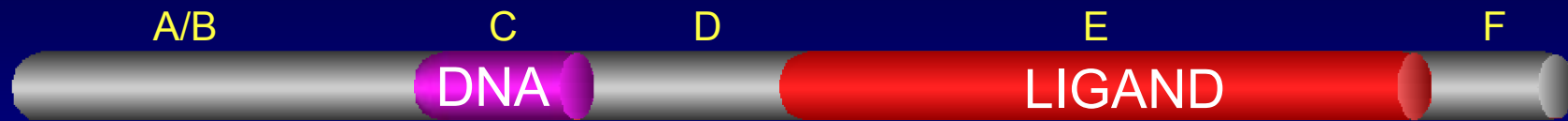


From Nature Medicine 10, 355 - 361 (2004)

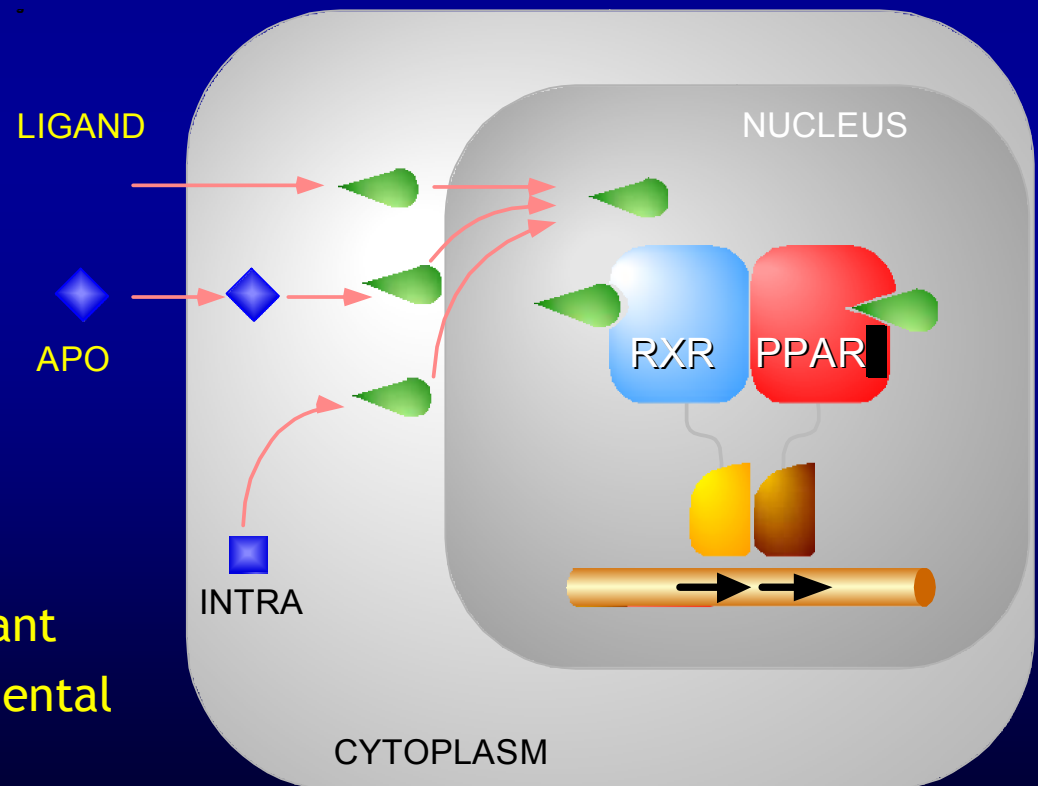
Endocrine Disrupting Chemicals (EDCs)

- Endocrine disrupter - a compound that mimics or blocks the action of endocrine hormones, either directly or indirectly
- Often persistent pollutants or dietary components
- Disturb development, physiology and homeostasis
- Frequently act through nuclear hormone receptors
 - Environmental estrogens
 - Anti-androgens
 - Anti-thyroid
- Are disturbances in endocrine signaling pathways involved in adipogenesis and obesity ?

Nuclear Receptors - A Large Family of Ligand Modulated Transcription Factors



- Bind to specific DNA targets - Hormone Response Elements
 - Most are activators
 - Some constitutive
 - Few inactivate
- Ligands are small lipophilic molecules that freely enter cells
 - Diffuse from source & penetrate to a target
- Respond to low levels of hormone
 - Parts per billion levels
 - Regulation of levels is important
 - Can be disrupted by environmental contaminants



The Nuclear Hormone Receptor Superfamily



Known Receptors

Classical receptors (from biochemistry)

GR	cortisol
MR	aldosterone
→ AR	testosterone
PR	progesterone
→ ER α, β	estradiol
VDR	1,25-(OH) ₂ vit D3
→ TR α, β	triiodothyronine
EcR	20-OH ecdysone

Adopted (EX) Orphans

RAR α, β, γ	all- <i>trans</i> retinoic acid
→ RXR α, β, γ	9- <i>cis</i> retinoic acid
→ PPAR α, β, γ	fatty acids, eicosanoids
LXR α, β	oxy-sterols
FXR α, β	bile acids
BXR α, β	benzoates

Nearly adopted orphans (natural ligands?)

CAR	androstanes, xenobiotics
→ SXR/PXR	steroids, xenobiotics

Orphan Receptors

Vertebrate

TR-2 α, β
NGFI-B α, β, γ
ROR α, β, γ
Rev-erb α, β
SF-1 α, β
COUP α, β, γ
HNF-4 α, β
Tlx α, β

Drosophila

DHR78
DHR38
DHR3
E75, E78
FTZ-F1 α, β
<i>svp</i>
HNF-4
<i>tll</i>

No known homologs

ERR α, β, γ	knirps
DAX-1	knirps-related
SHP	egon
GCNF	DHR96

C. elegans

~250 nuclear receptors

D. melanogaster

~20 nuclear receptors

H. sapiens

~48 genes

Arabidopsis

no family members

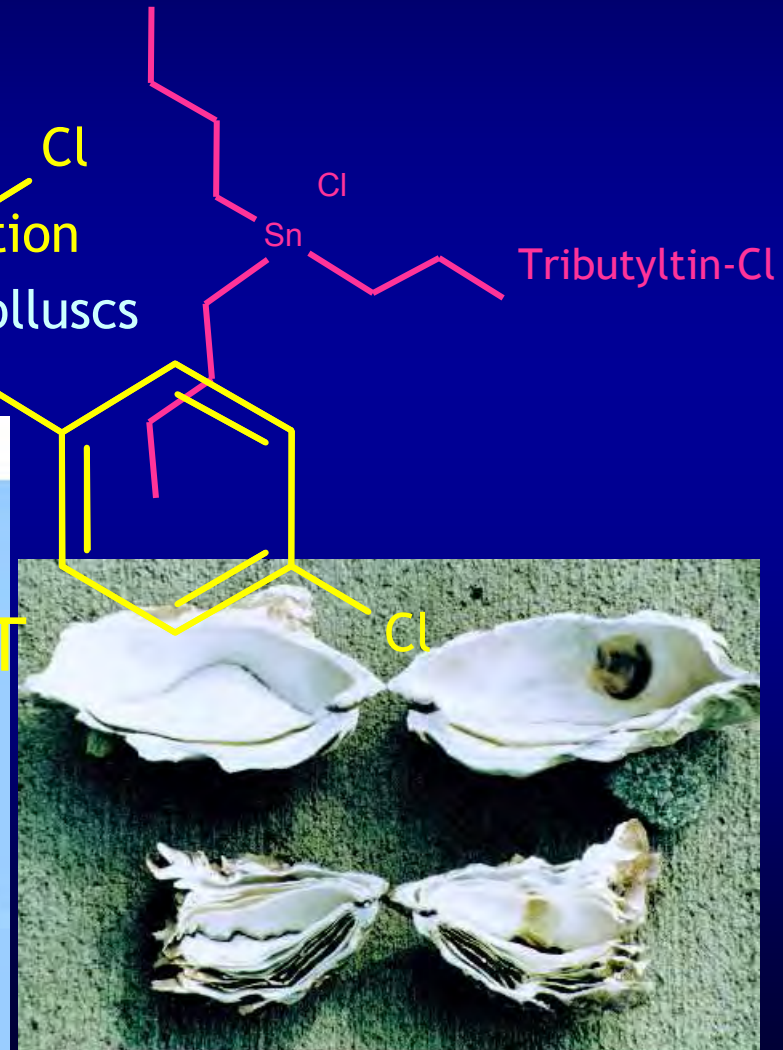
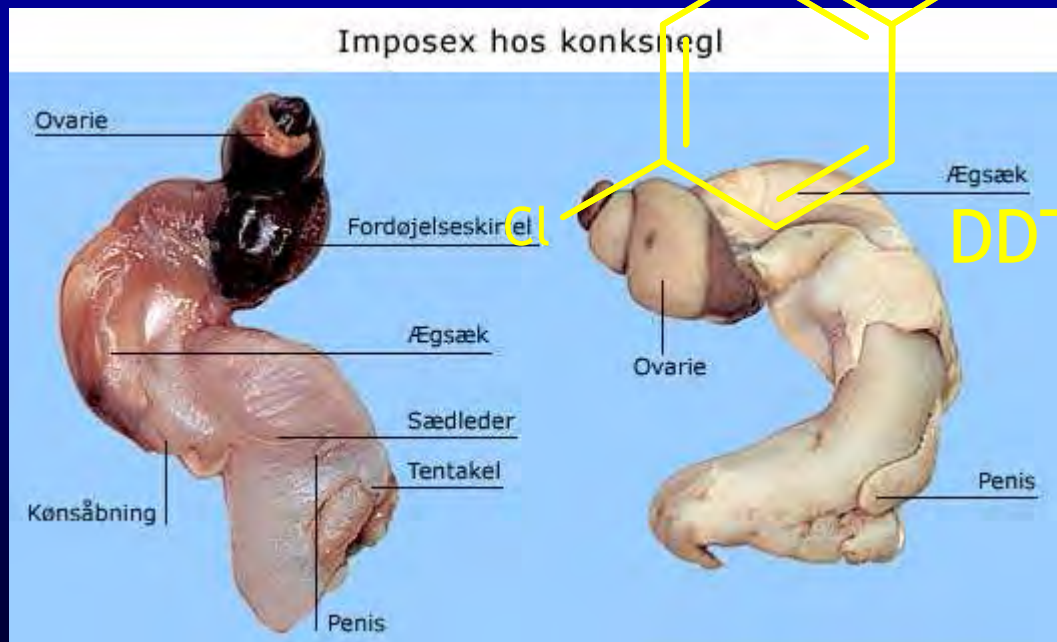
The obesogen hypothesis

- *Obesogens* - chemicals that inappropriately stimulate adipogenesis and fat storage, exist and contribute to obesity epidemic
- Pre- and postnatal exposure to environmental estrogens (ER) increases weight
 - Bisphenol A, alkylphenols, DES, genistein
- Thiazolidinedione anti-diabetic drugs (PPAR γ)
 - Increase fat storage and fat cell size at all ages in humans
- Phthalates, PFOA cause fat cell differentiation in vitro (PPAR γ)
- What about human epidemiology ?
 - Urinary phthalates correlate with waist diameter and insulin resistance in humans

Endocrine disruption by organotins - invertebrates

Endocrine disrupter - a compound that mimics or blocks the action of endocrine hormones, either directly or indirectly

- Organotins -> imposex in molluscs
- Direct inhibitory effect on aromatase (CYP19) enzymatic activity
 - Also blocks testosterone esterification
- Alters shell development in bivalve molluscs

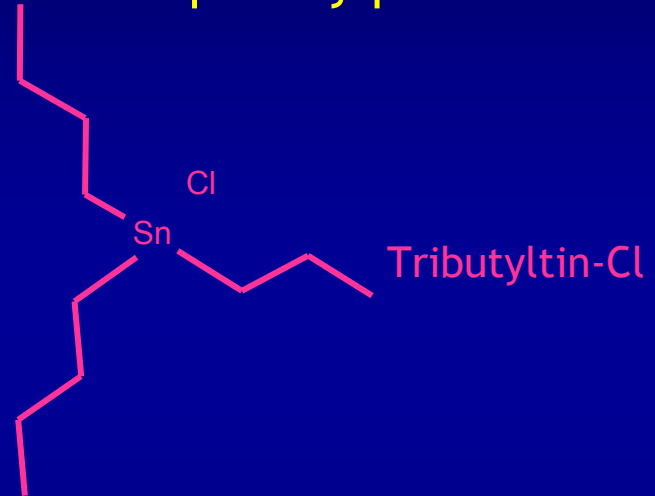


Organotin effects on vertebrates

- Spermatotoxic
 - Sperm lack flagella or have impaired motility (fish, rat)
- Sex reversal in fish (*Danio rerio* and Japanese flounder)
 - Increased % males
 - Masculinization of genetically female flounder (~25-30%)
- Mild effects on mammalian sex characteristics
 - Inhibits aromatase in cultured granulosa cells
 - Reduced seminal vesicle weights in male rats
- Immunotoxic (human exposure limits based on these effects)
 - induces neutrophil apoptosis
 - Inhibit cytotoxic function of NK cells
- Hepatotoxic, neurotoxic
 - Trimethyl and triethyltins are potent specific neurotoxins

Routes of exposure to organotins

- Marine ship paints
 - Trialkyltins are potent biocidal antifouling agents for molluscs
 - Widely used 1960-1970s, regulated but incompletely phased out
 - Contaminates seafood
- Other uses and routes of exposure
 - Fungicide on high value food crops (potatoes, rice, celery, pecans)
 - Wood preservative
 - Catalysts for organic synthesis
 - Heat stabilizers in manufacture of polyolefin plastics (PVC)
- Bioaccumulative
 - BCF of ~2-11K in oysters, 17K-350K in mussels
 - BCF of ~2.5K-12K in fishes
 - BCF of ~30K in algae



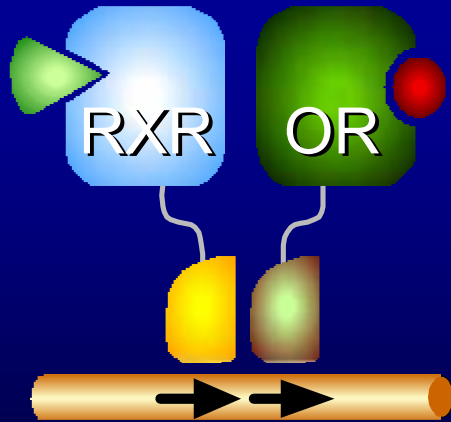
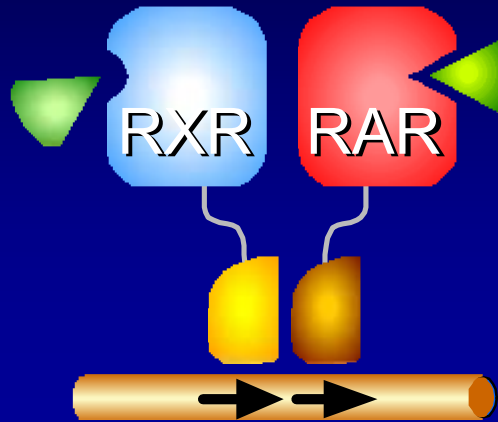
How do organotins cause endocrine disruption?

- Direct inhibition of aromatase (CYP19) activity (μM concentrations)
 - Inhibition of testosterone storage
- Transcriptional effects on aromatase expression
 - CYP19 in human ovarian granulosa cells is sensitive to inhibition by TBT, RXR- and PPAR γ -specific ligands
- TBT alters sex determination - typically female -> male
 - TBT effects are seen at nM doses and below
 - Sex determination requires sex steroids at critical times in development
 - Sex steroids act through nuclear receptors
- *Hypothesis* - TBT alters the activity of one or more nuclear receptors, thereby causing endocrine disruption
 - Test nuclear receptors for activation or inhibition by organotins
 - Expect effect on steroid receptor activity

Nuclear receptor LBD activation by TBT

<i>Construct</i>	<i>Fold activation (60 nM TBT)</i>	<i>Permissive RXR heterodimer?</i>
→ RXR α (human)	60	Yes
→ RXR α (xenopus)	25	Yes
→ RXR γ (xenopus)	7.0	Yes
NURR1 (human)	7.0	Yes
LXR α (human)	2.1	Yes
PPAR α (mouse)	0.7	Yes
→ PPAR γ (human)	5.3	Yes
PPAR δ (human)	1.7	Yes
RAR α (human)	0.7	No
TR β (human)	0.4	No
VDR (human)	0.5	No
SXR (human)	1.0	No

RXR is a key partner for many pathways



Known ligands

RAR α, β, γ

TR α, β

VDR

PPAR $\alpha, \beta / \delta, \gamma$

LXR α, β

FXR α, β

BXR α, β

EcR

all-trans RA

thyroid hormone

1,25-(OH)₂-VD₃

fatty acids, eicosanoids

oxysterols

bile acids

benzoates

ecdysteroids

Activatable Orphans

SXR/PXR

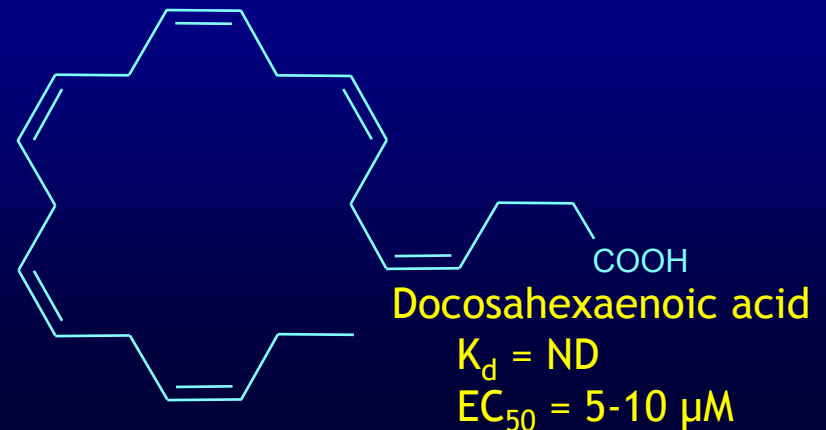
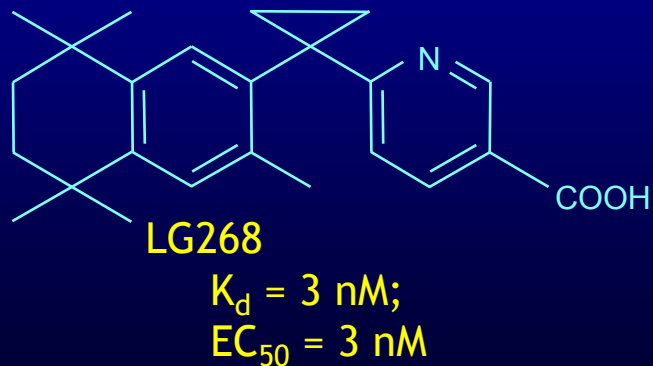
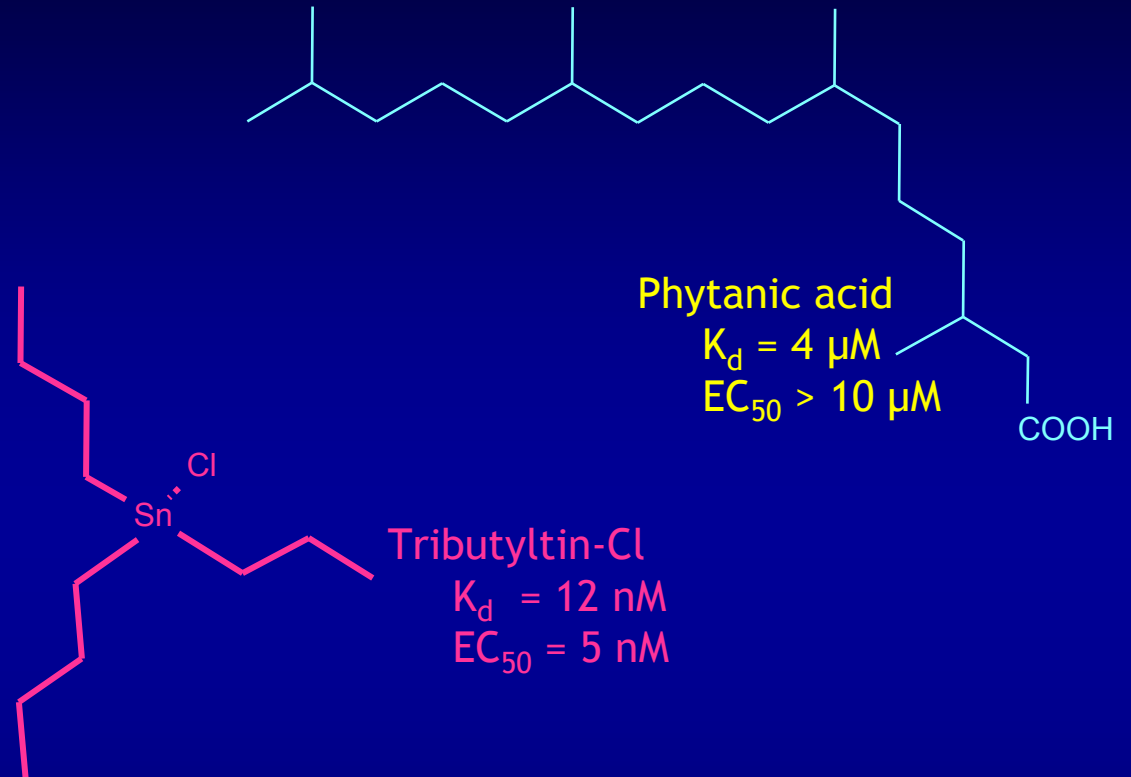
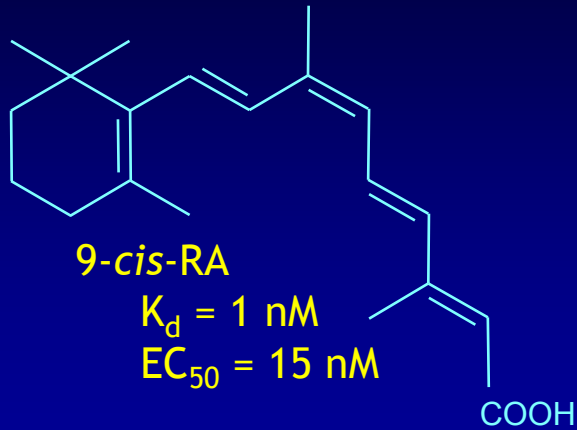
CAR

steroids, xenobiotics

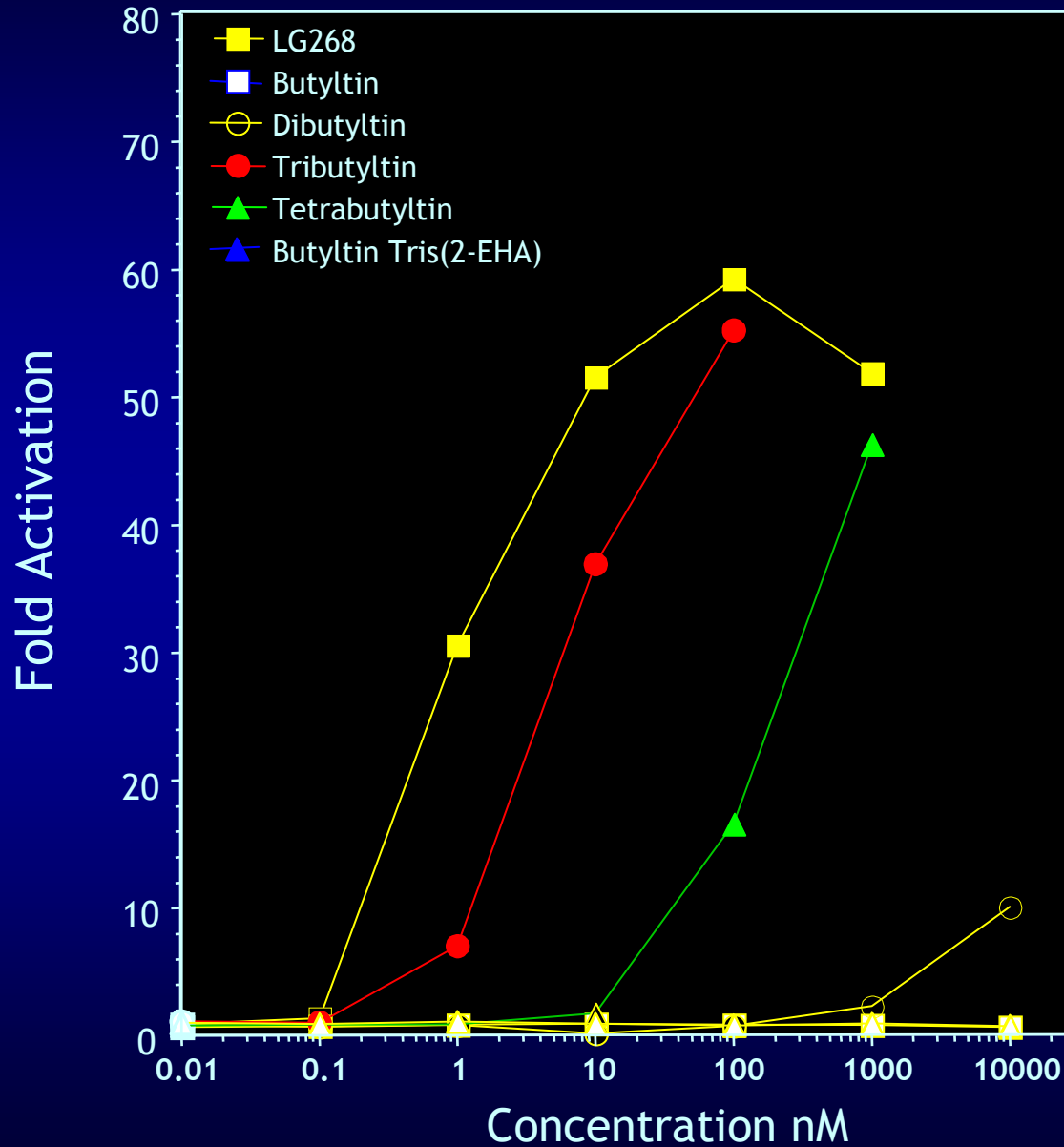
xenobiotics, androstanes

Inappropriate RXR activation may be expected to cause wide ranging disturbances in the body's homeostatic hormonal controls

Structures of RXR-specific agonists

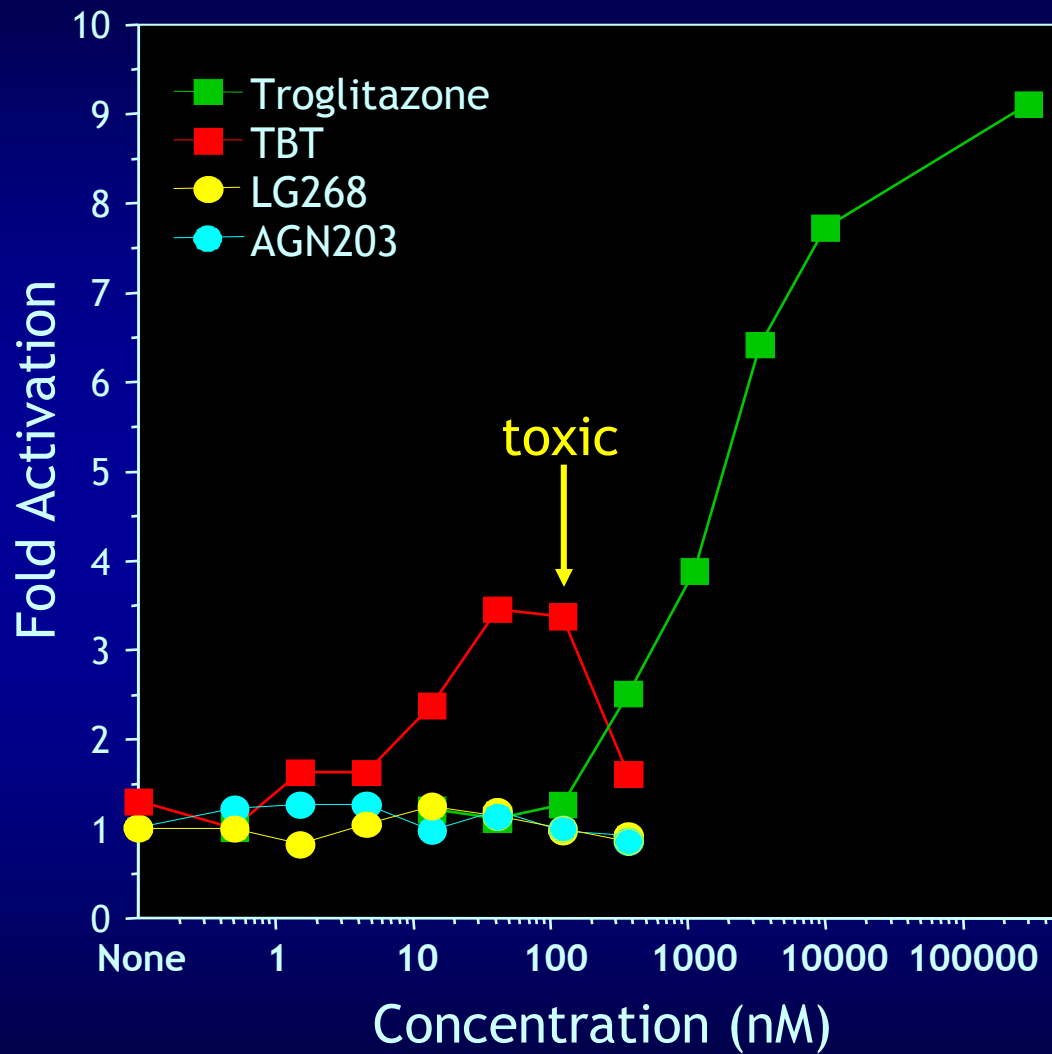


Organotins activate hRXR α



	EC ₅₀
DBT	> 2800 nM
TBT	5 nM
4BT	150 nM

TBT activates PPAR γ



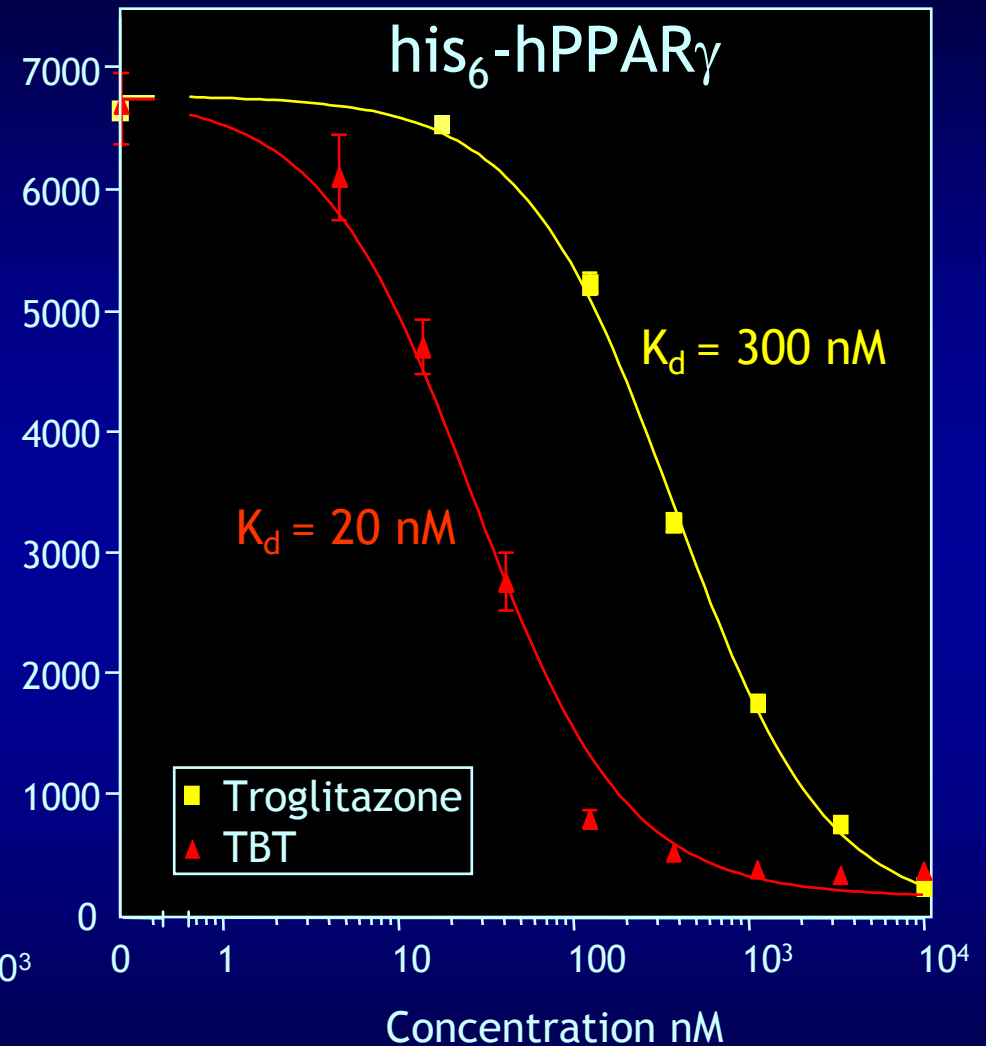
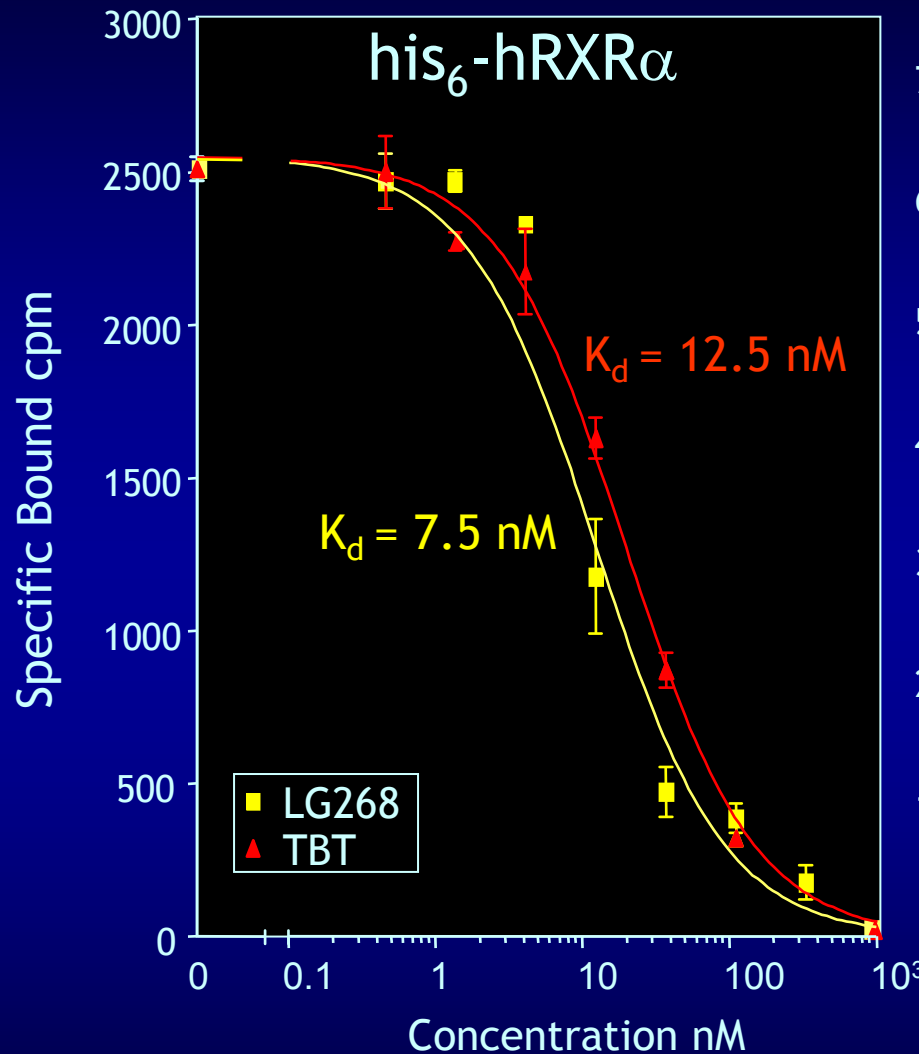
PPAR γ -regulates lipid metabolism and adipocyte differentiation

Nuclear receptor activation by organotin

Ligand	Nuclear Receptor LBD EC ₅₀ nM		
	hRXRα	hRARα	hPPARγ
LG268	2-5	na	na
AGN203	0.5-2	na	na
9- <i>cis</i> RA	15		na
all- <i>trans</i> RA	na	8	na
Butyltin chloride	na	na	na
Dibutyltin chloride	3000	na	na
Tributyltin chloride	3-8	na	20
Tetrabutyltin chloride	150	ND	ND
Di(triphenyltin) oxide	2-10	na	20
Butyltin-tris (2-ethylhexanoate)	na	ND	ND
Troglitazone	na	na	1000

Organotins are highly potent nuclear receptor agonists
Do they bind to the receptors?

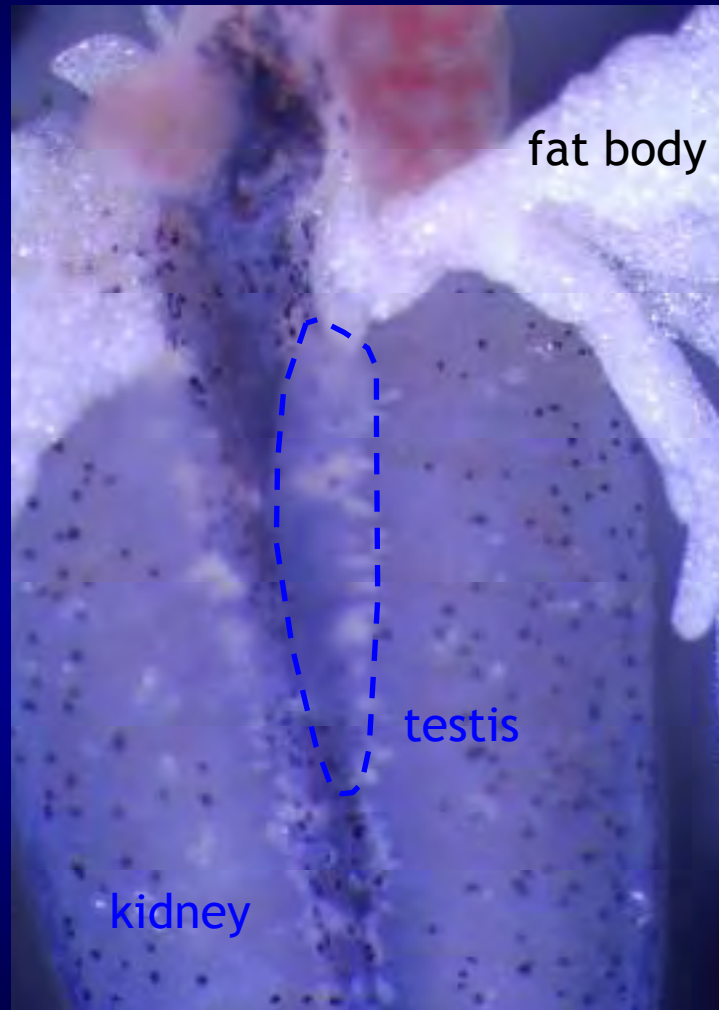
Competitive Binding of TBT



- TBT binds to and activates RXR and PPAR γ with high affinity
- What effect does it have on sex determination?

Xenopus laevis Gonads/Kidney ± TBT

Vehicle (DMSO)

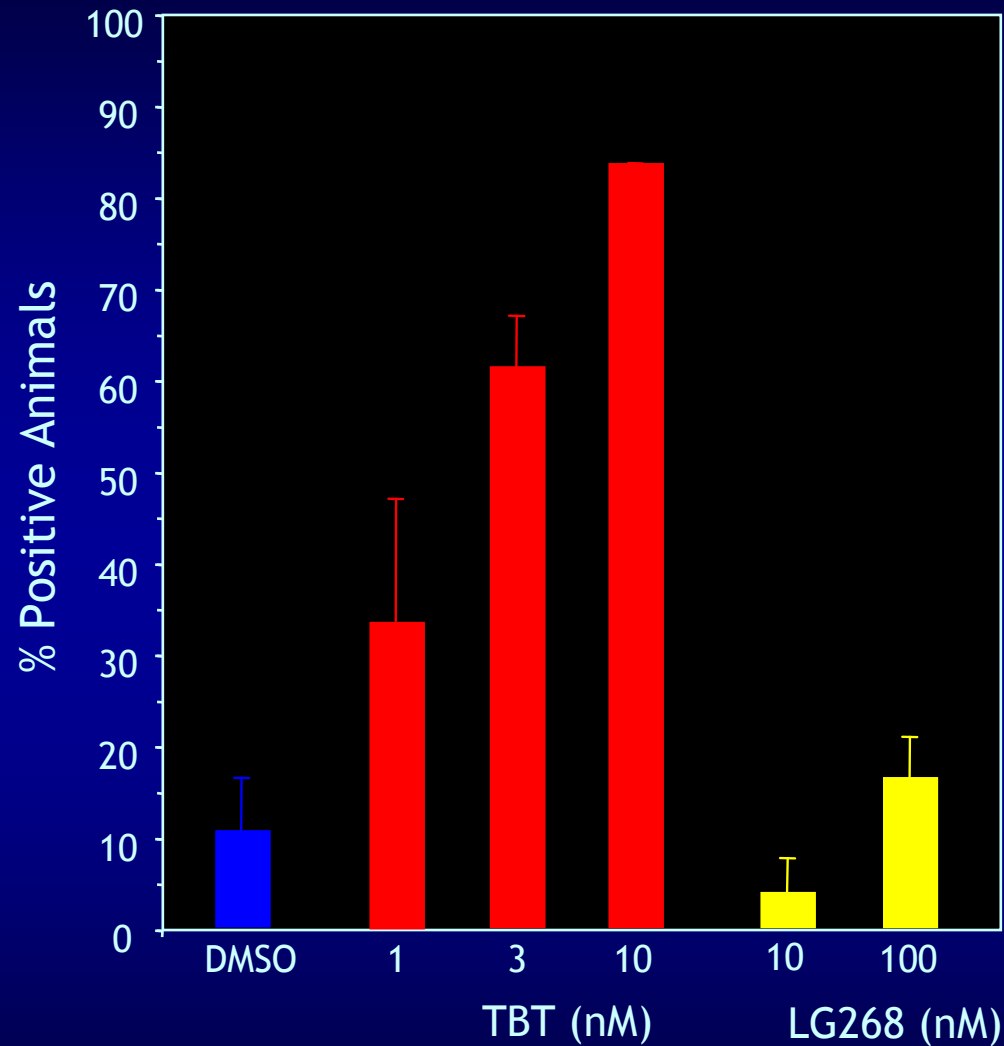


TBT



X. laevis tadpoles st.52/53 (gonadogenesis) were exposed to vehicle only (DMSO) or 1, 3, 10 nM TBT by weekly dosing under static renewal conditions until st.65 (metamorphosis).

TBT Induces Gonadal Adipocytes in Xenopus



Perhaps adipogenesis is an important effect of TBT treatment?

Is adipogenesis is an important target of TBT?

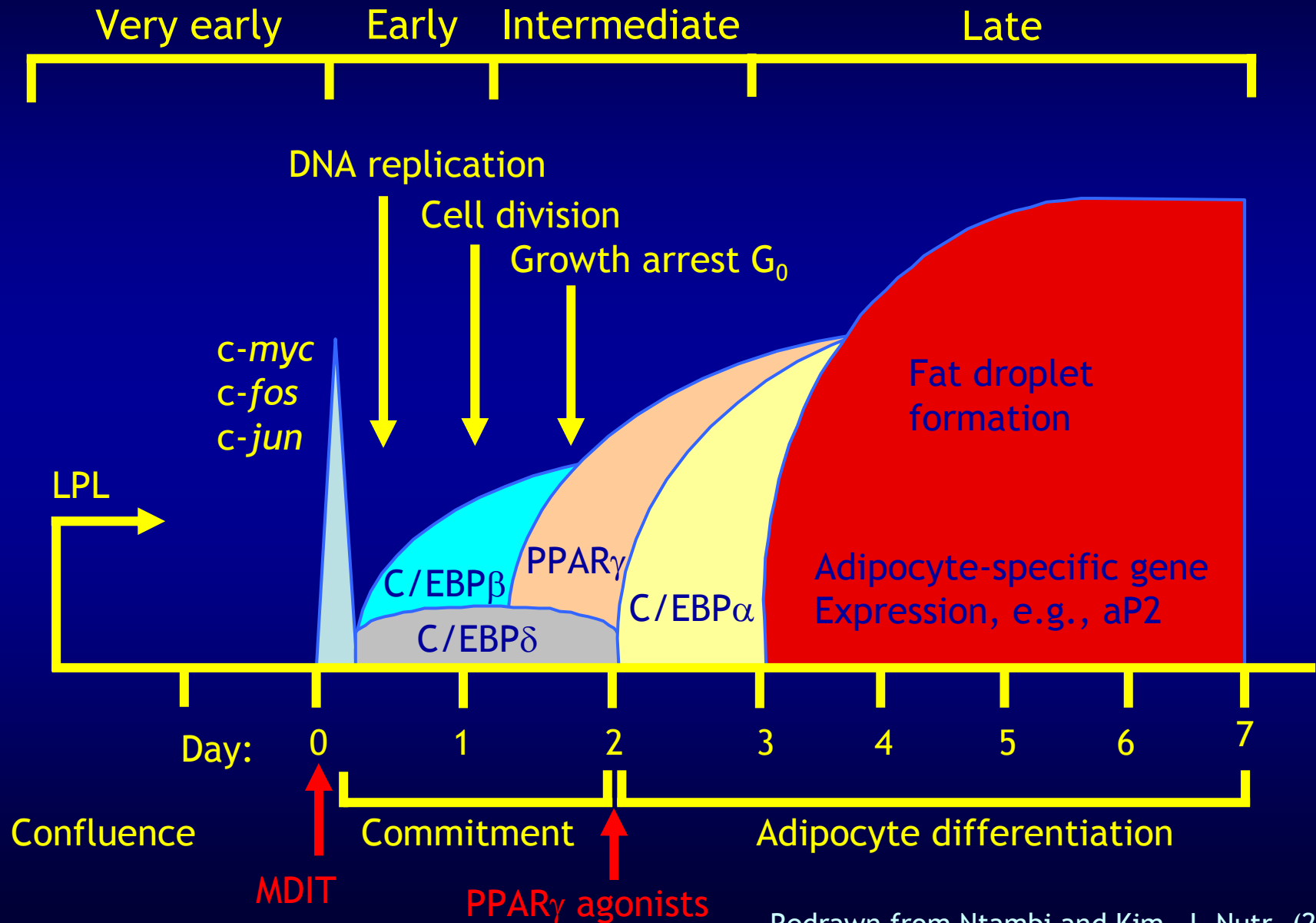
- If so, effects should be reflected in predictably altered gene expression
- Toxicogenomic analysis of mouse tissues
 - Affymetrix GeneArrays employed
- Two systems tested
 - Prenatal exposure to test effects on developing organism at P0
 - Percellome and QPCR analysis of adult exposure
 - Acute exposure over 4-24 hours

TBT-regulated genes in mouse liver

- Early response/proliferation transcription factors (c-jun, Atf3 etc.)
- Master regulators of adipogenesis (C/EBPs, DBP, PBP, lipin)
- PPAR γ target genes
- Nuclear receptor related - NRs, NR repressor (COUP-TFII), co-activators and co-repressors.
- Lipid/cholesterol metabolism (Insig2, VLDLR, LPL, lipin, CYPs)
- Insulin related signaling
- CYP450s

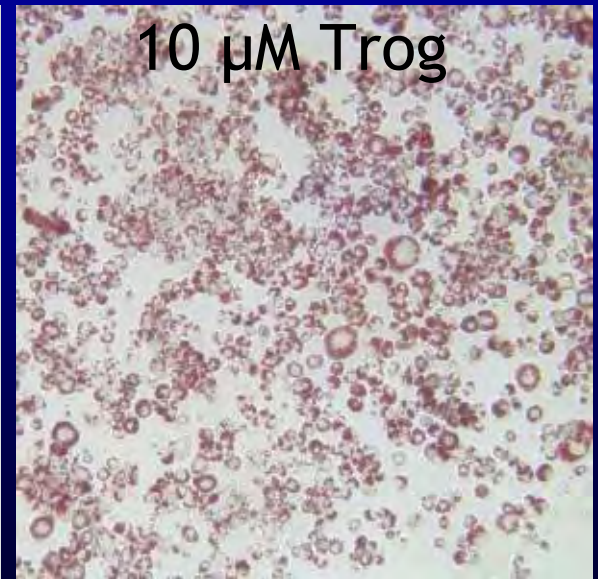
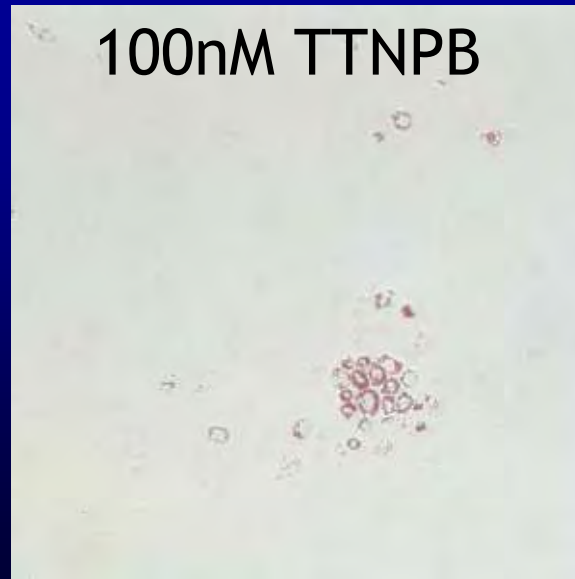
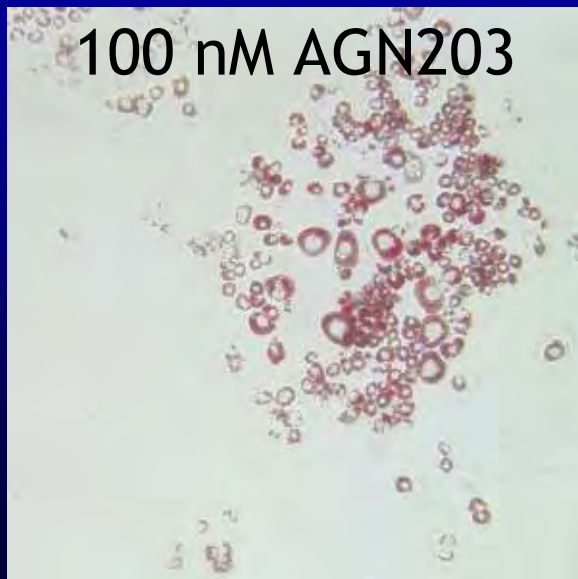
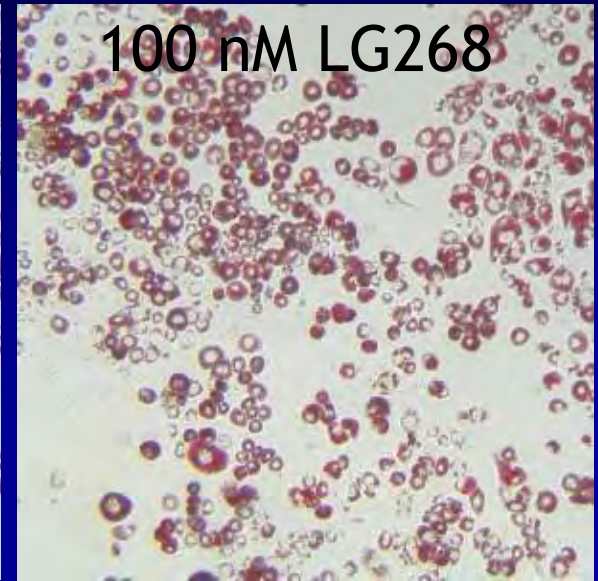
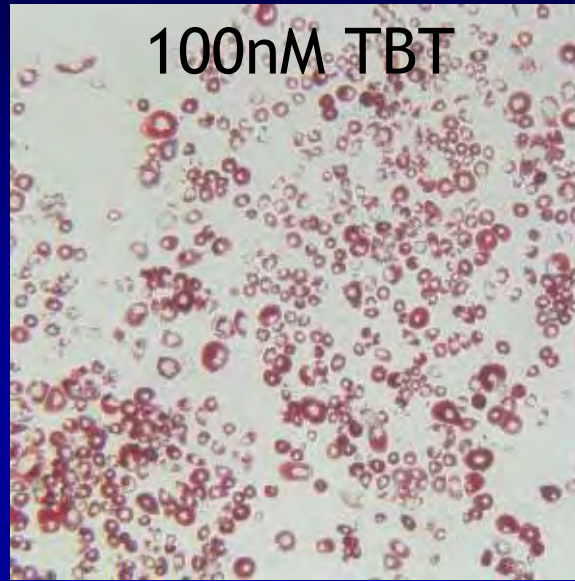
- What is the effect of TBT treatment in adipogenic models?
 - Cells
 - Animals

Model of 3T3-L1 adipocyte differentiation

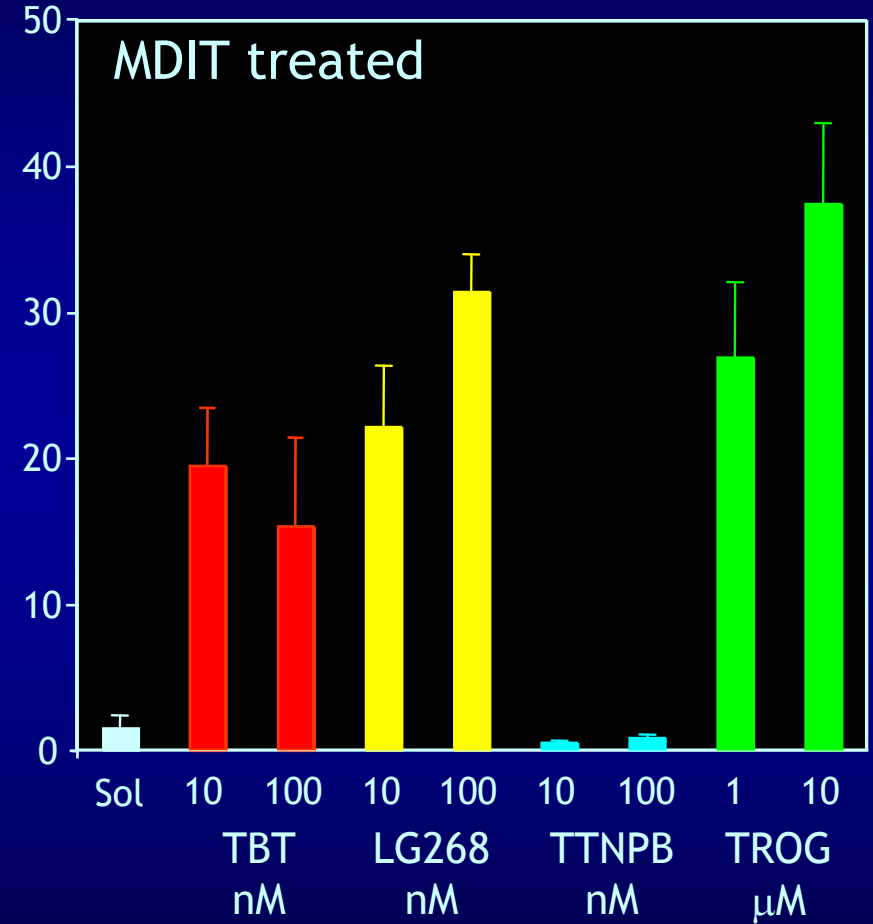
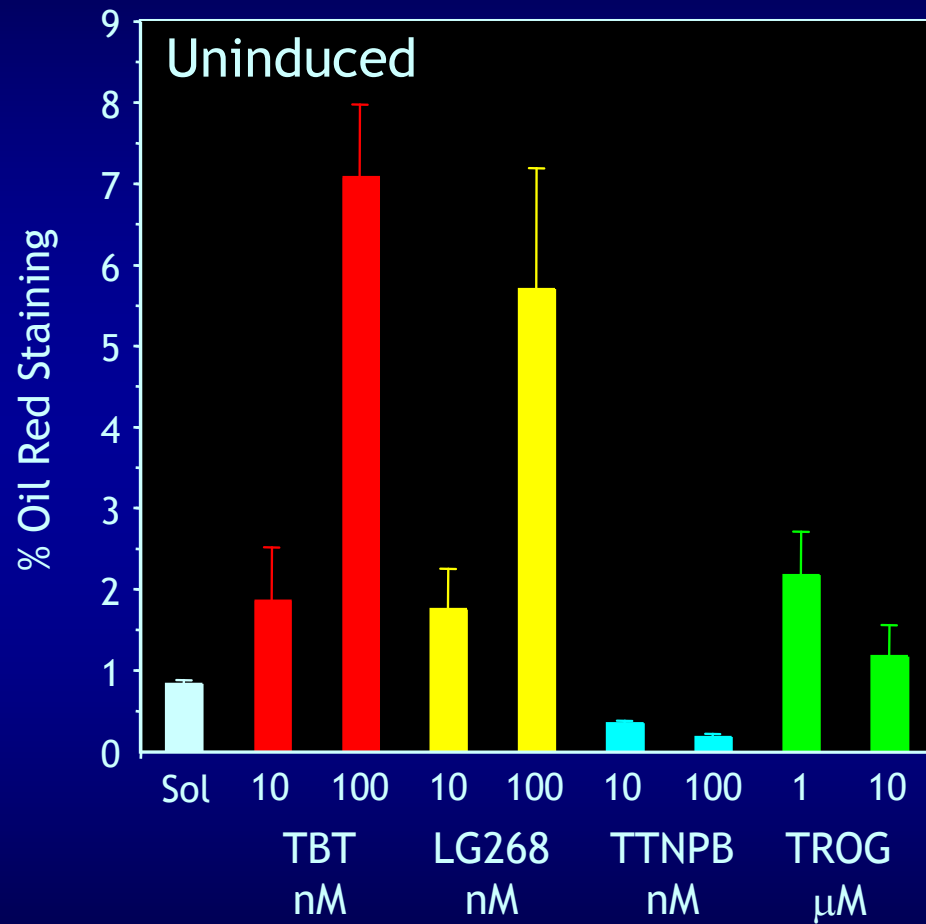


Redrawn from Ntambi and Kim, J. Nutr. (2000)

3T3-L1 Cells \pm RXR or PPAR γ Ligands

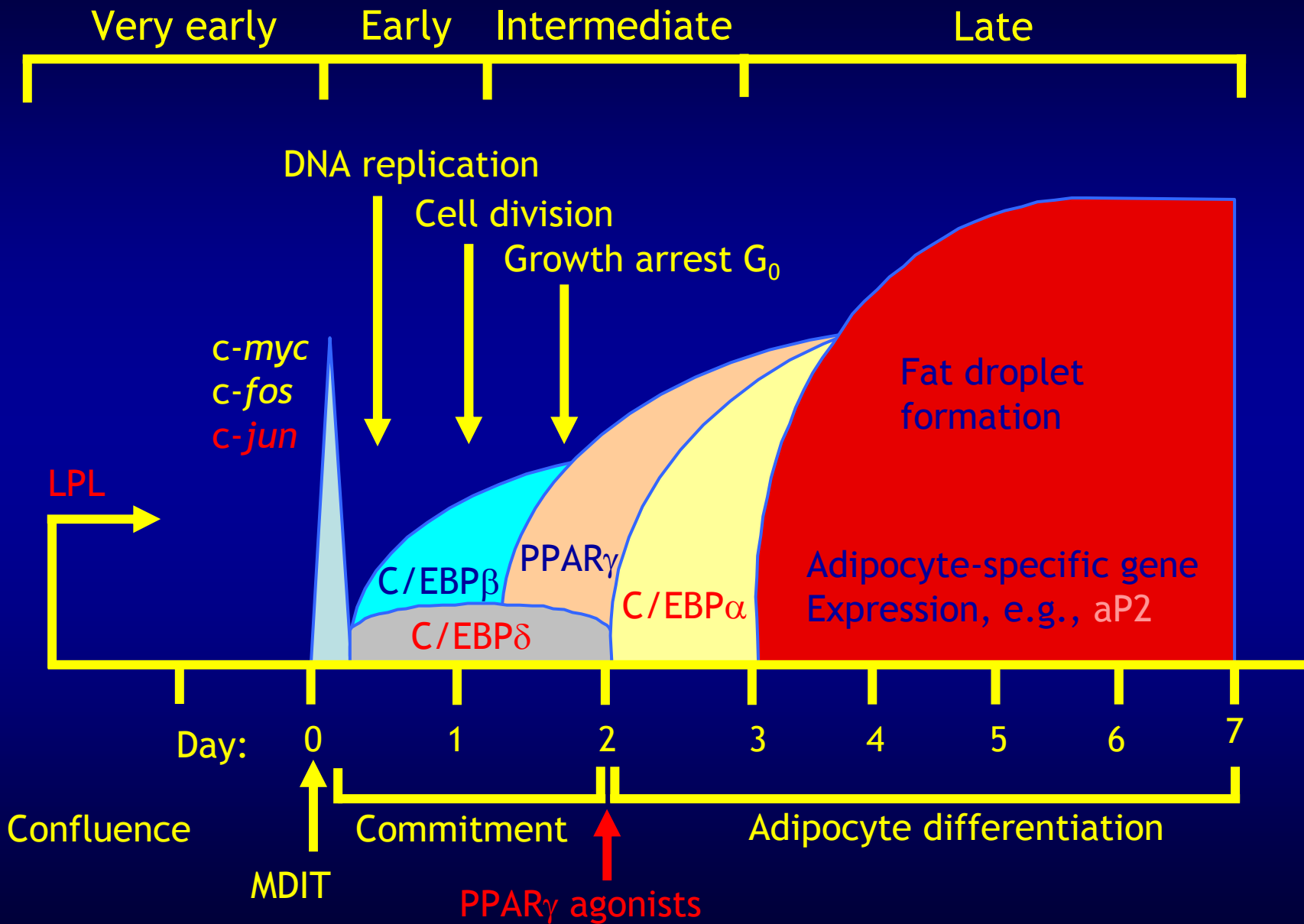


TBT Induced 3T3-L1 Adipocyte Differentiation



Ligand

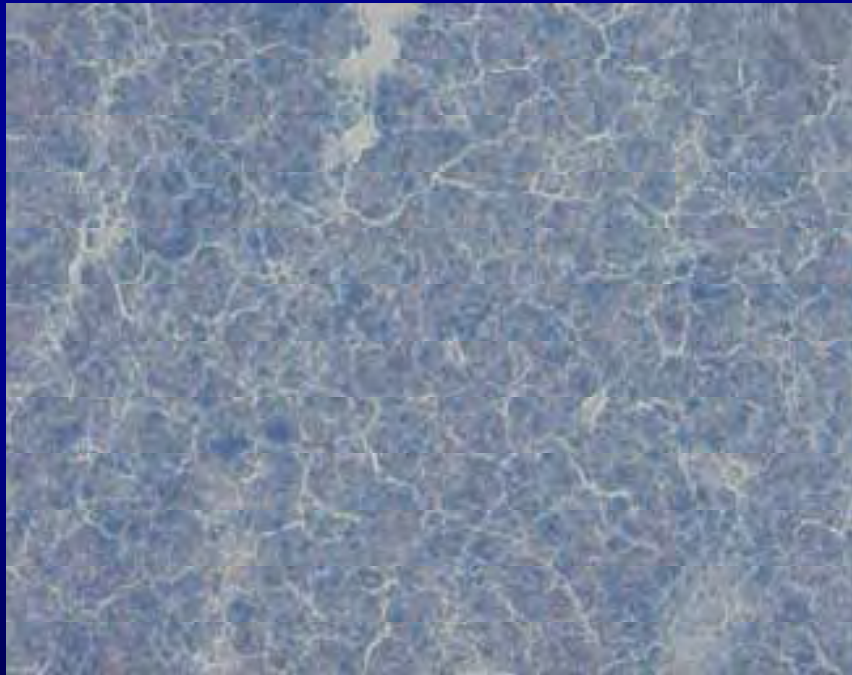
TBT induces important adipogenic genes



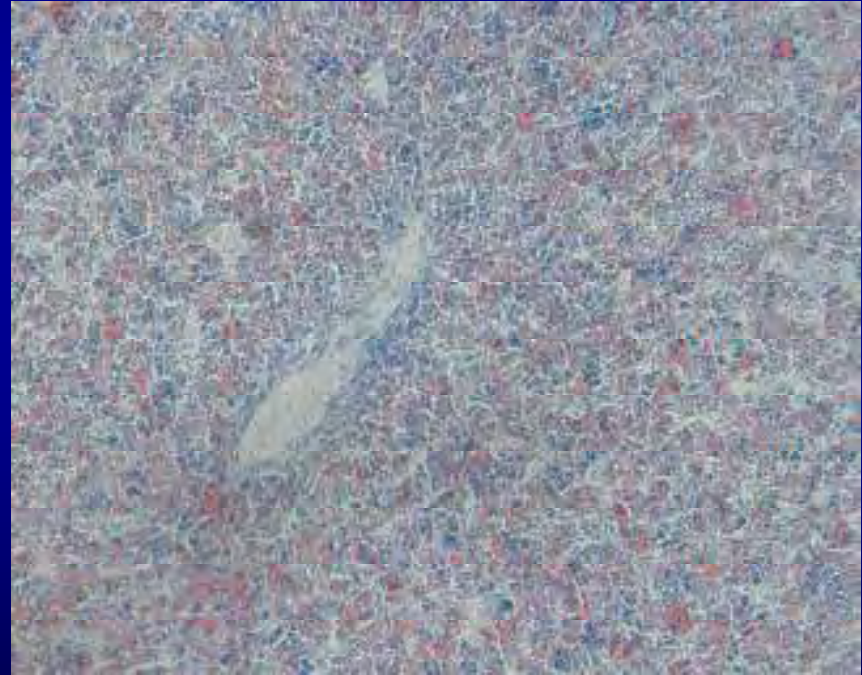
What is the effect of TBT treatment, in vivo?

Newborn Liver \pm TBT (*in utero*)

Vehicle (corn oil)



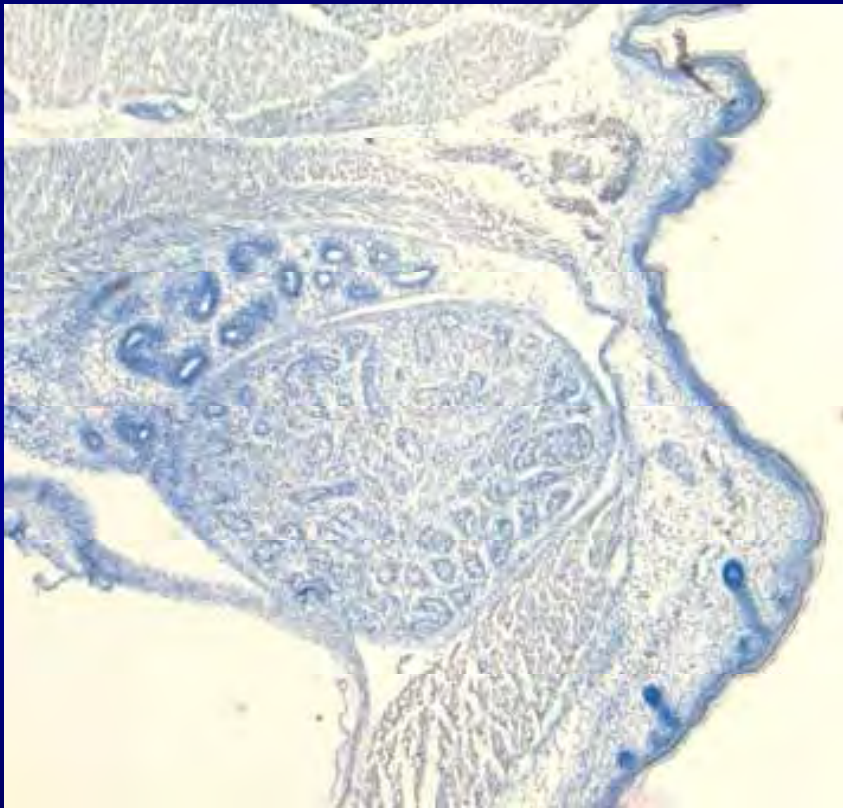
TBT



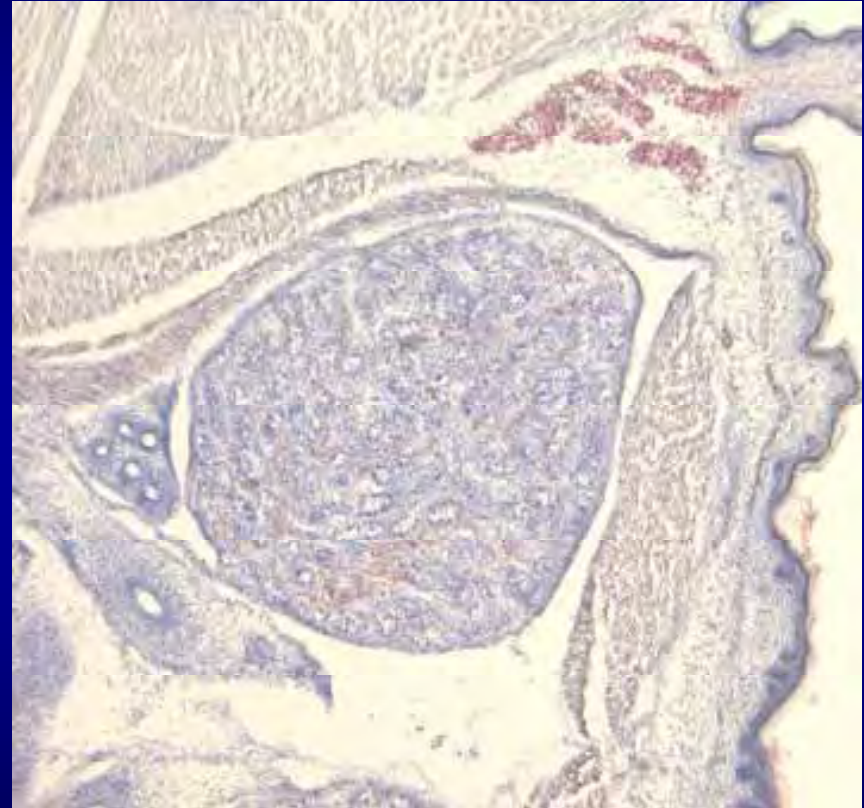
Pregnant C57BL6 dams dosed with TBT (0.5 mg/kg i.p.) from E12-E18. 20 μ m tissue cryosections were prepared from newborn pups and stained with Oil Red O (lipid stain) and counterstained with eosin/hematoxylin.

Newborn Testis \pm TBT (*in utero*)

Vehicle (corn oil)

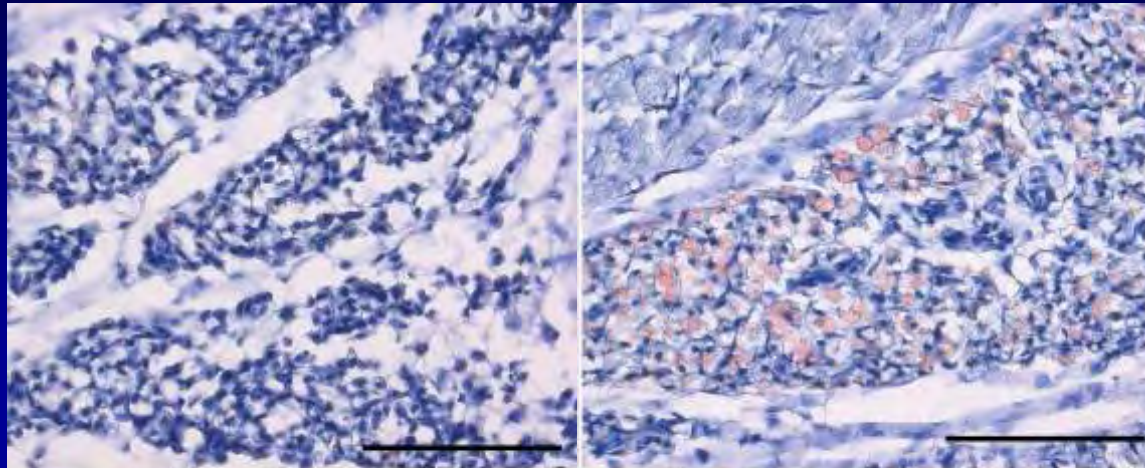


TBT

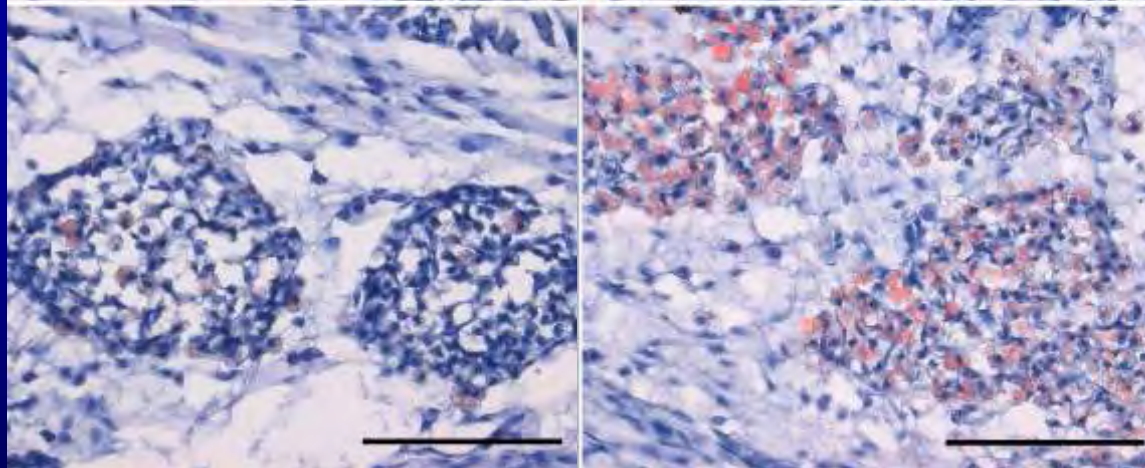


TBT effects on fat depots

Inguinal
Fat pad



Mammary
Adipose
tissue

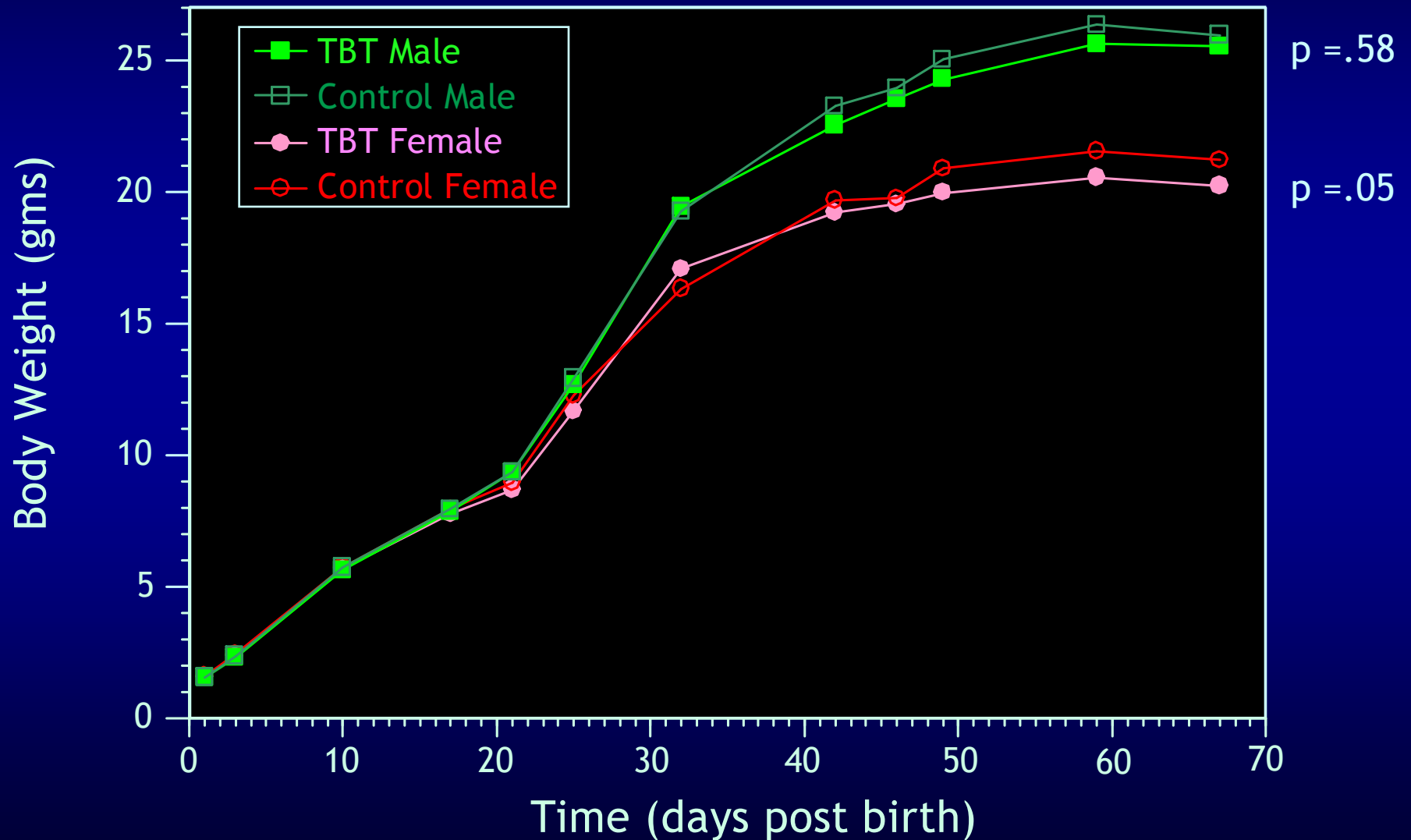


vehicle

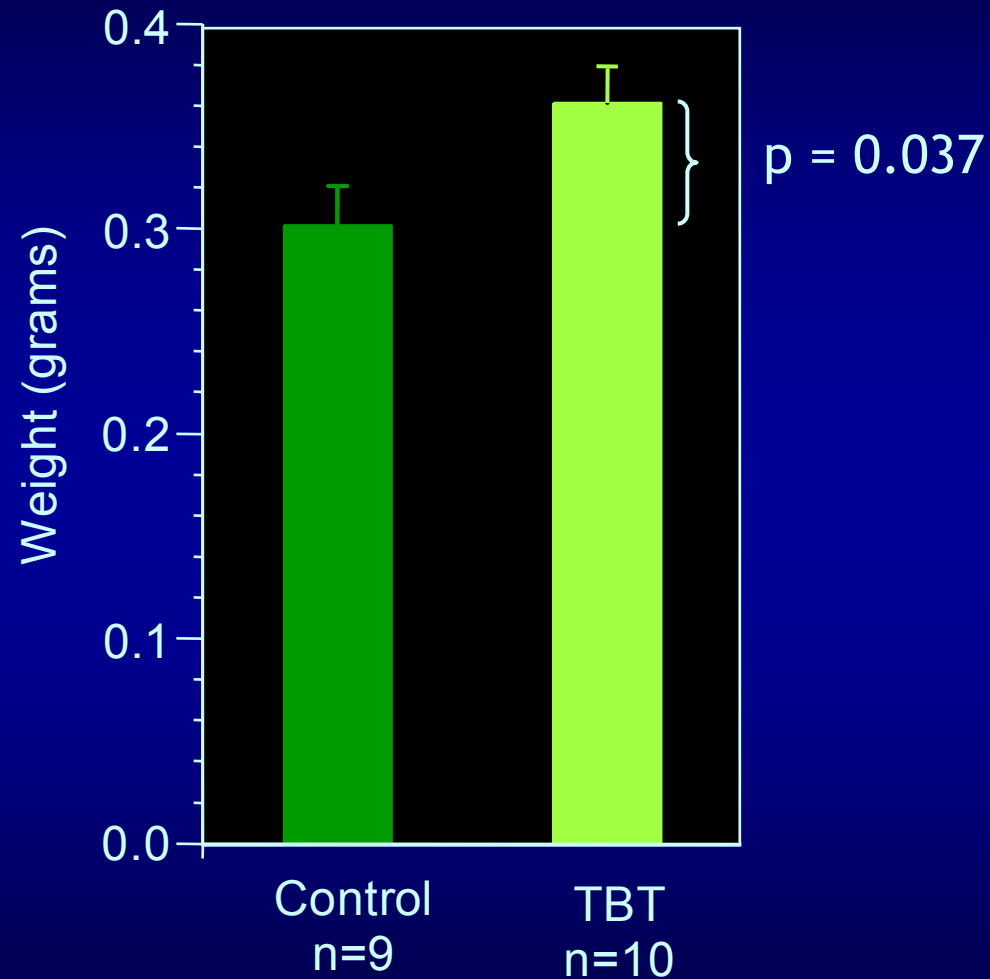
TBT

What is the effect of prenatal TBT exposure on adult animals?

Growth following prenatal TBT exposure

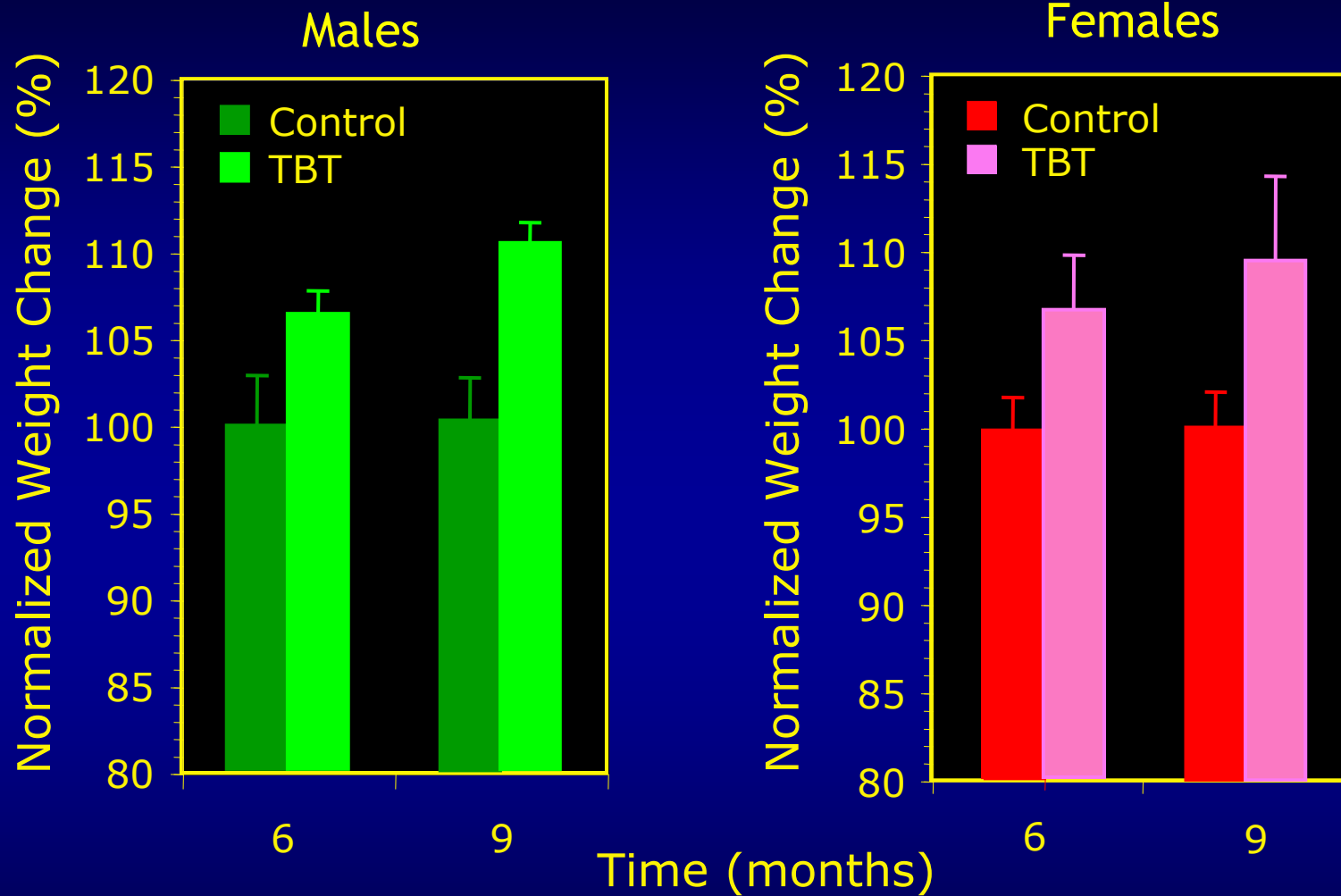


TBT increases testis fat pad weight at 10 weeks



Fat depot size increases at the expense of overall body mass

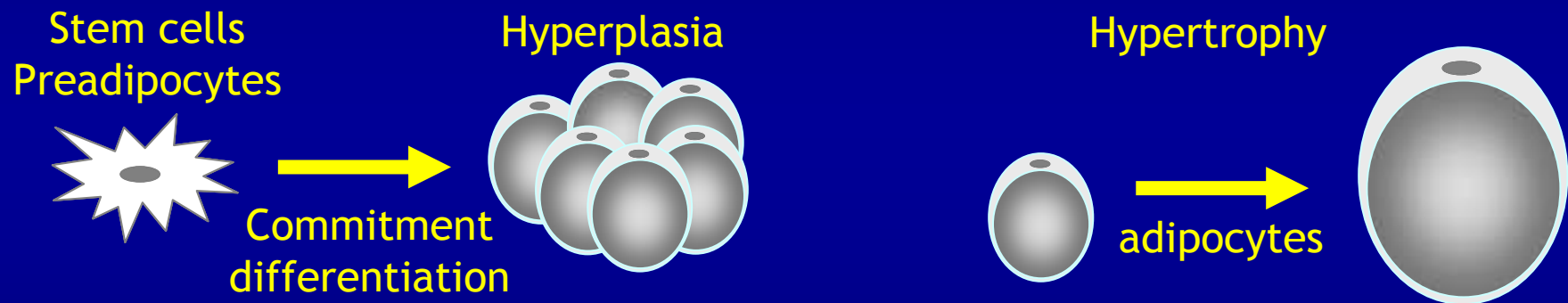
C57BL6 Weight Gain: 6-9 months



Prenatal TBT exposure causes permanent physiological changes resulting in predisposition to weight gain

How does prenatal TBT exposure cause weight gain?

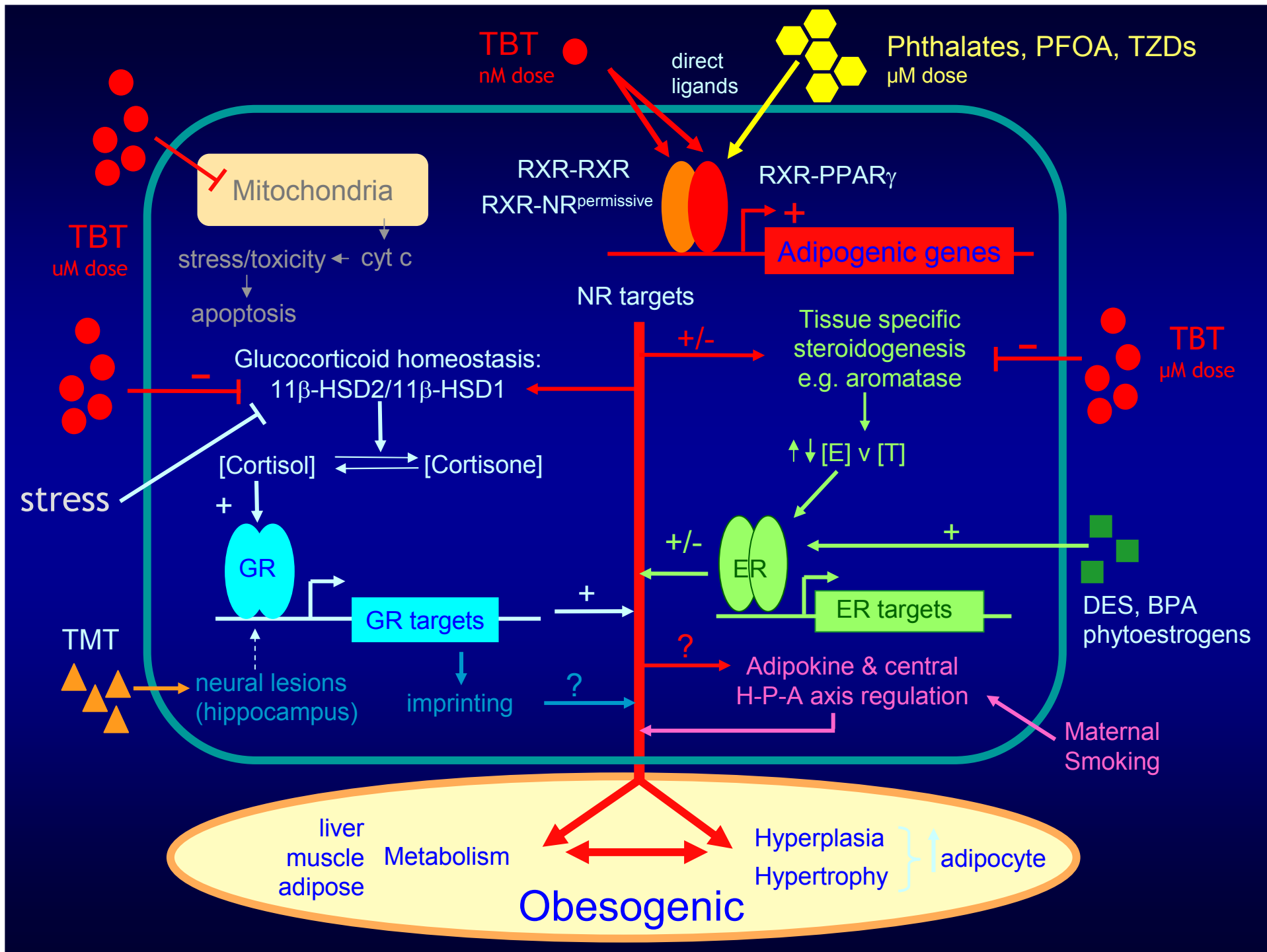
- Increased number of pre-adipocytes?
- Altered ability of adipocytes to process and store lipids?
- Changes in the hormonal control of appetite and satiety?
- Disturbances in stem cell pool?



- Mesenchymal stem cells (MSCs) are precursors to multiple cell lineages including bone, cartilage, and adipose.
 - **MSCs differentiate into adipocytes following rosiglitazone exposure**
- **Hypothesis:** TBT induces adipogenesis in hMSCs.

Conclusions - organotins and nuclear receptors

- Organotins are exceptionally potent agonists of RXR and PPAR γ
 - ~5 nM EC₅₀, 12.5 nM K_d on RXR α
 - ~20 nM EC₅₀ and K_d on PPAR γ
- TBT drives adipocyte differentiation in murine cell cultures
 - Independent of induction by MDIT
- TBT exposure during development induces adipogenesis in two vertebrate species: mouse and *Xenopus*
 - TBT induces expression of expected RXR/PPAR γ 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 γ -RXR
 - Aromatase expression/function - estradiol levels
 - Glucocorticoid levels
 - Other stressors?



Obesogens - Just the Tip of the Iceberg ?

Tributyl Tin

Estradiol

Genistein

Organophosphate
pesticides

Phthalates

DES

Nicotine

PCBs ?

PFOA

Bisphenol A

PBDEs?

- **What don't we know yet?**
 - **Body burdens in population**
 - **Molecular targets of action beyond RXR-PPAR γ**
 - **Critical windows of exposure**
 - **How does prenatal exposure alter adult phenotype ?**
 - **Endpoints to study**

Conclusions - organotins and obesity

- Is organotin exposure a contributing factor for obesity?
 - Neonatal exposure permanently alters adult phenotype
 - Adult exposure rapidly induces adipogenic genes
 - Drugs that activate PPAR γ increase obesity
 - 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
 - Prevalent contaminants in dietary sources
 - Average blood level of 27 nM in 32 random people tested
- Human exposure to organotins may reach levels sufficient to activate high affinity receptors
 - 1000 x lower dose than natural dietary RXR and PPAR γ ligands

Is the environment making us fat?

- UCI - Blumberg Lab
 - Stephanie Casey
 - Connie Chow
 - Naman Dalal
 - Daniel Diamond
 - Felix Grün
 - Amanda Janesick
 - Kameron Johnson
 - Tiffany Kieu
 - Séverine Kirchner
 - Leah Parilla
 - Trang Pham
 - Rand Relatores
 - Denise Stephens
 - Mao Taketani
 - Suman Verma
 - John Ycaza
- UCI - labs
 - Edward Nelson
 - David Fruman

- Former lab members
 - Lauren Maeda
 - Matt Milnes
 - Jason Shiotsugu
 - Michelle Tabb
 - Zamaneh Zamanian
 - Changcheng Zhou

- NINS - Okazaki, Japan
 - Taisen Iguchi
 - Hajime Watanabe
- NIHS - Tokyo, Japan
 - Jun Kanno
- City of Hope
 - Barry Forman



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