

# **Xenobiotic Obesogens: Organophosphate pesticides and the Obesity Epidemic.**

**David N. Collier, MD, PhD, FAAP**

Assistant Professor of Pediatrics, Brody School of Medicine @ ECU  
Director, Pediatric healthy Weight Research and Treatment Center  
Associate Director, Metabolic Institute

# Xenobiotics and environmental exposures – a role in obesity?

More!

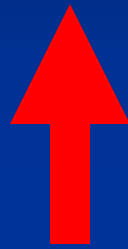
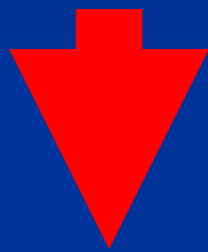


- Obesity and energy balance
- Fetal programming
- Xenobiotics as obesogens?
  - Organophosphate pesticides
  - Bisphenol A
  - phthalates,
  - organotins,
  - Perfluoroalkyl acids.

Picture from: Geoff Price “Understanding Capitalism Part IV: Capitalism, Culture and Society” Feb 4, 2005 @ rationalrevolution.net

***E intake***

***E expenditure***



Environmental/genetic influences



- $E_{in} \approx E_{exp}$ .
- Weight neutral

- $E_{in} > E_{exp}$ .
- Obesity

- $E_{in} \approx E_{exp}$ .
- Obesity

# Morbid Obesity due to Leptin Signaling Defects



**Ob-/Ob- leptin deficient**

**LRb T1138S  
leptin receptor defect**



# Leptin Deficits

Farooqi and O'Rahilly



- Leptin deficiency
  - Early onset morbid obesity
  - High fat mass
  - Infertility
  - T-cell defects recurrent infections
  - Rare: autosomal recessive
  - Readily diagnosed
  - Rx with recombinant leptin
- Leptin receptor deficiency
  - Milder phenotype
  - No specific Rx

# Defects in MC4R Receptor

Farooqi and O'Rahilly

- Loss of  $\alpha$ -MSH mediated anorexia
- Phenotype
  - Hyperphagia
  - Accelerated linear growth
  - Increased bone density
  - $\uparrow$  adipose and lean tissue mass
- Multiple alleles
  - Codominant
  - Homozygous > heterozygous
- Prevalence
  - $\approx$  6% in severe childhood onset obesity
  - 1/2000 general population
- Dx/Rx
  - DNA sequence from WBC
  - Athena Diagnostics - \$810
  - No specific treatment

450 FAROOQI ■ O'RAHILLY



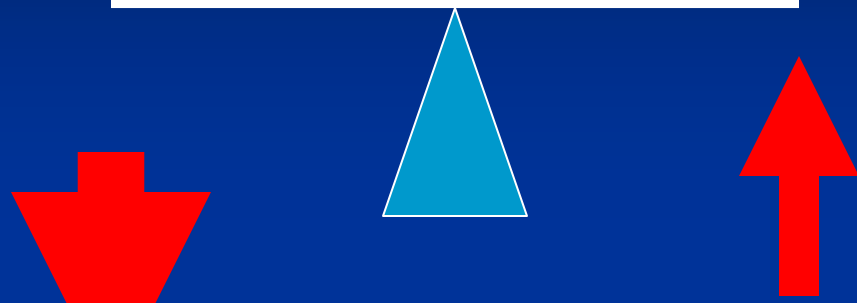
**Figure 3** Clinical phenotype of MC4R deficiency. MC4R mutations result in a dominantly inherited obesity syndrome. *Left:* 9-year-old with MC4R mutation. *Right:* 16-year-old sibling with normal MC4R.

***E intake***

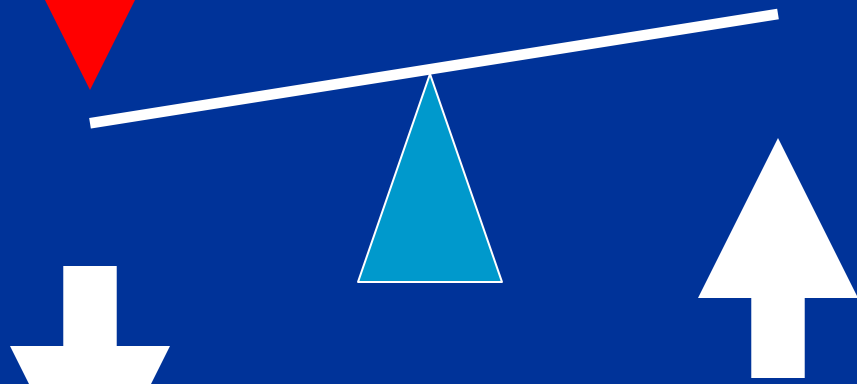
***E expenditure***



- $E_{in} \approx E_{exp}$ .
- Weight neutral



- $E_{in} > E_{exp}$ .
- Obesity



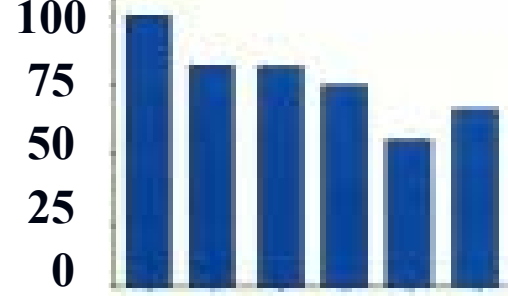
- $E_{in} \approx E_{exp}$ .
- Obesity

Environmental/genetic influences

# Higher risk adult diseases with lower birth size

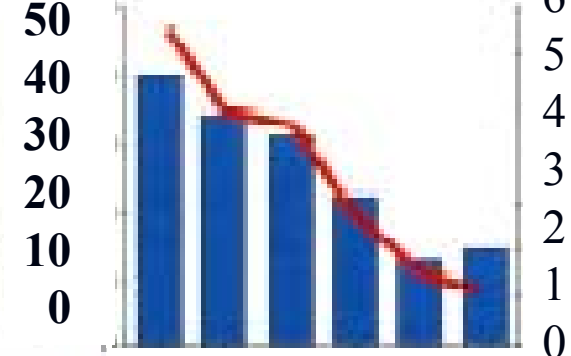
Barker DJP, Mol Med Today 1995:418-423

**Standard mortality ratio for deaths from CVD**



<5.5  
5.6-6.5  
6.6-7.5  
7.6-8.5  
8.6-9.5  
>9.5

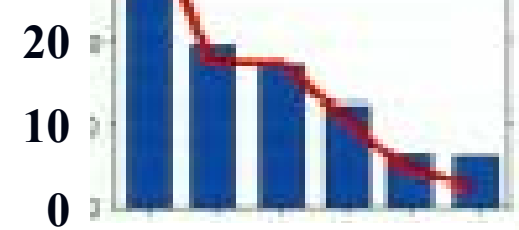
**Prevalence IGT or DM**



Odds ratio

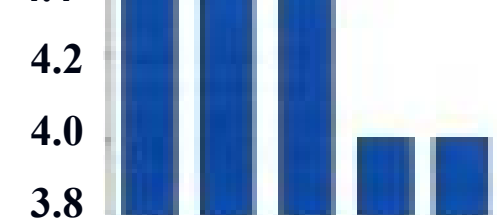
<5.5  
5.6-6.5  
6.6-7.5  
7.6-8.5  
8.6-9.5  
>9.5

**% insulin resistant**



<5.5  
5.6-6.5  
6.6-7.5  
7.6-8.5  
8.6-9.5  
>9.5

**LDL (mmol/l)**



<11.5  
11.6-12.0  
12.1-12.5  
12.6-13.0  
>13



# Maternal nutrition imbalance and programming of the metabolic syndrome

- Thrifty phenotype hypothesis
  - Nutritional imbalances in utero foreshadow postnatal deprivation
  - Fetal phenotype is “programmed” for efficiency
    - AKA: adaptive response
  - Mismatch between pre/post natal environment results in disease
- ↓ birth weight and catch up growth associated with ↑ risk of central adiposity and the metabolic syndrome
  - Dutch famine vs. siege of Leningrad in WWII
    - Present in all ethnicities examined
  - Animal models
    - Placental insufficiency and hypoxia
    - Caloric restriction
    - Macro or micronutrient restriction
    - Macronutrient imbalances

# *In utero* programming and post-natal susceptibility to obesogenic diets.

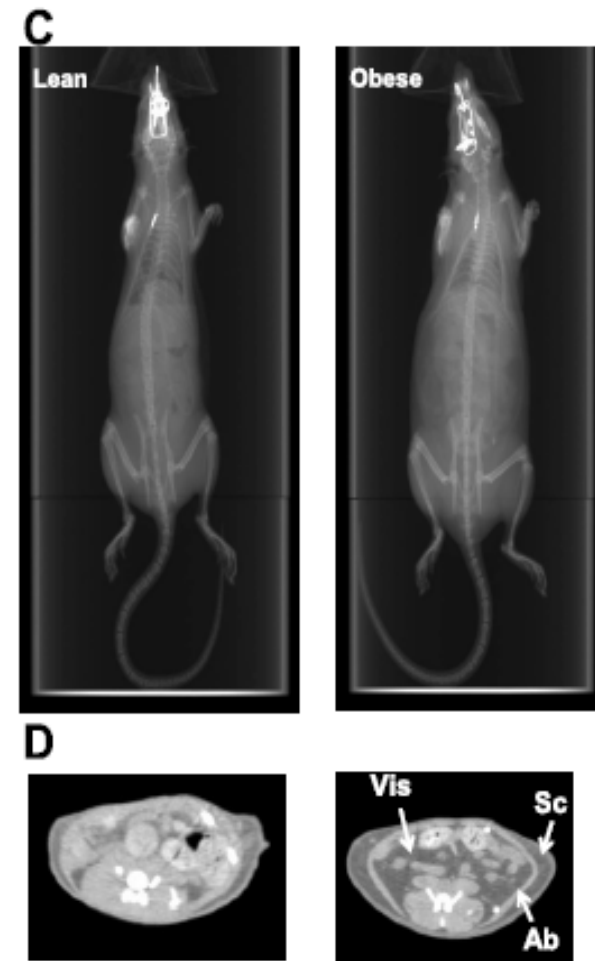
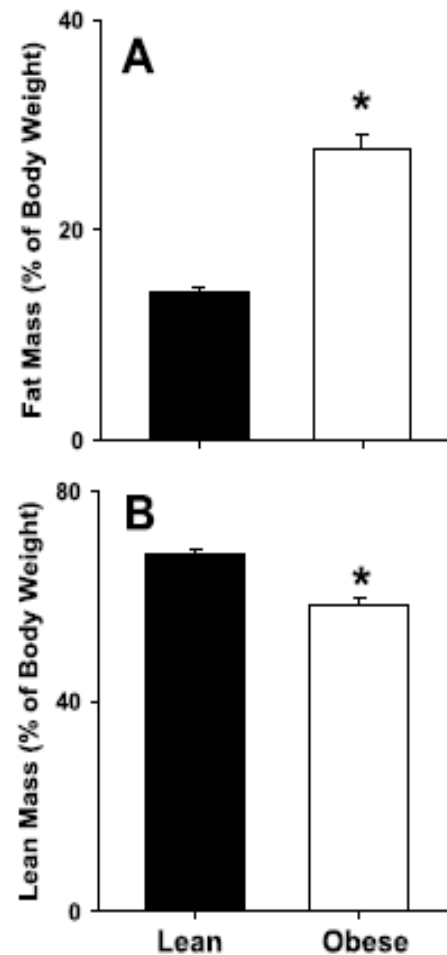
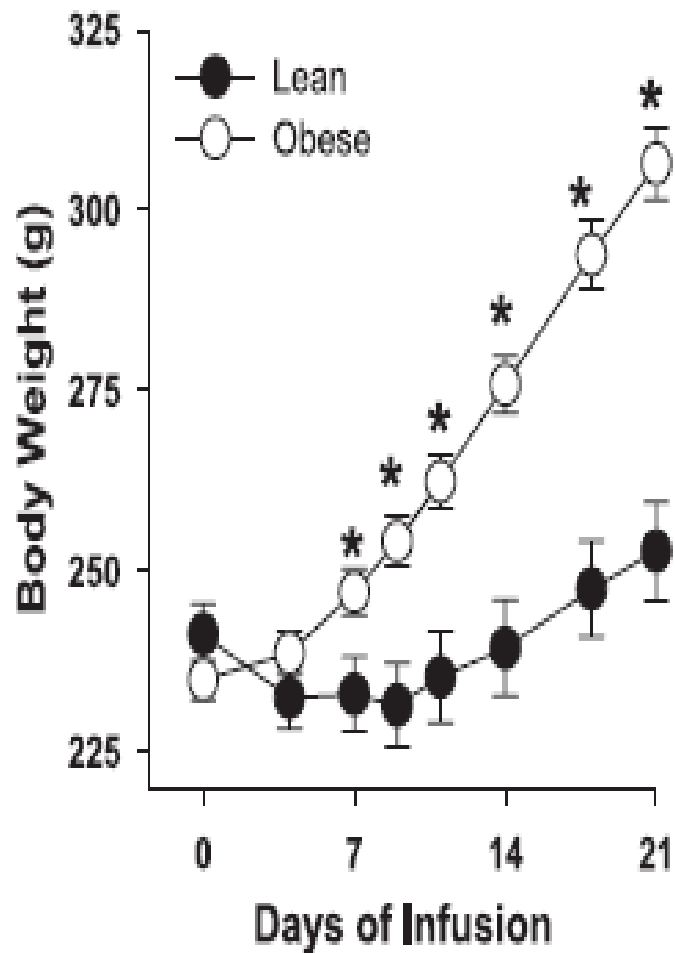
Prenatal exposure  
(maternal diet)

Postnatal exposure

	chow	“cafeteria diet”
chow low protein	normal ↓ wt, fat mass	normal ↑ wt, fat mass
chow low calorie	chow normal +/- ↓ wt, fat mass	high fat normal ↑ wt, fat mass
chow high fat	chow normal ↑ wt, fat mass	high fat normal ↑↑ wt, fat mass

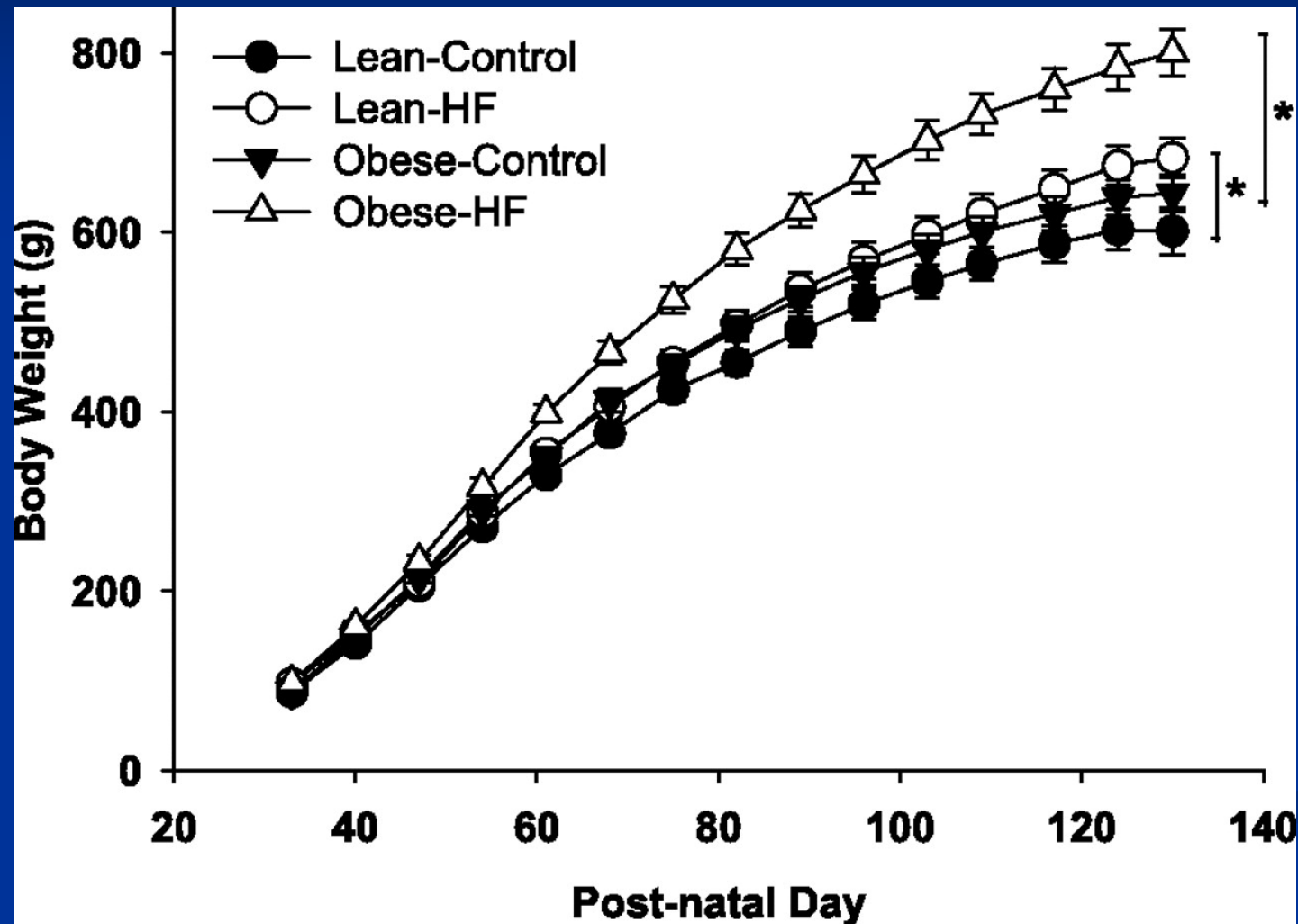
# Maternal Obesity @ Conception Programs Obesity in the Offspring

Shankar K et al, Am J Physiol Regul Interg Comp Physiol 2008;294:R528-38



# Maternal Obesity @ Conception Programs Obesity in the Offspring

Shankar K et al, Am J Physiol Regul Interg Comp Physiol 2008;294:R528-38



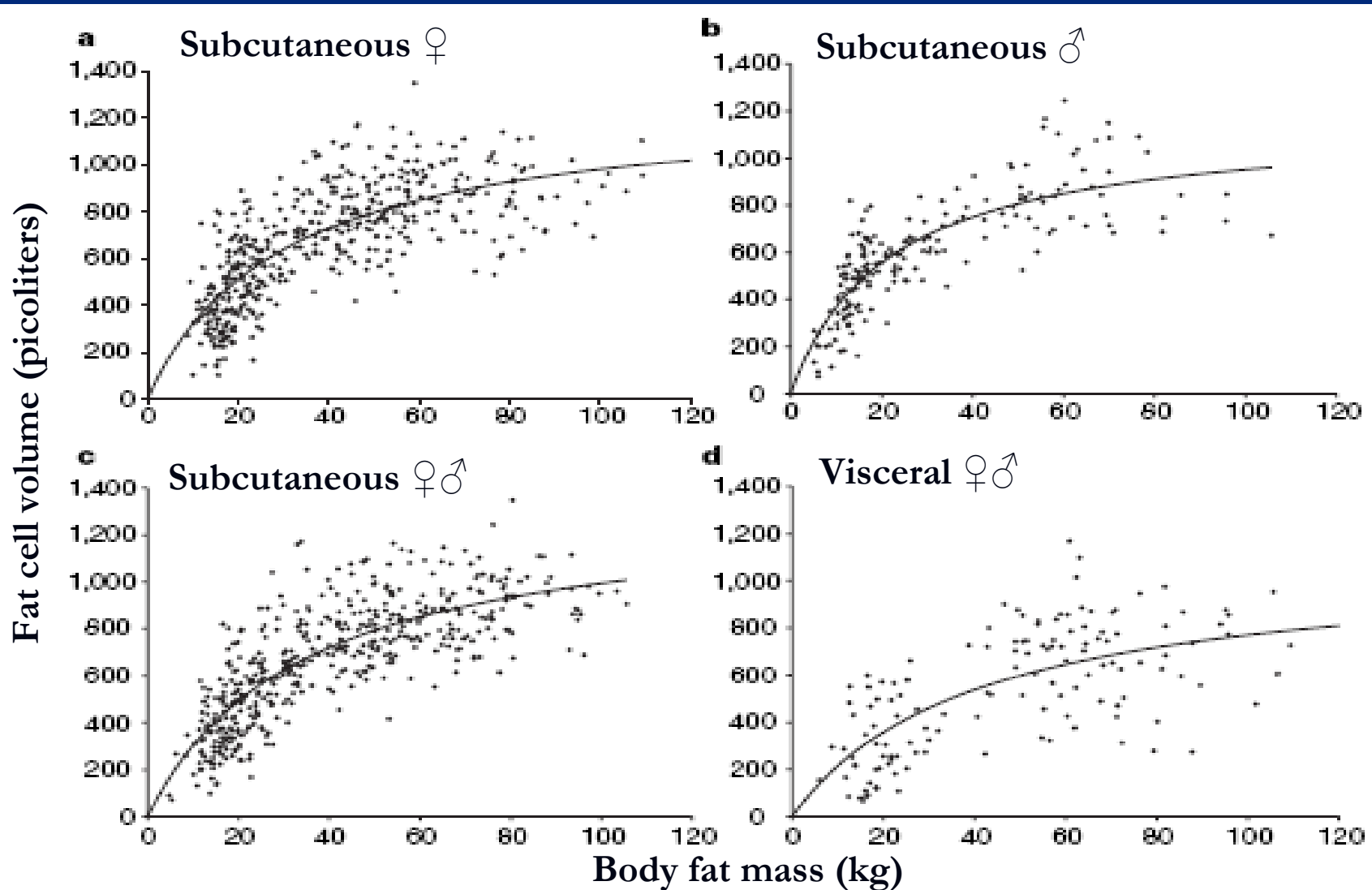
# Excessive Gestational Weight Gain and Child's Weight Status

Birth Wt. Status	Gestational Weight Gain		
	Inadequate	Adequate	Excessive
SGA <10 <sup>th</sup>	22%	50%	28%
AGA 10-90 <sup>th</sup>	14%	36%	50%
LGA > 90 <sup>th</sup>	7%	26%	66%
Child BMI @ age 3			
85-94 <sup>th</sup>	1.0	2.09	2.03
≥ 95 <sup>th</sup>	1.0	3.8	4.4

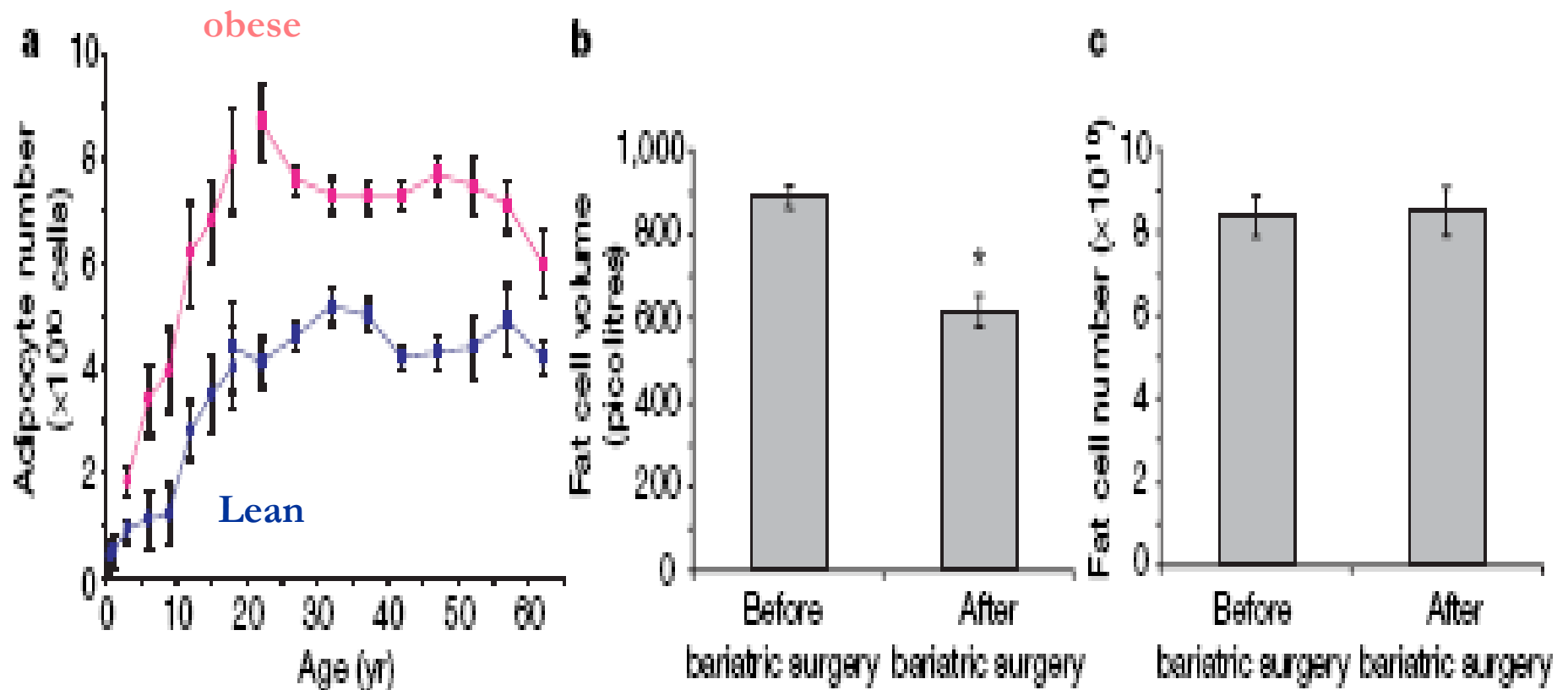
Oken E et al, *Am J Ob Gyn* 2007;322e1-8

# Adult fat mass is determined by both adipocyte size and number

Spalding KL *Nature* May 5 2008



# Number of adipocytes is determined during childhood/adolescence and is stable in adulthood even with significant weight loss



# Adipocyte hyperplasia

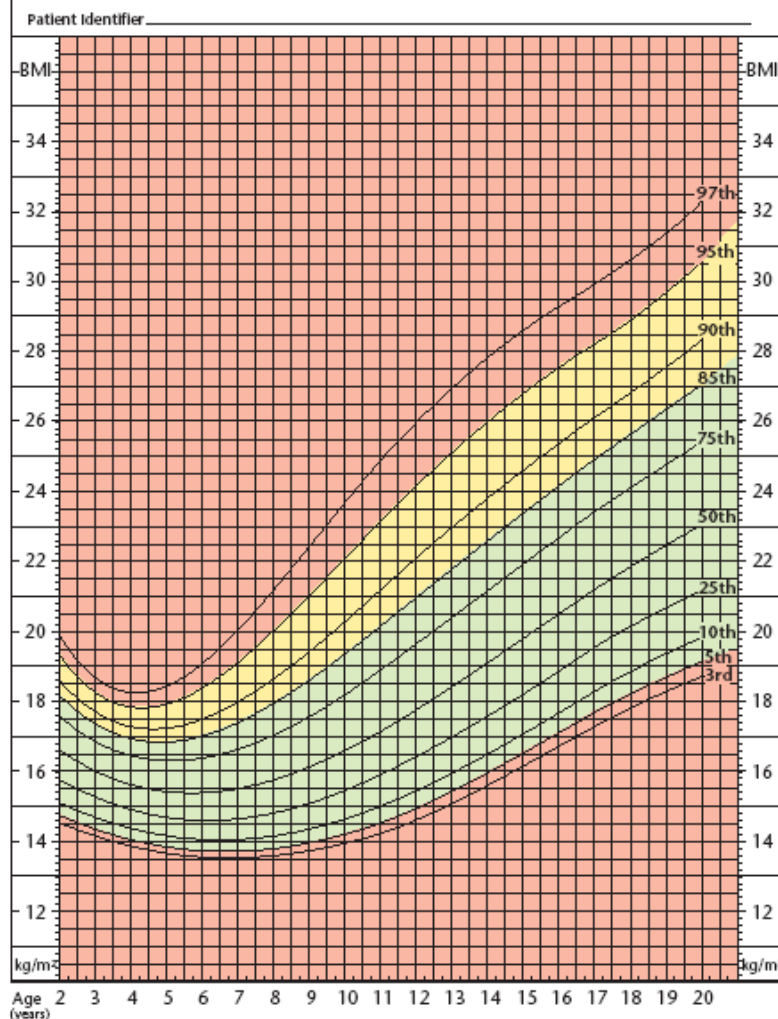
- Adipocyte hyperplasia (number expansion) begins earlier in obese subjects
  - 5.7 vs. 2.1 years of age
- Degree of hyperplasia higher in obese subjects
- Number, but not volume, of adipocytes fixed by early adulthood
- Adipocyte differentiation/recruitment potential mode of action for obesogens?
  - Thiazolidinediones as proof of concept
    - Weight gain (subcutaneous fat) associated with pioglitazone and rosiglitazone stimulation of PPAR $\gamma$  and adipocyte differentiation





# Body mass index 2 to 20 years

# BOYS



**To calculate BMI:**  
 Kilograms and meters:  
 $\text{weight (kg)} / [\text{height (m)}]^2$   
 Pounds and inches:  
 $\text{weight (lb)} / [\text{height (in)}]^2 \times 703$

**BOYS: 99th percentile cut-points**

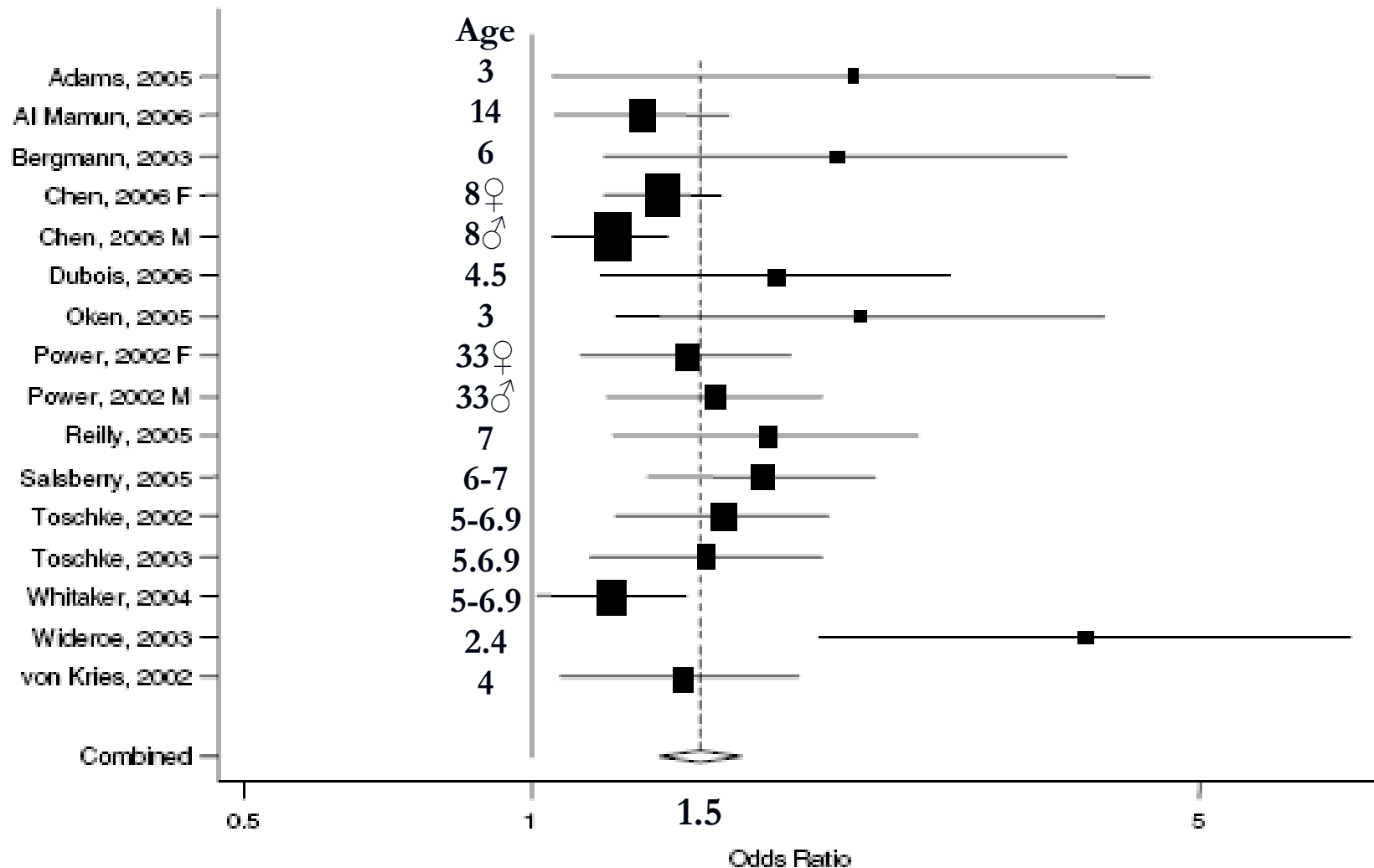
AGE	BMI
5	20.1
6	21.6
7	23.6
8	25.6
9	27.6
10	29.3
11	30.7
12	31.8
13	32.6
14	33.2
15	33.6
16	33.9
17	34.4

From National Initiative for Children's Healthcare Quality ([www.nichq.org](http://www.nichq.org))

Color coding of the 2000 CDC BMI charts by UNC's Department of Pediatrics and Center for Health Promotion and Disease Prevention (CDC Cooperative agreement U48-DP-000059) for research and clinical purposes

# Effect of maternal smoking during pregnancy on child overweight (n= 84,563, @ 3-33 years of age)

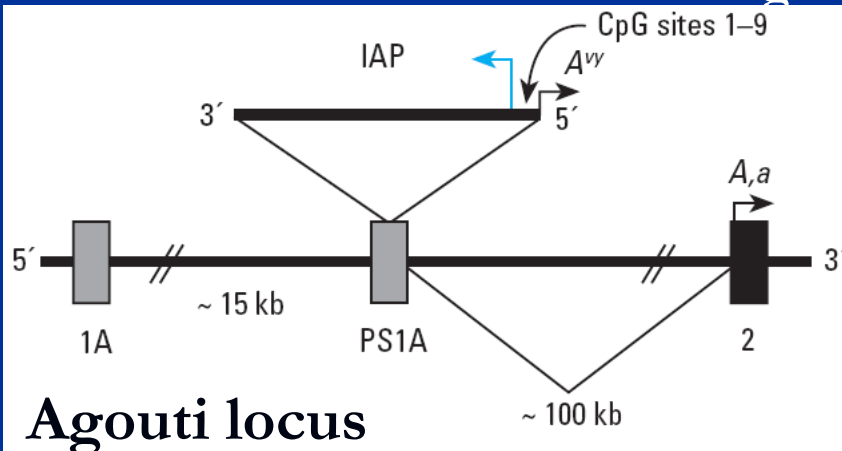
Oken E, Levitan EB, Gillman MW Int J Obesity 2008;32:201-210



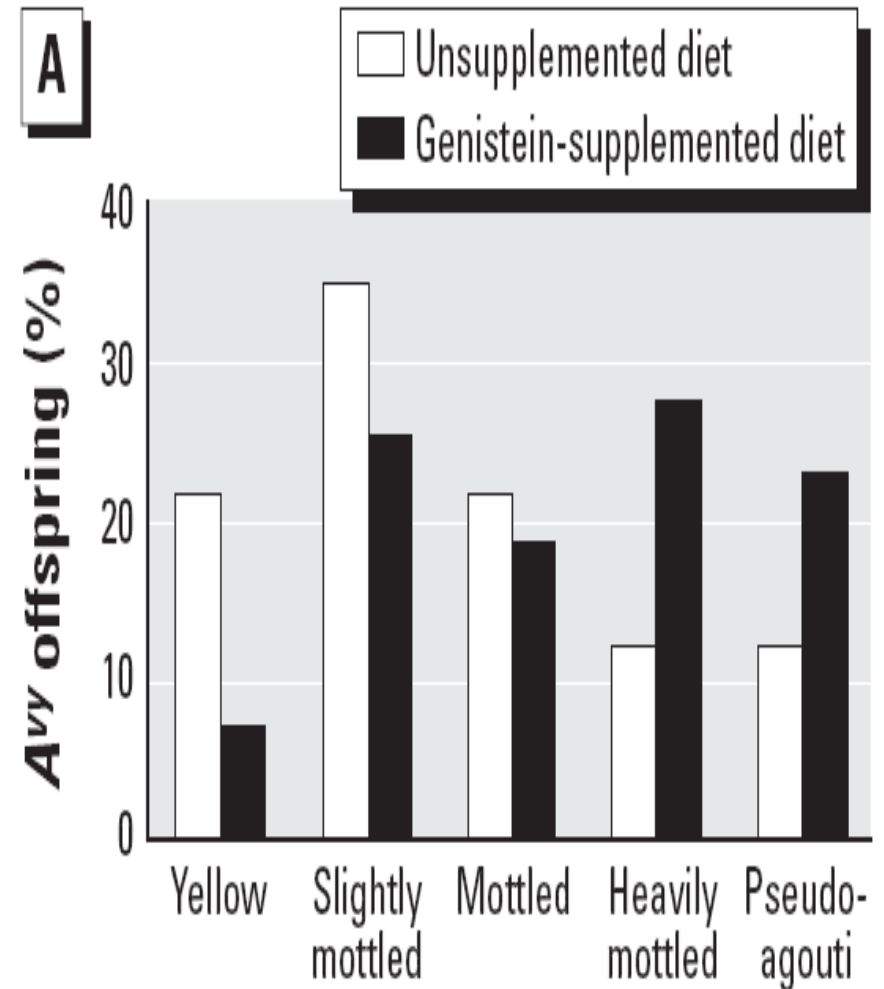
# Maternal Genistein Consumption Promotes Hyper-methylation and in utero programming



Low ← → High



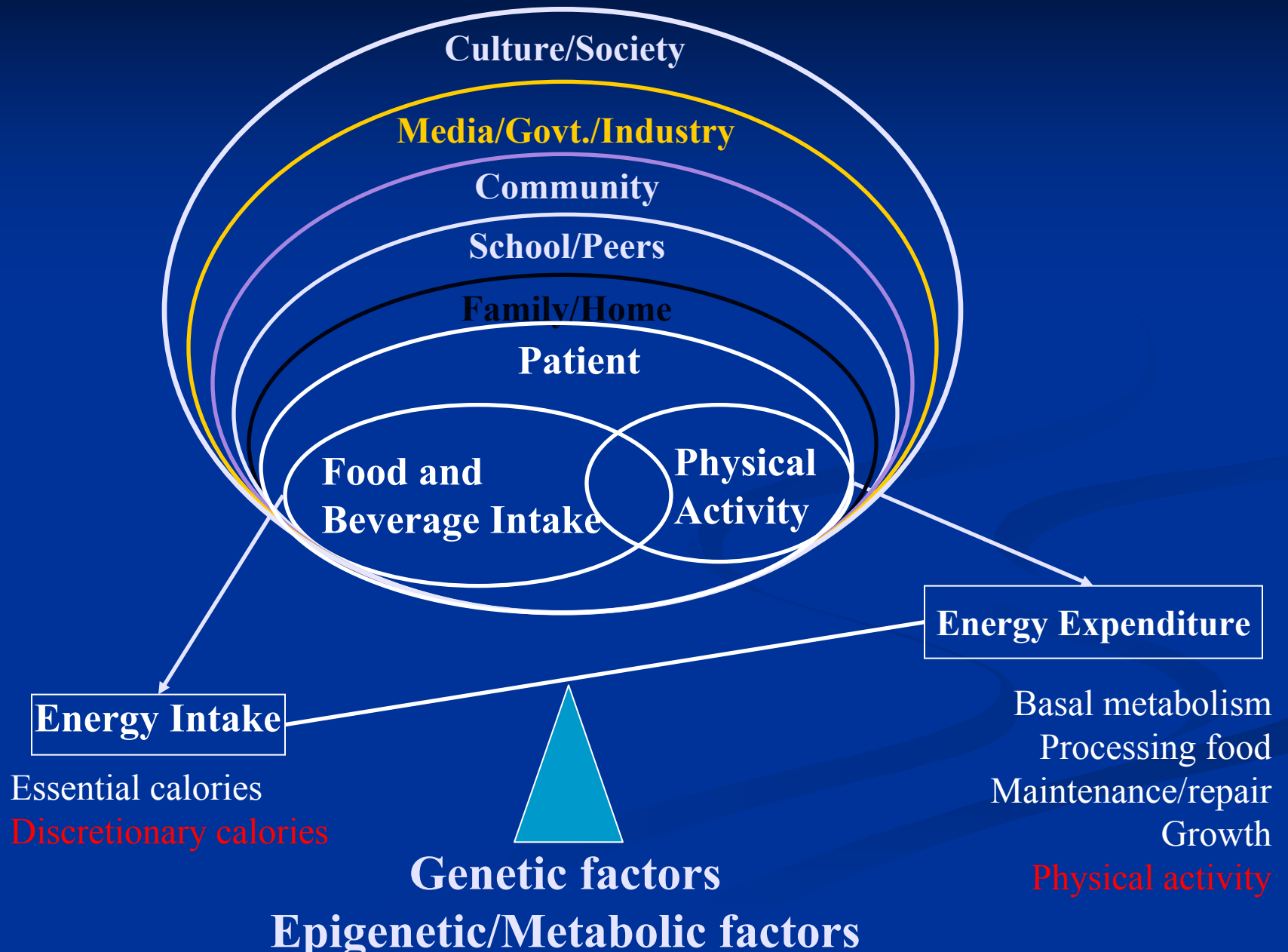
Agouti locus



Dolinoy et al, *EHP* 2006;114:567

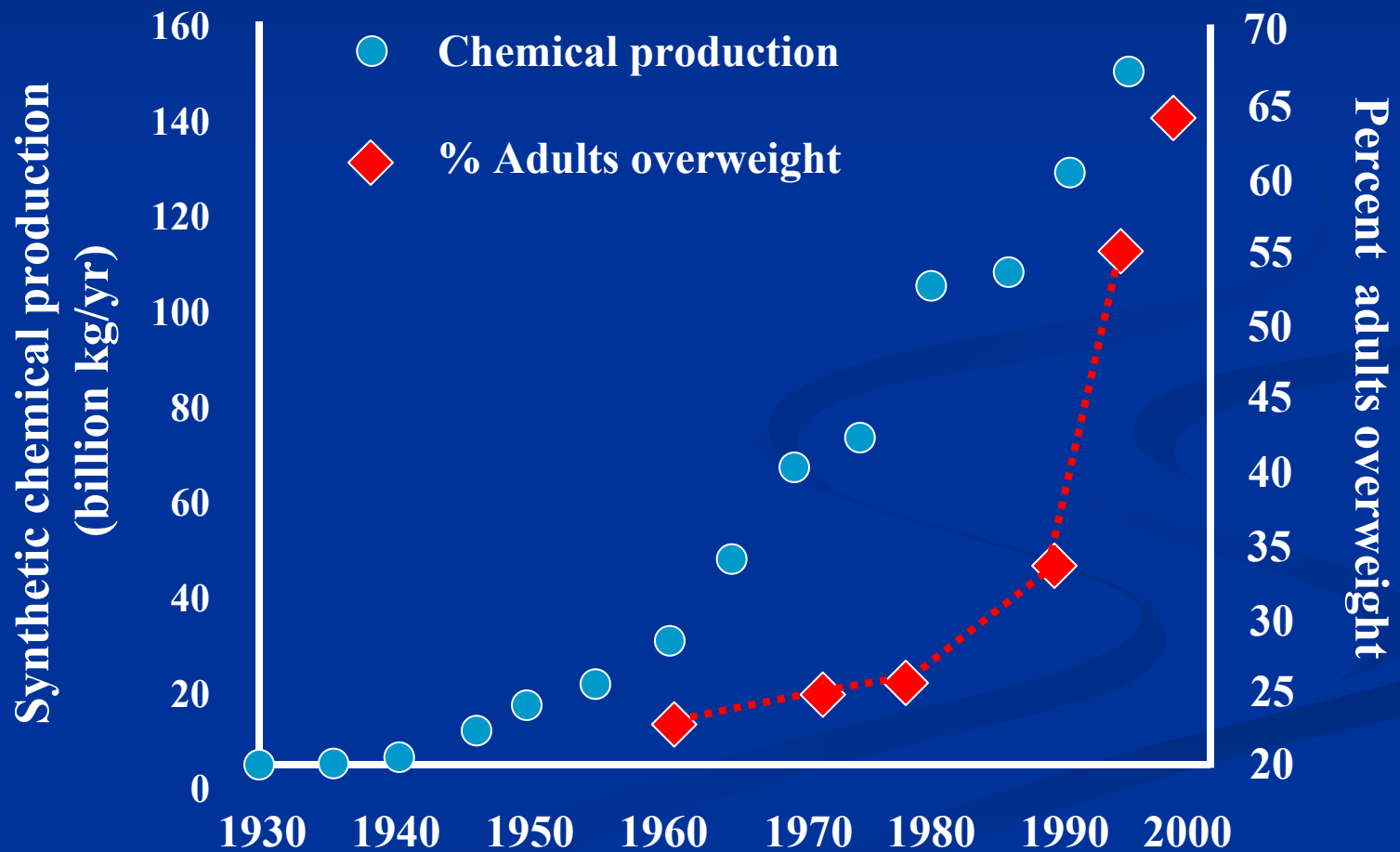
# Ecological Systems Theory Model

Davison KK, Birch LL *Obes Rev* 2001;2:159-71



# Synthetic chemical production and overweight in the US

Baille-Hamilton PF. J Alt Comp Med 2002;2:185-192



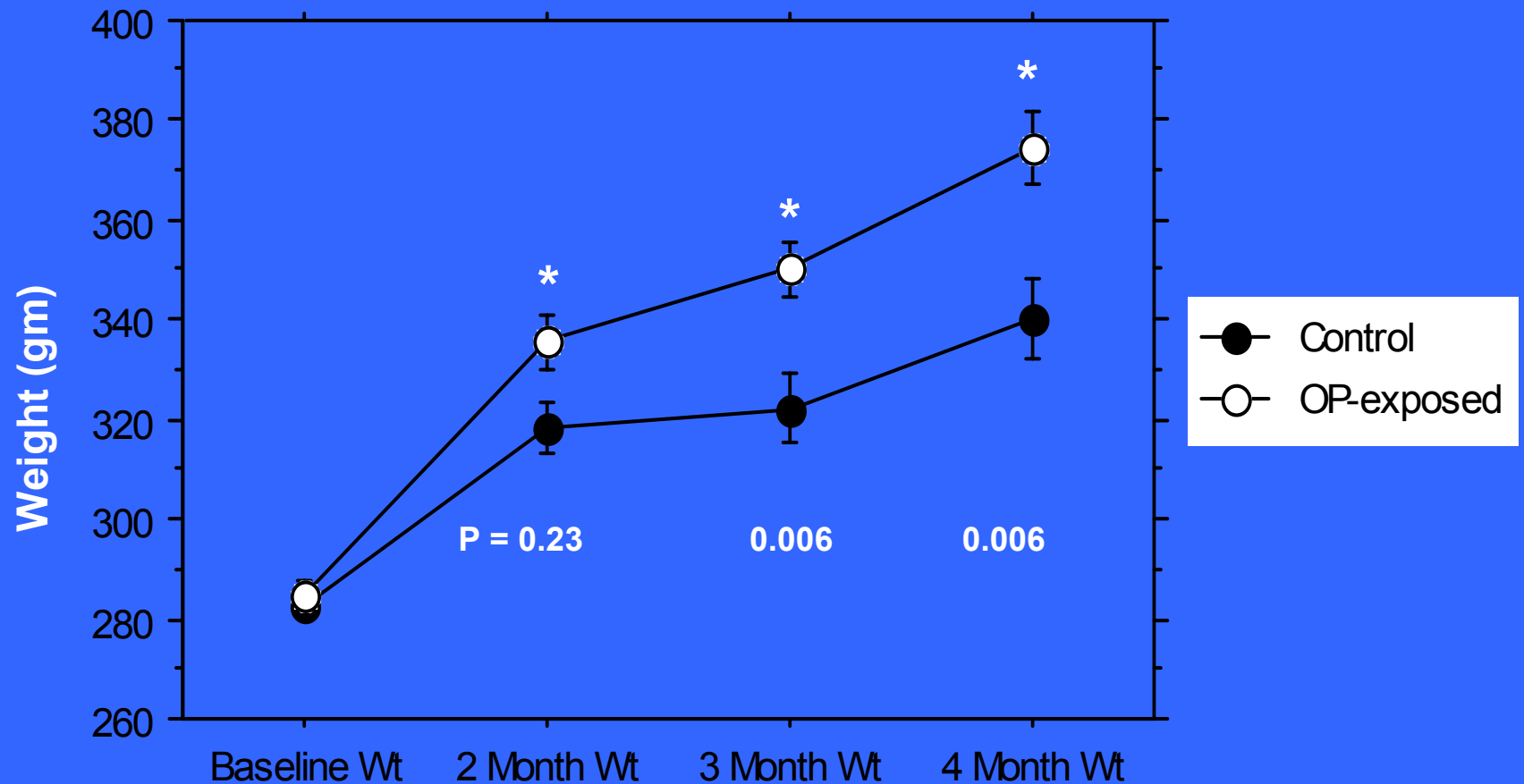
# Synthetic compounds reported to promote weight gain

Baille-Hamilton PF. J Alt Comp Med 2002;2:185-192

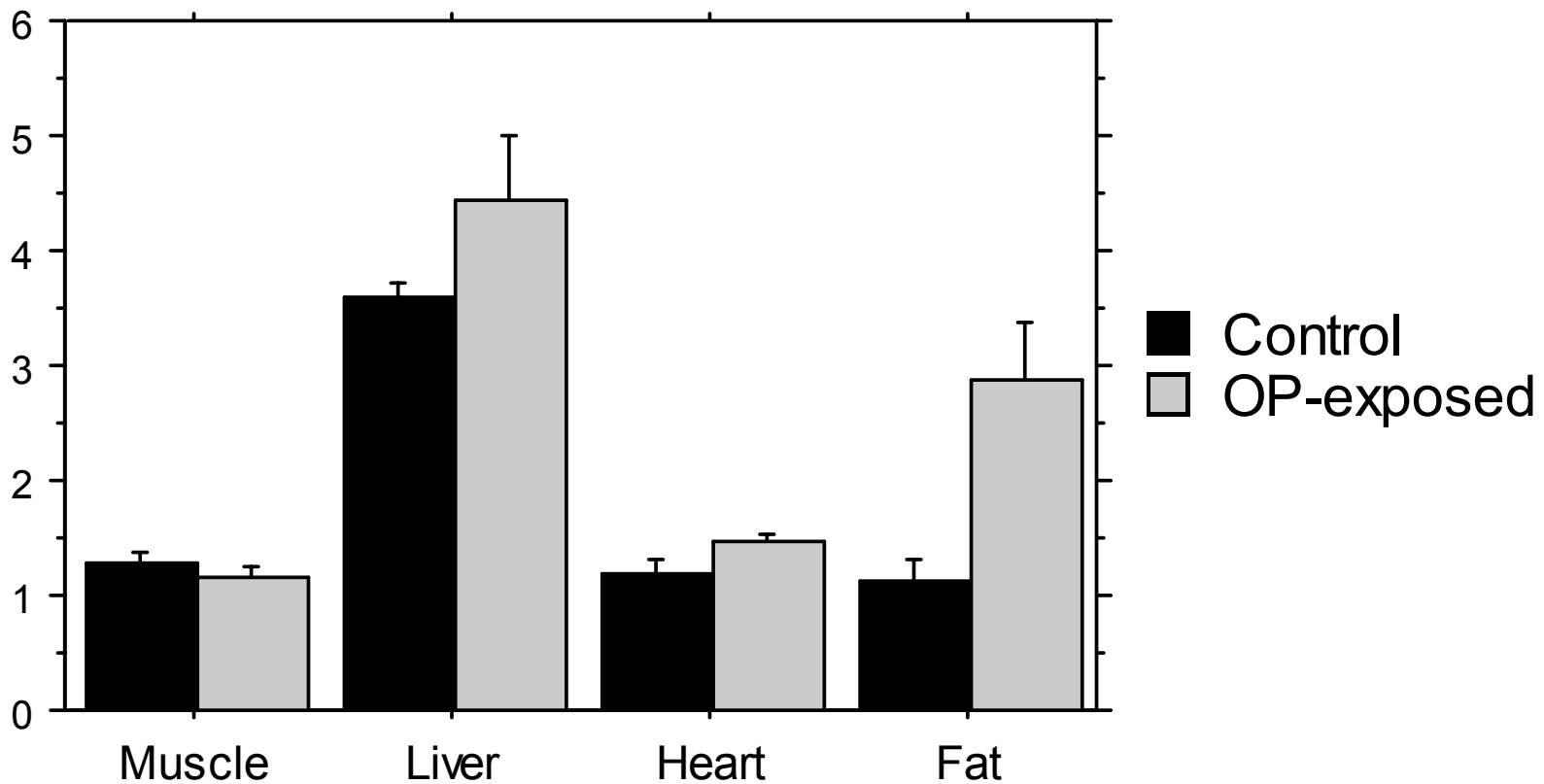
- Pesticides
  - Organophosphates
  - Organochlorines
  - Carbamates
- Polychlorinated/brominated biphenyls
- Perfluoralkyl acids
- Components of plastics
  - Bisphenol-A
  - Phthalates
- Metals
  - Cadmium, lead, organotins
- Solvents

# Effect of chronic chlorpyrifos exposure on growth of female Long-Evans rats (5 mg/kg day<sup>-1</sup>, ≈ 5% LD50)

Meggs, Brewer and Collier

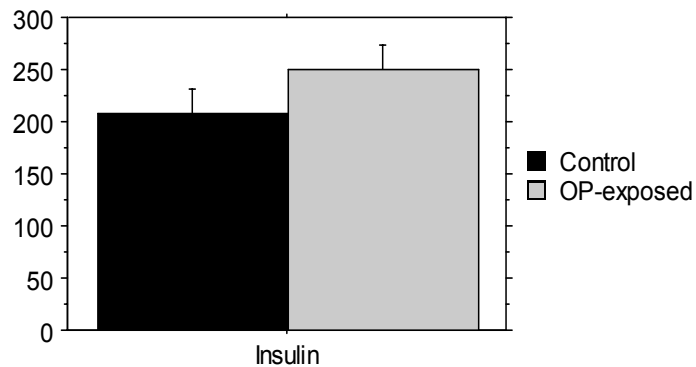
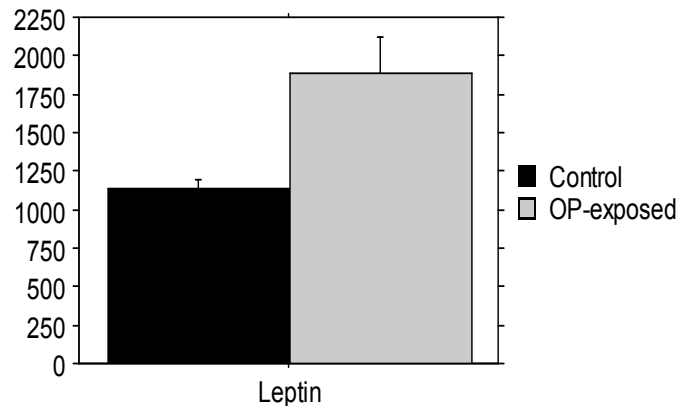
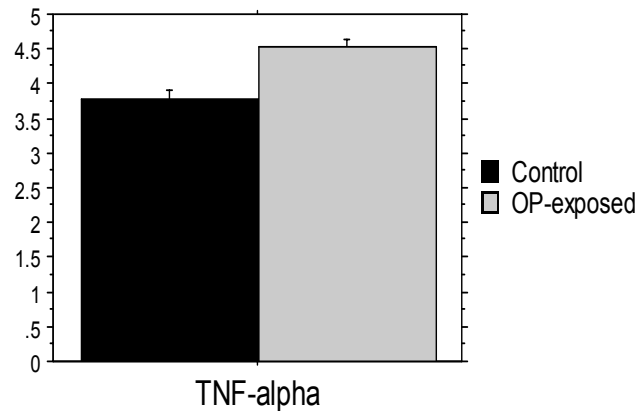


# Effect of chronic chlorpyrifos exposure on organ weights in rats

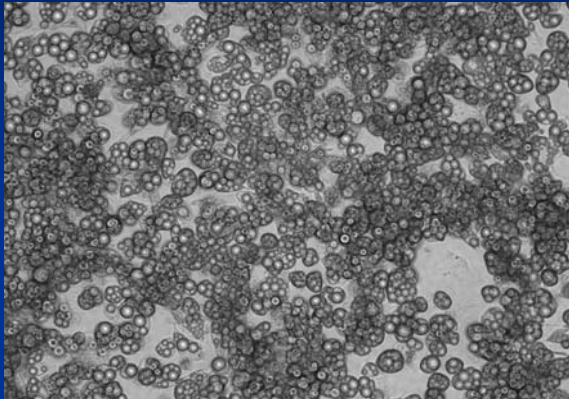




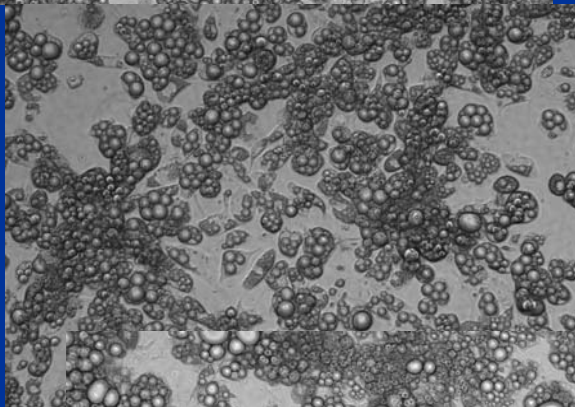
# TNF-alpha, leptin and insulin levels in rats after 2 weeks of continuous exposure to chlorpyrifos



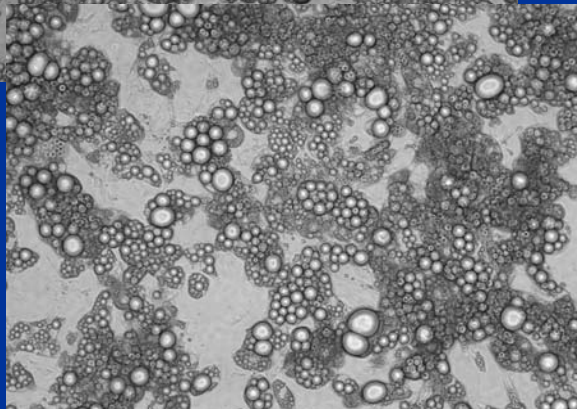
# Effect of chlorpyrifos on pre-adipocyte differentiation in vitro



MDI (stimulates differentiation)



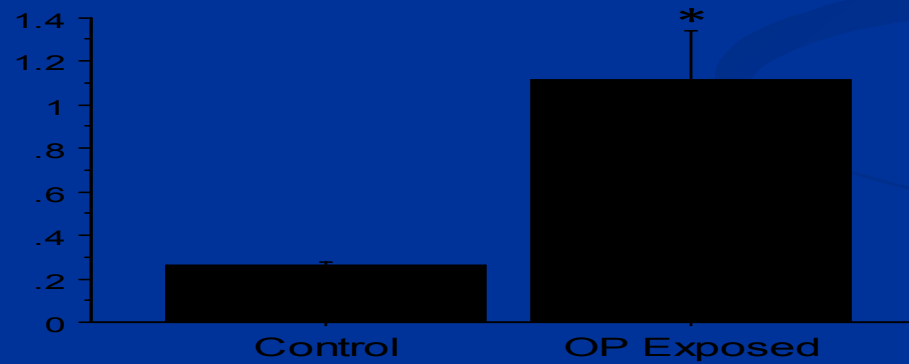
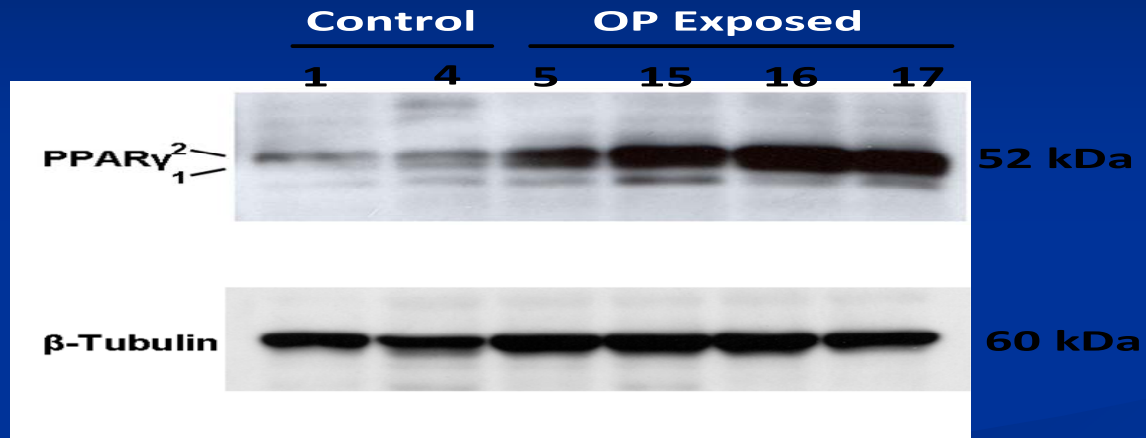
MDI + DMSO (vehicle)



MDI + DMSO  
+ chlorpyrifos @ 0.004 mg/ml

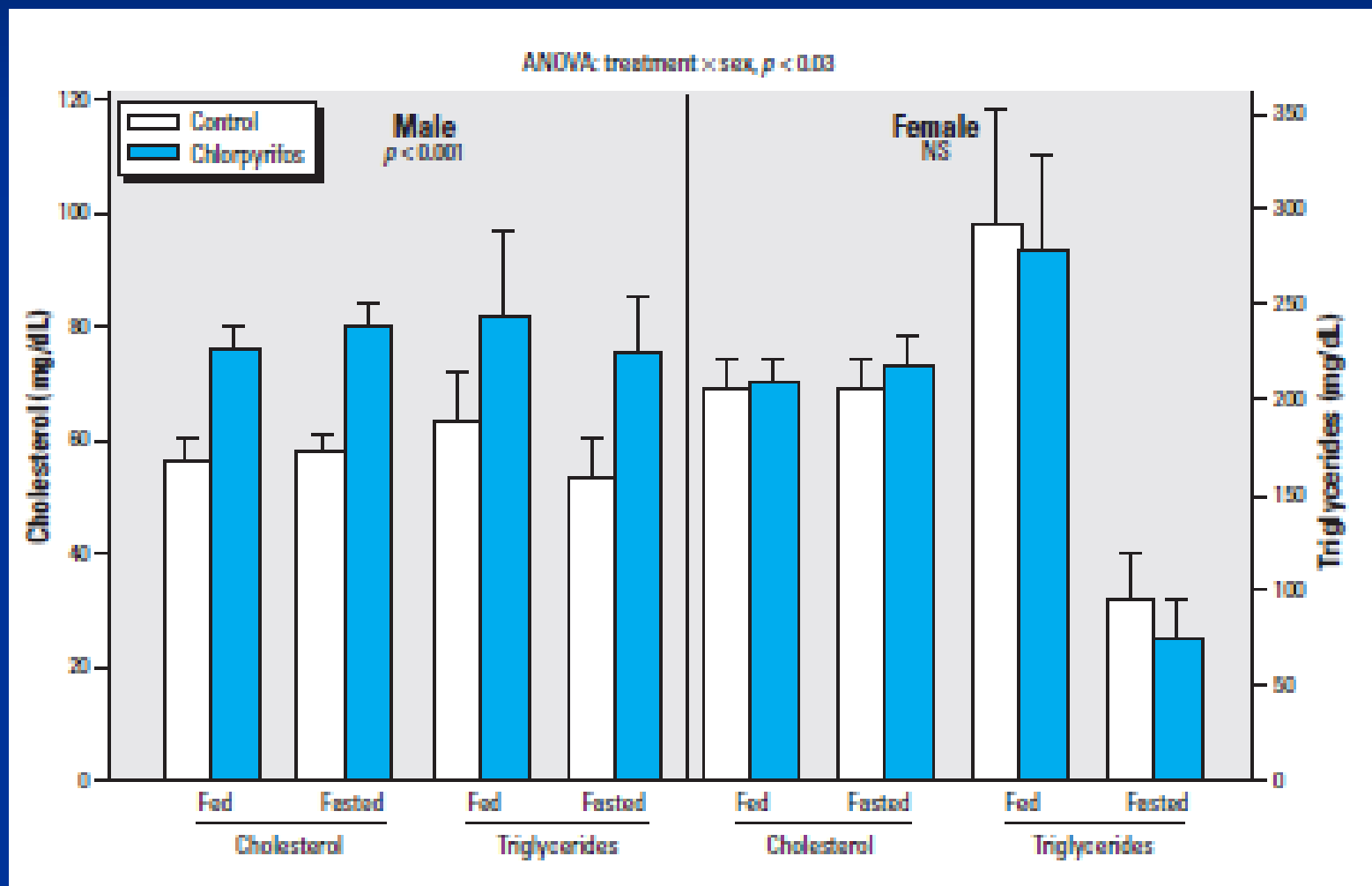
FIGURE 1

# Chlorpyrifos exposure up-regulates PPAR $\gamma$ expression in adipose tissue



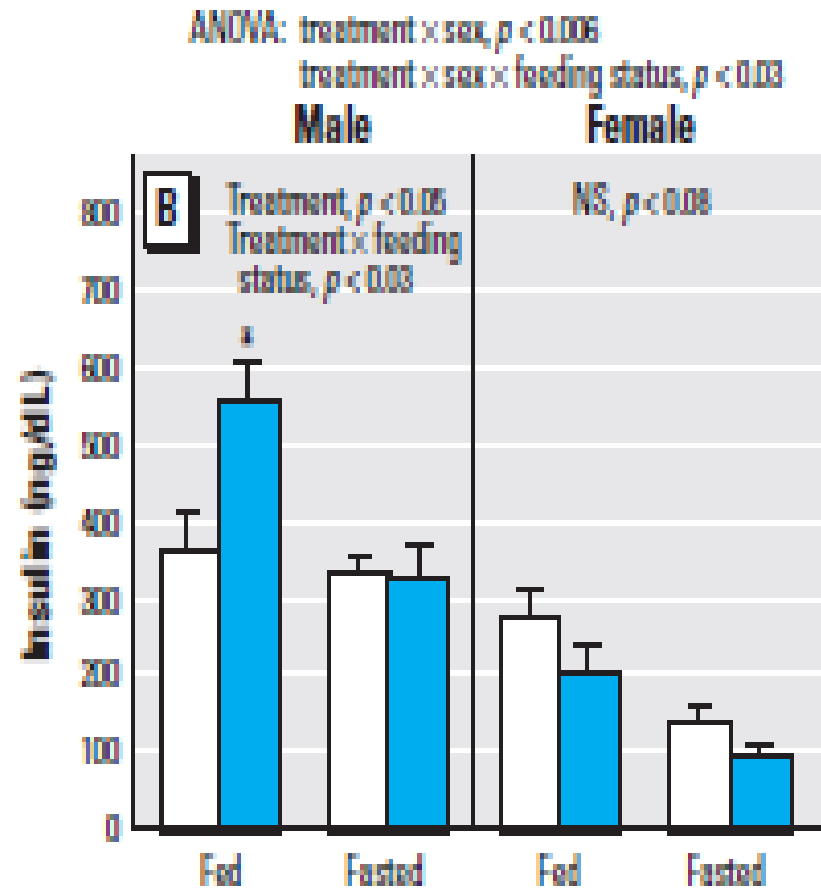
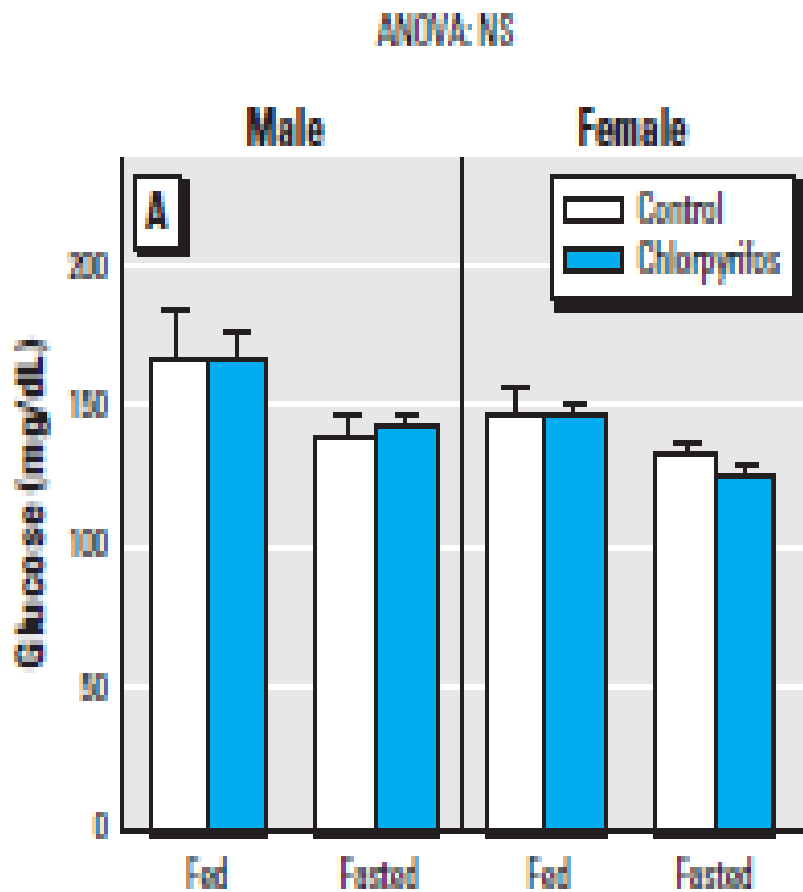
# Perinatal Exposure to Chlorpyrifos (1 mg/kg) Results in Dyslipidemia in Adult ♂ Off-Spring

Slotkin, Brown, Seidler EHP 2005;113:1291-94



# Perinatal Exposure to Chlorpyrifos (1 mg/kg) Results in Insulin Resistance in Adult ♂ Off-Spring

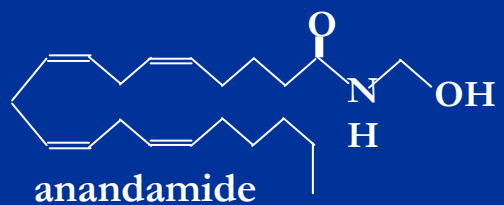
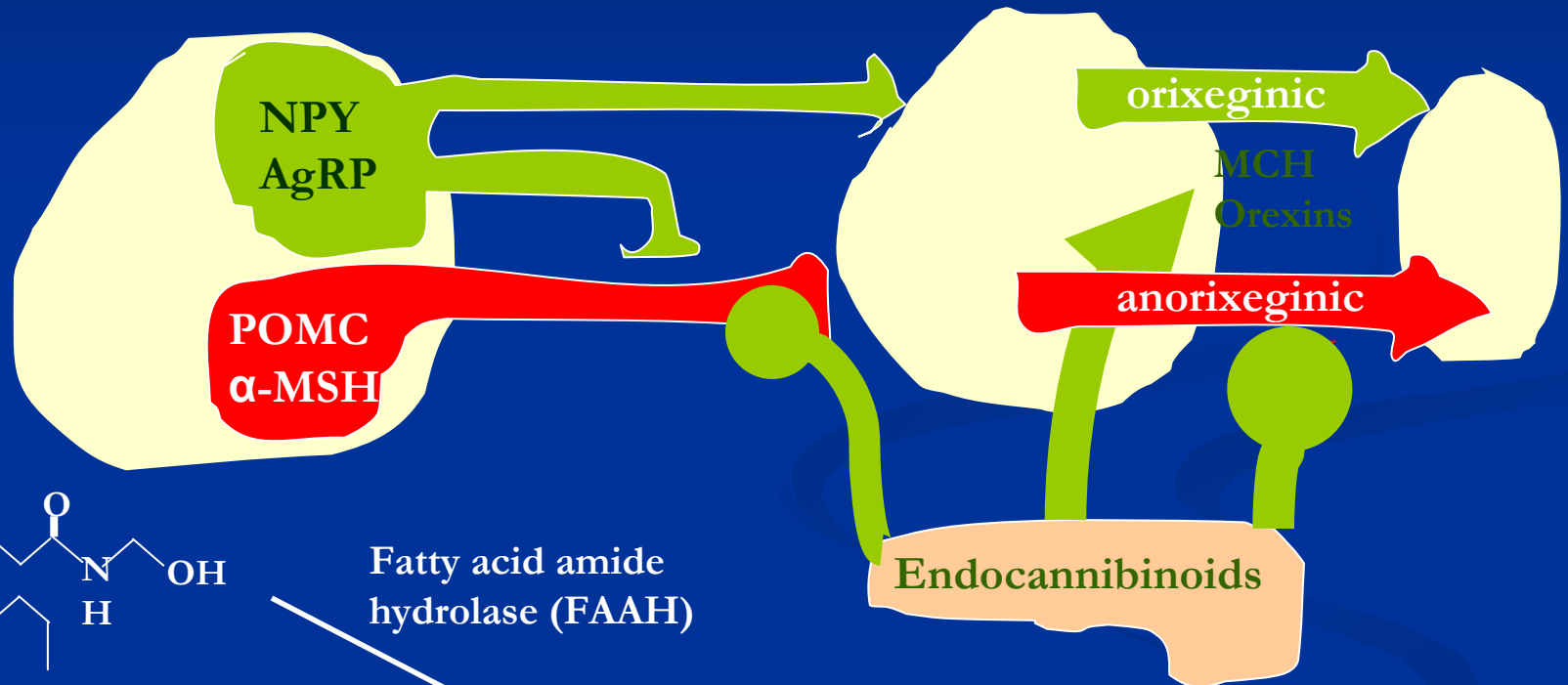
Slotkin, Brown, Seidler EHP 2005;113:1291-94



# Is the Endocannabinoid System Responsible for Organophosphate Mediated Obesity?

Arcuate Nucleus

Paraventricular Nucleus



Fatty acid amide  
hydrolase (FAAH)

X



Monoacyl glycerol  
Lipase (MAGL)

X



organophosphates

2-arachidonylglycerol

# Acknowledgements

- OP work at ECU done by:
  - Kori Brewer PhD
  - Bill Meggs MD, PhD
  - Phil Pekala PhD
  - David Collier MD, PhD
  - Garrett Franklin BS
  - Brian Whitfield BS

# Bisphenol A

vom Saal and Hughes EHP 2005;113:926-33

- Building block of polycarbonate
  - Bottles, can liners, dental resins
  - 6.4 billion pounds/year
- Readily leaches
  - Extremes of pH, temperature and age, detergents
- Potent “endocrine disruptor”
  - Gestational exposure (maternal consumption of ppb):
    - permanent androgen mediated prostate hypertrophy in rodents
    - Decreased sperm count
    - aneuploidy
    - Intraductal hyperplasia and carcinoma in situ in rats
- Other effects
  - Cognitive-behavioral
    - Excessive aggression in males
    - Hyperactive/inattentive

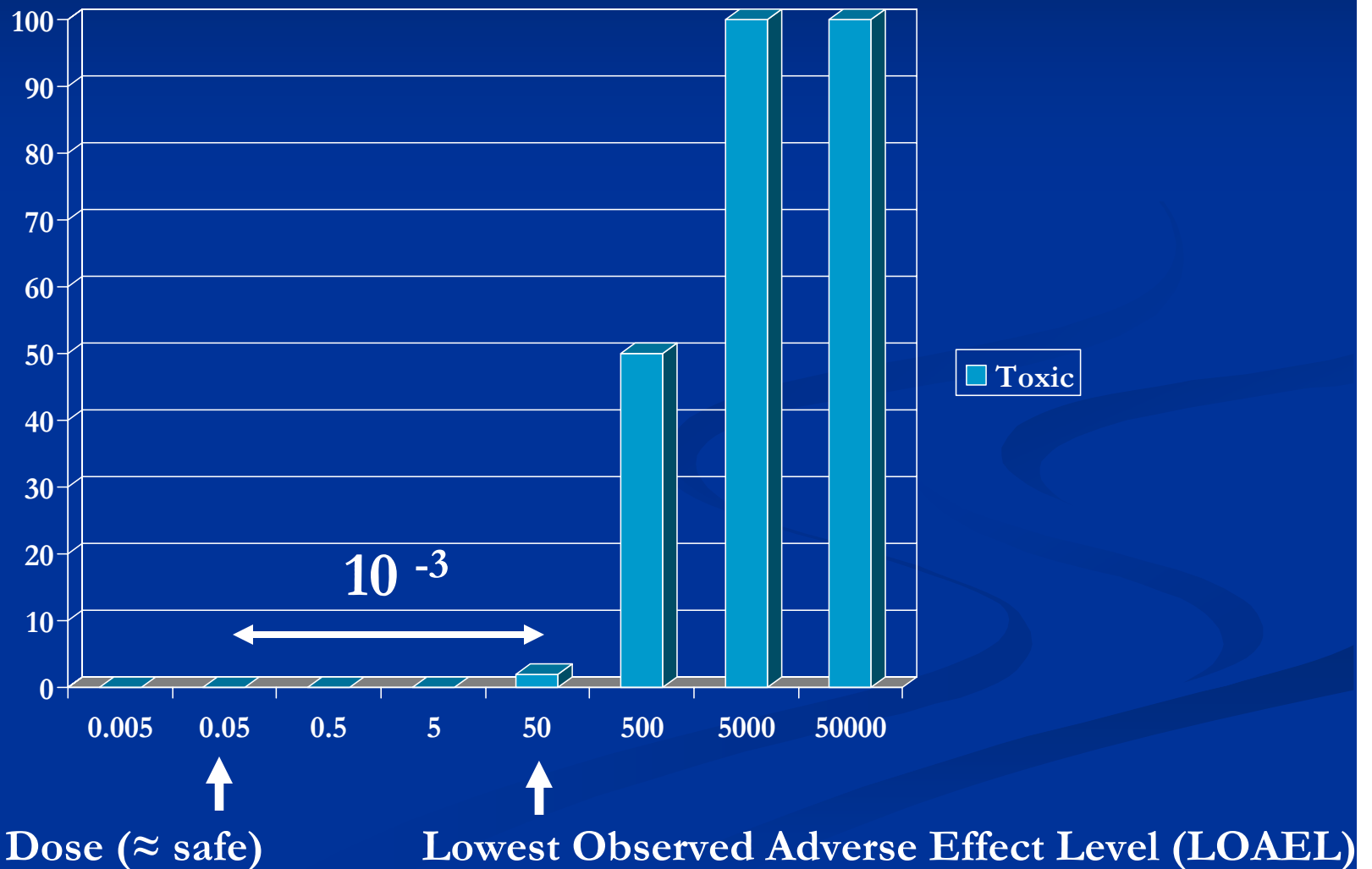


# Bisphenol A

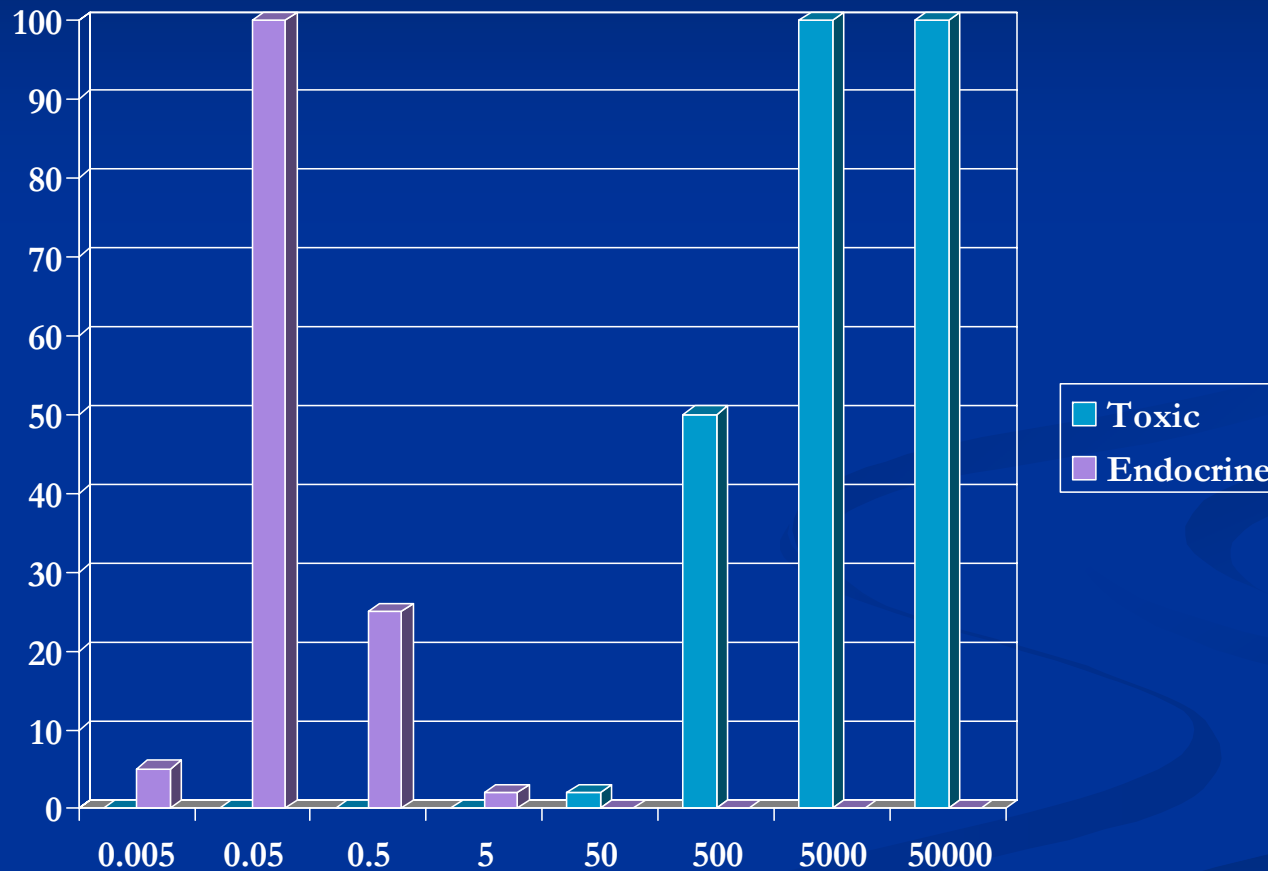
vom Saal and Hughes EHP 2005;113:926-33

- Common in humans
  - 95% of urine samples +
  - Maternal/fetal plasma, placenta, breast milk
  - Amniotic fluid levels 5X maternal plasma
  - Biologically relevant concentrations
    - Median BPA levels in humans exceed levels required to cause adverse effects in mice
    - 94/115 published in vivo studies demonstrate biological effects at low levels (below the LOAEL of 50 mg/kg/day)
    - 40/115 show effects below reference value (50 ug/kg/d)

# LOAEL vs. Reference Dose for BPA (mg/kg day<sup>-1</sup>)



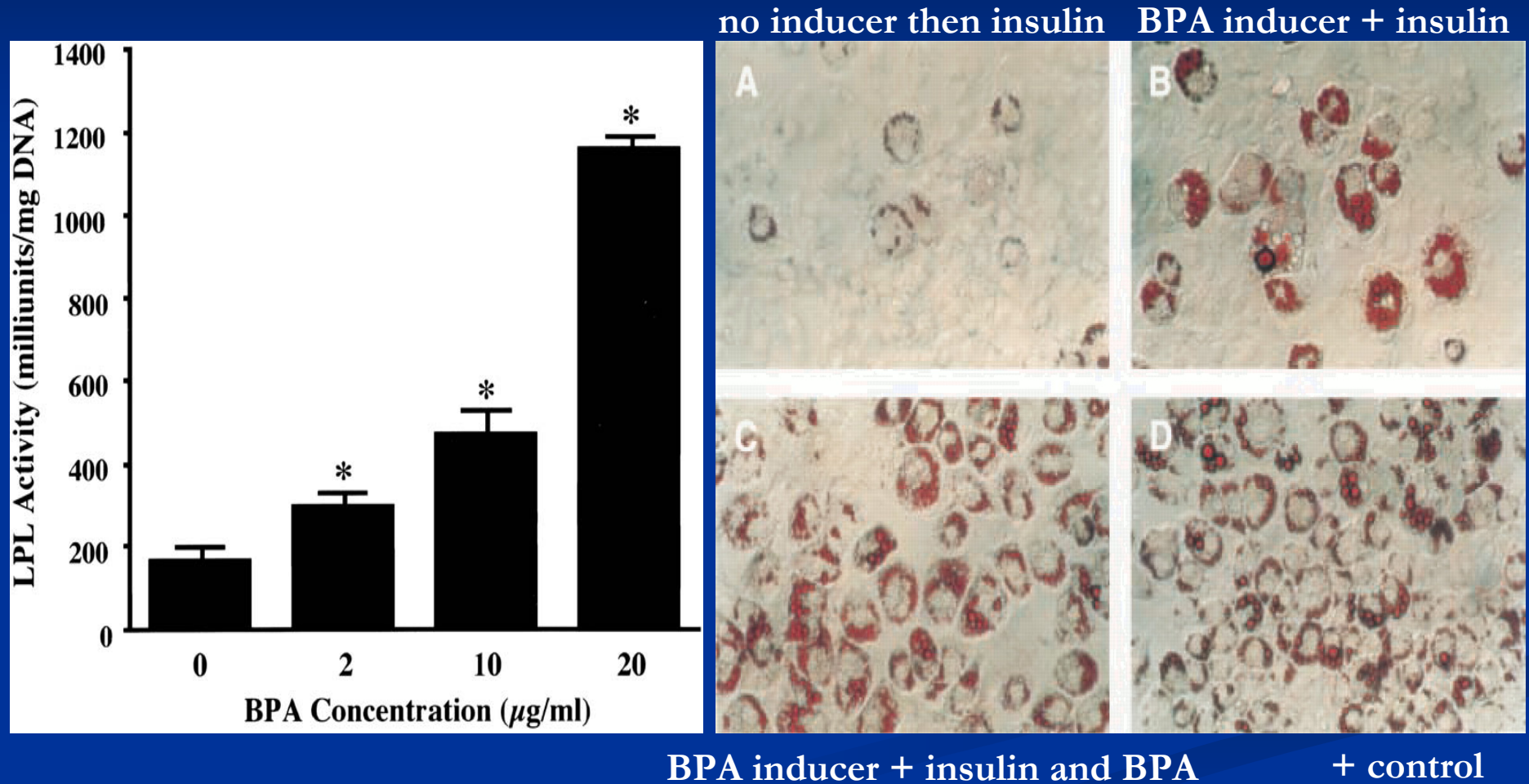
# Toxic and endocrine disruption dose response curves for BPA are distinct



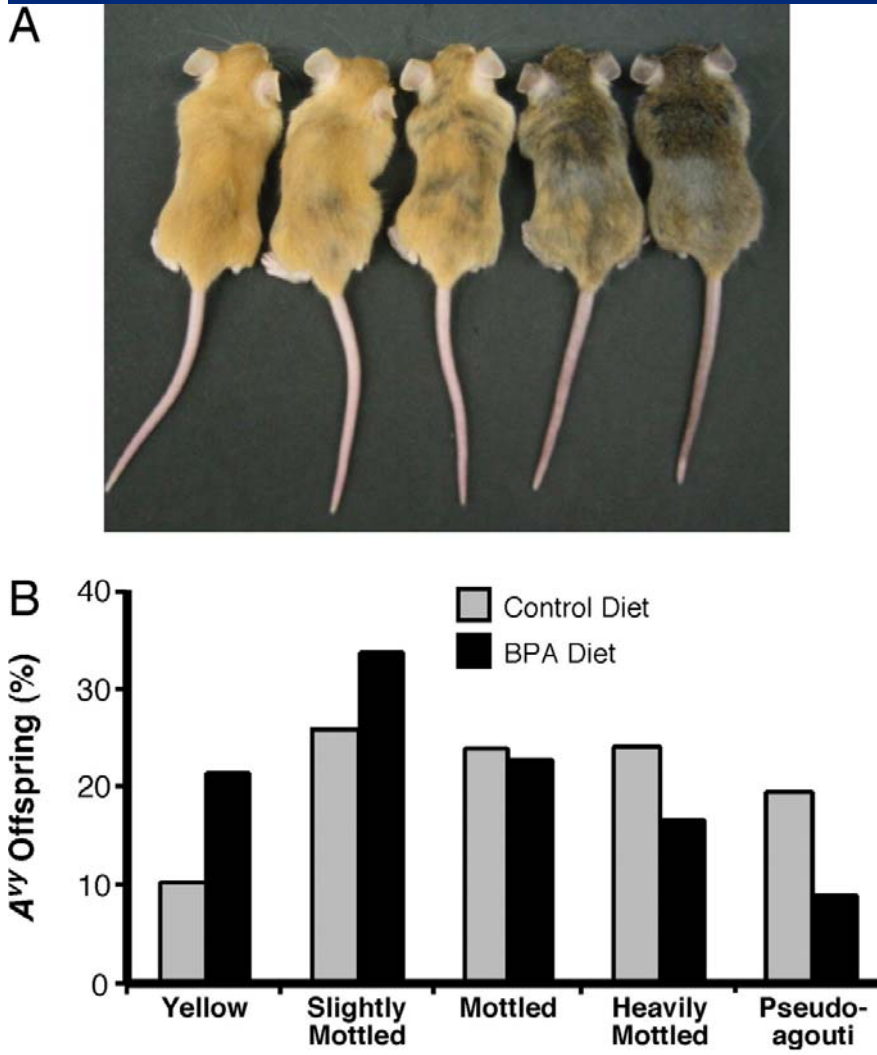
Reference Dose ( $\approx$  safe)

Lowest Observable Adverse Effect (LOAE)

# BPA accelerates the differentiation of 3T3 cells into adipocytes



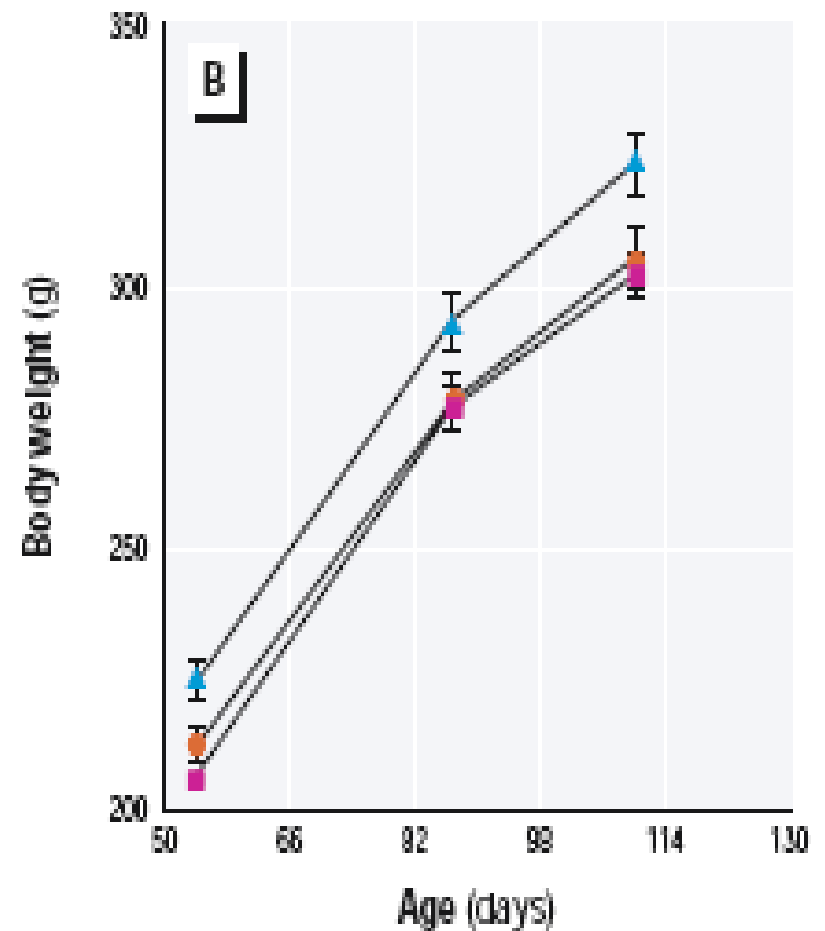
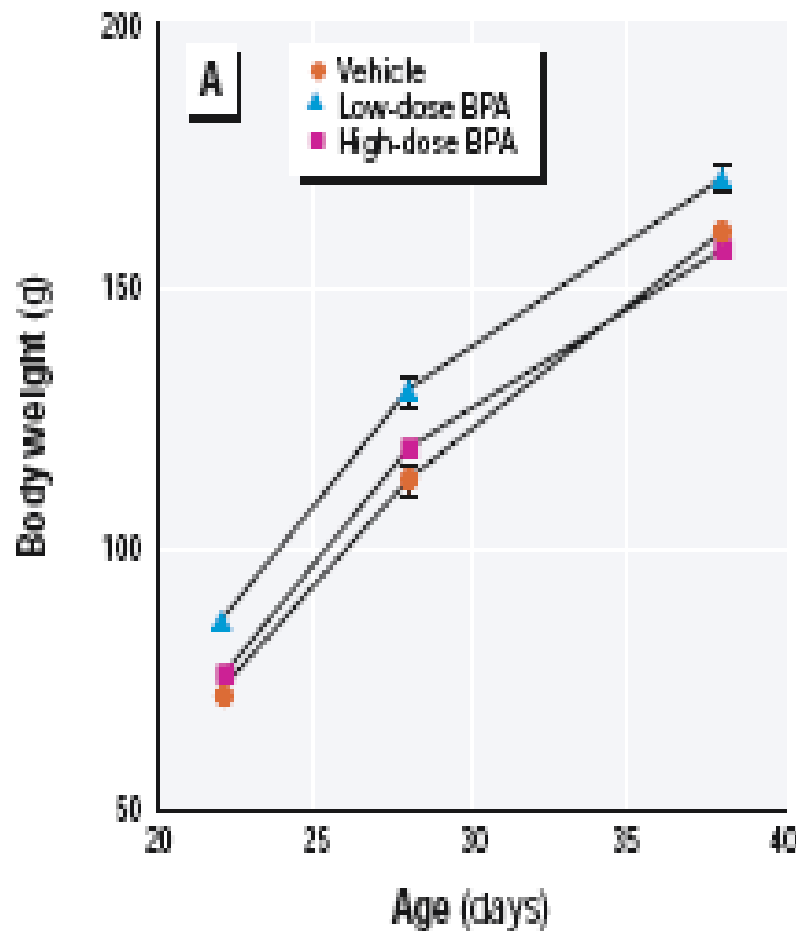
# Maternal exposure to BPA



- Oral exposure (50mg/kg) in dams
- Shifts coat color of offspring to yellow
- Shifts offspring towards obese
- Associated with hypo-methylation of target DNA
- Shows that in-utero exposure to xenobiotic influences chemical modification and expression of DNA

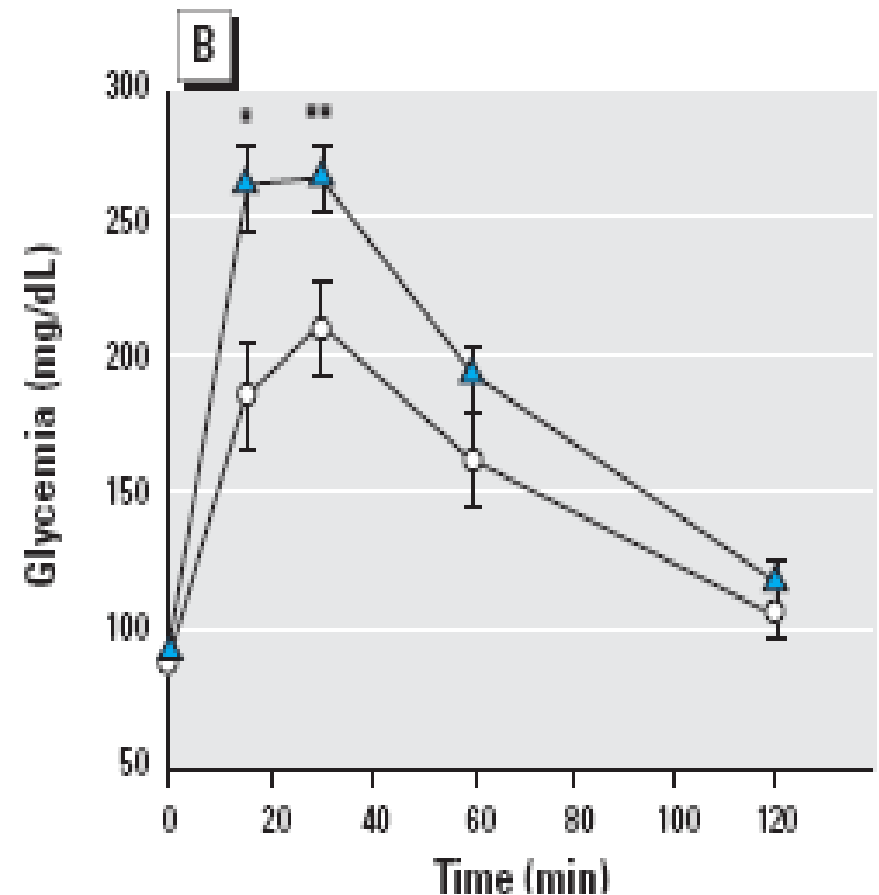
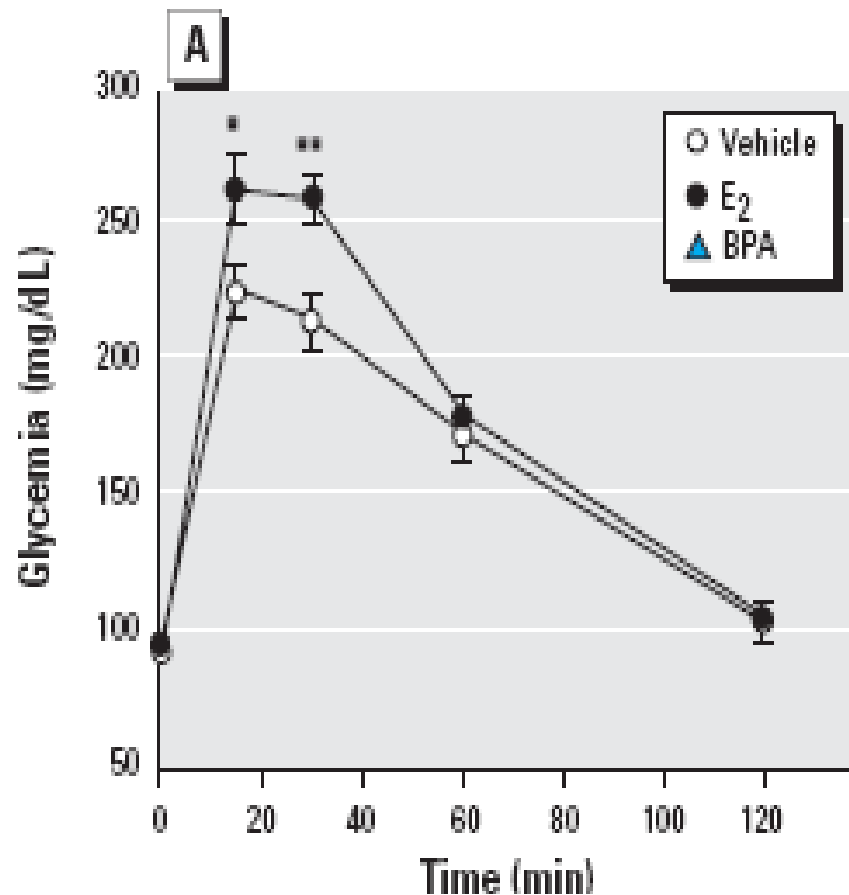
Dolinoy, Huang, Jirtle PNAS 2007

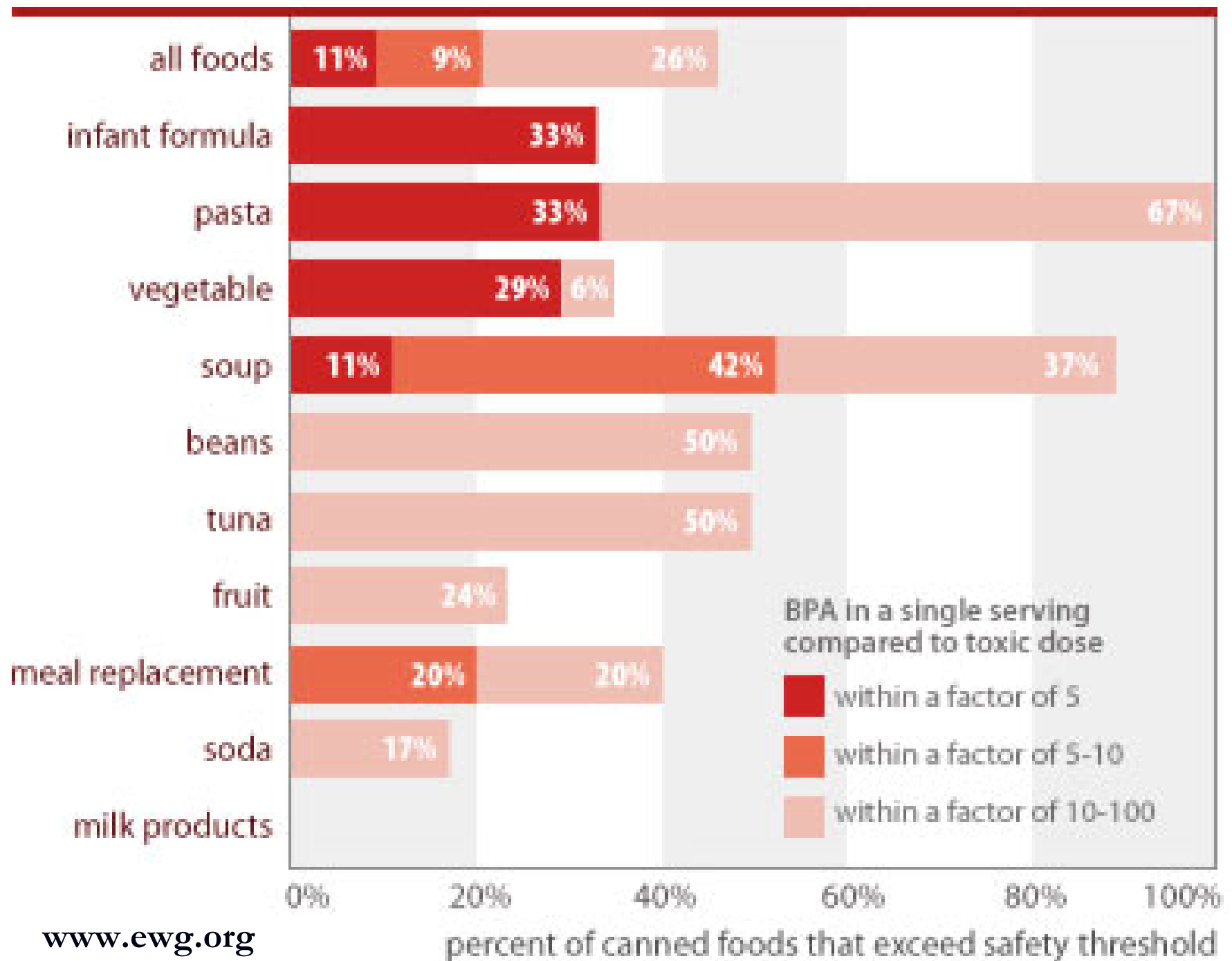
# Effect of in utero exposure to bisphenol-A on weight gain in offspring (rats)



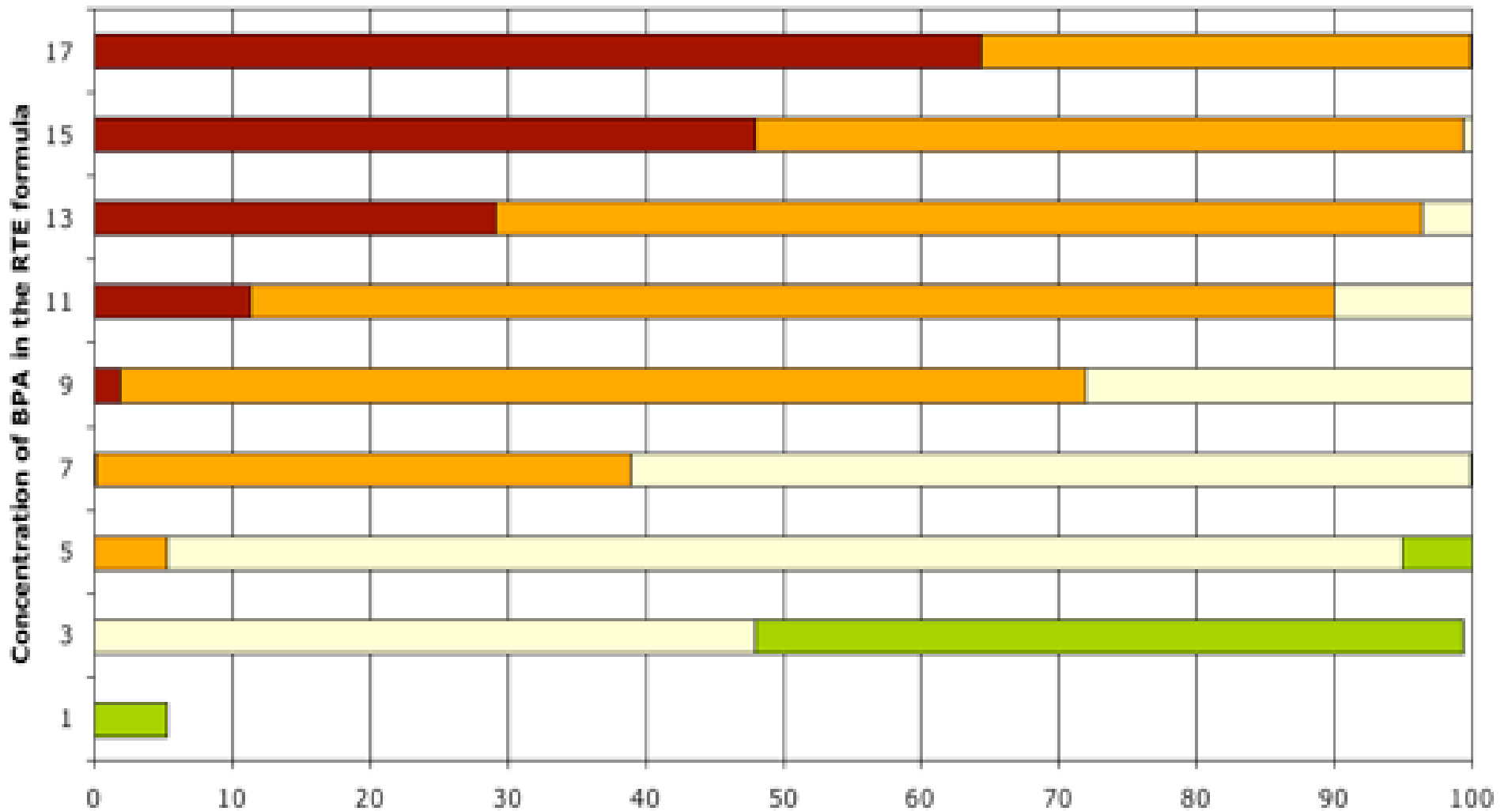
# “Chronic” exposure to bisphenol-A induces insulin resistance and glucose intolerance (Alonso-Magdalena P, Env Health Pers 2006;114:106-12)

- 4 day oral intake
- 100  $\mu\text{g}/\text{kg}/\text{day}$
- Only 2X U.S. EPA’s reference dose and 1/500<sup>th</sup> LOAEL









% infants fed ready-to-eat formula who are exposed above the given dose

Infants exposures to BPA versus the toxic dose

■ % of infants exposed above the toxic dose

■ Within a factor of 2

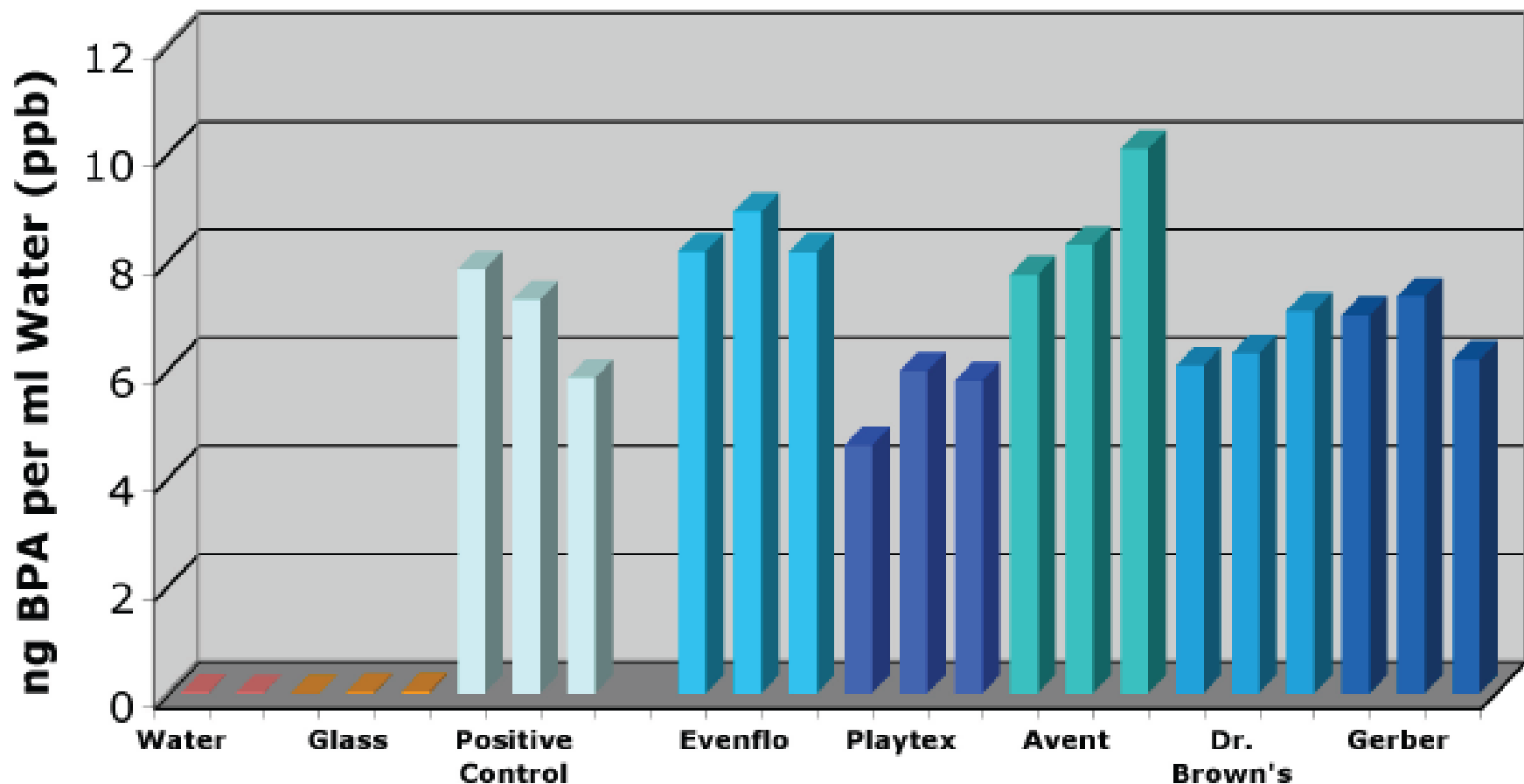
■ Within a factor of 5

■ Within a factor of 10

[www.ewg.org](http://www.ewg.org)

# Rates of BPA leaching from off the shelf baby bottles (24 hrs @ 80° C)

Figure 4: Bisphenol A Extracted from Polycarbonate Baby Bottles



# Phthalates

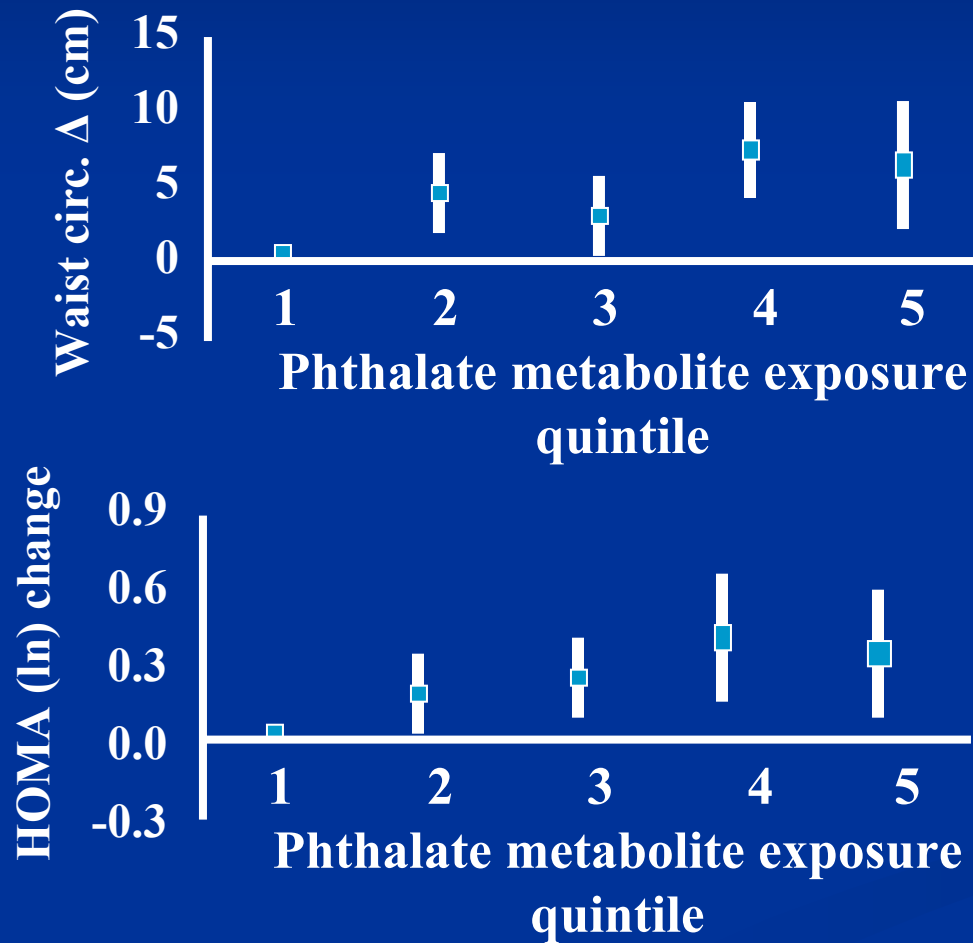
- Commonly used (diesters of phthalic acid)
  - Plasticizers that are non covalently bound to substrates
  - Plastics, cosmetics, shampoos, toys, pacifiers, meds, blood bags
  - Common in people
    - 75% US adults  $\geq$  5 urinary metabolites
    - Present in breast milk, formula
    - NICU infants
- Known endocrine disruptors
  - Anti-androgenic in fetus
  - Levels in cord blood and breast milk positively associated with:
    - cryptorchidism and hypospadias
    - reduced sperm count and testosterone levels are correlated
- Low testosterone in men associated with:
  - Obesity
  - Insulin resistance
  - Diabetes



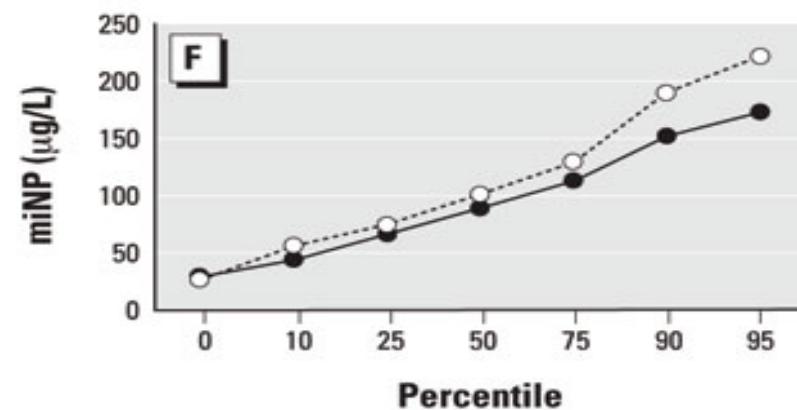
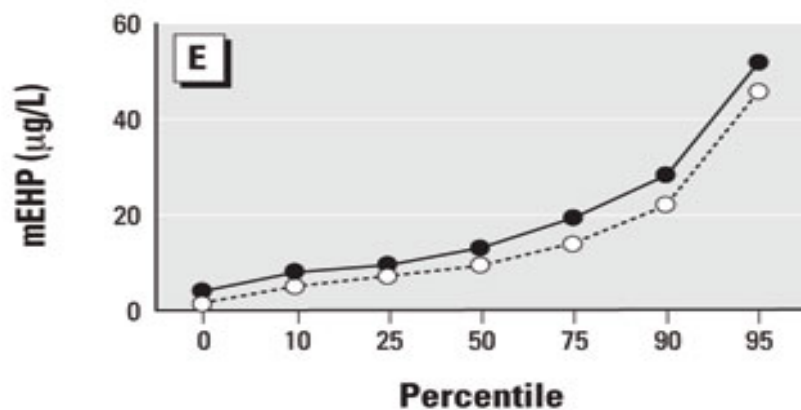
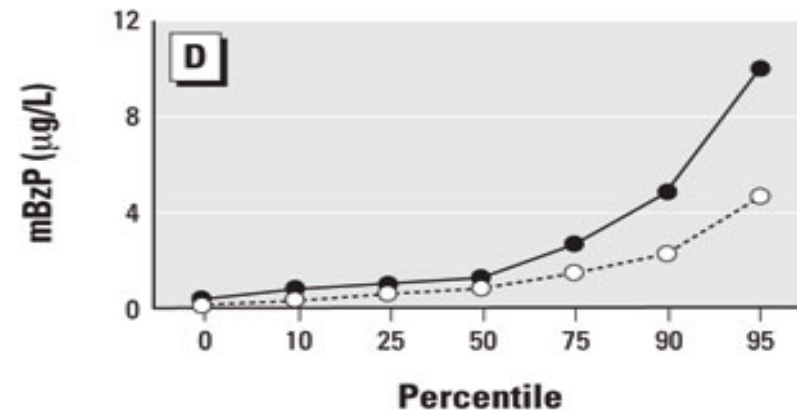
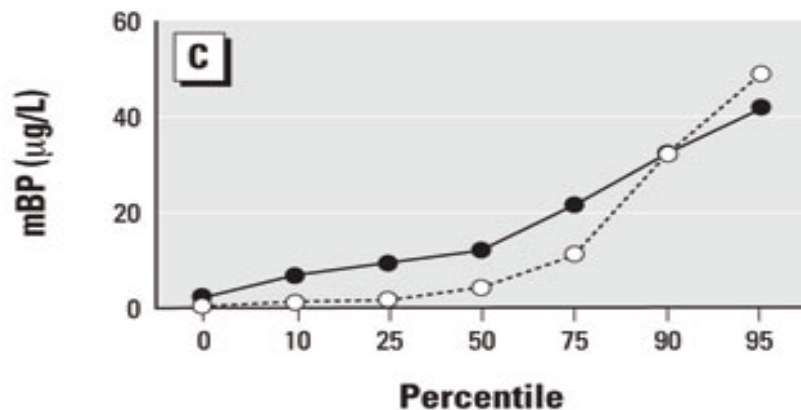
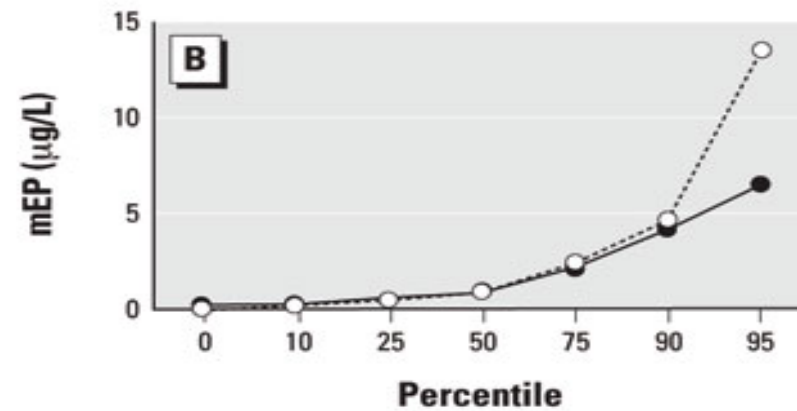
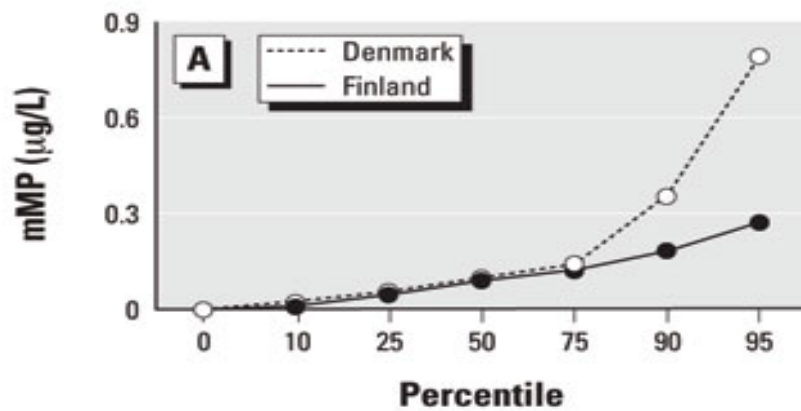
The normal testis, left, comes from a healthy male rat. The right tissue comes from a rat exposed to DEHP, a type of phthalate, while its reproductive tract was maturing. It is a small testis filled with fluid. The corresponding tissue from the other side of the same animal exhibits no visible testis or sperm-storing epididymis.

# Waist circumference, insulin resistance (HOMA) and urinary levels of phthalate metabolites

Stahlhut et al 2007 (poster)



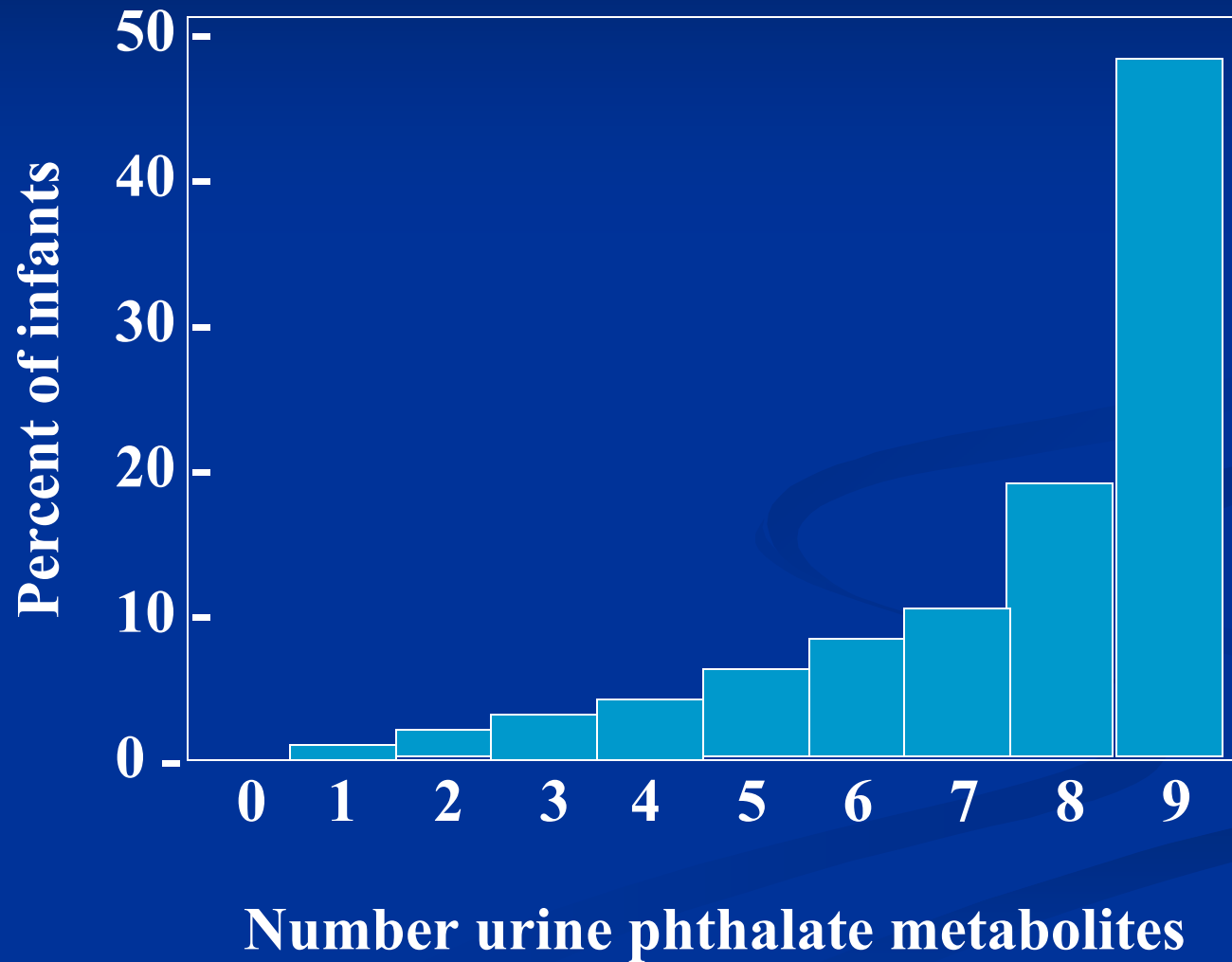
- Data from NHANES
  - 1292 adult ♂
- Similar relationship for 5 different metabolites
- [Phthalate metabolite] positively associated with obesity and insulin resistance



**Figure 1.** Concentration of six phthalate monoesters ( $\mu\text{g/L}$ ) in human breast milk samples from Denmark ( $n = 65$ ) and Finland ( $n = 65$ ), 1997–2001, collected between 1 and 3 months postnatally as additive aliquots. Data are given as percentile distribution. (A) mMP, (B) mEP, (C) mBP, (D) mBzP, (E) mEHP, (F) miNP.

# Number of phthalate metabolites in infant urine

Sathyanarayana S. *Pediatrics* 2008;121:e261-268

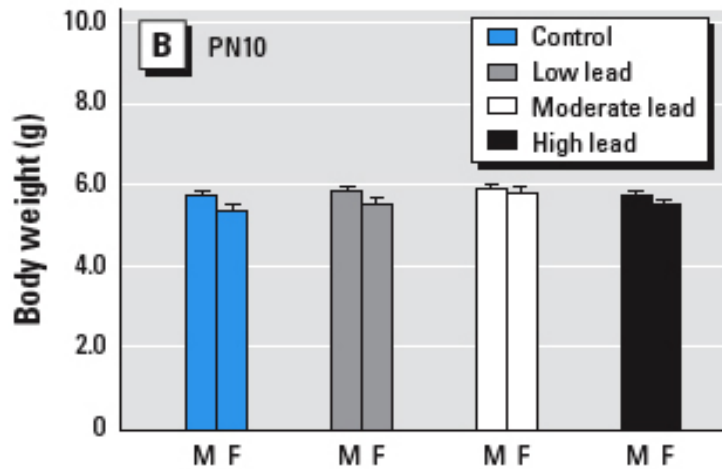


# Organotin

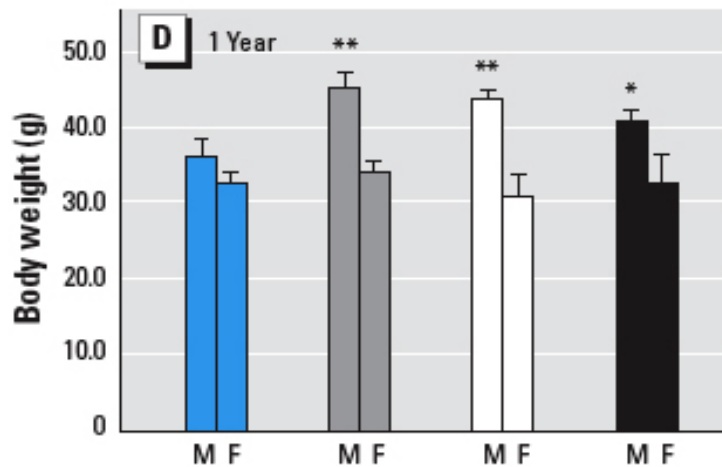
- Organic derivatives of tin (e.g. tributyltin)
- Antifungals, wood preservatives, PVC products
- Potent activators (1-10 nM) of:
  - PPAR $\gamma$  receptor (target of thiazolidinediones)
  - Retinoid X receptor
  - Adipocyte differentiation
- In utero exposure in animals:
  - Increased ectopic fat deposition in offspring
  - Hepatic steatosis
- Levels in human samples
  - Range 3-100 nM
  - Mean 27 nM
  - Low transfer to breast milk



# Lead (in utero)



10 days old



1 year old

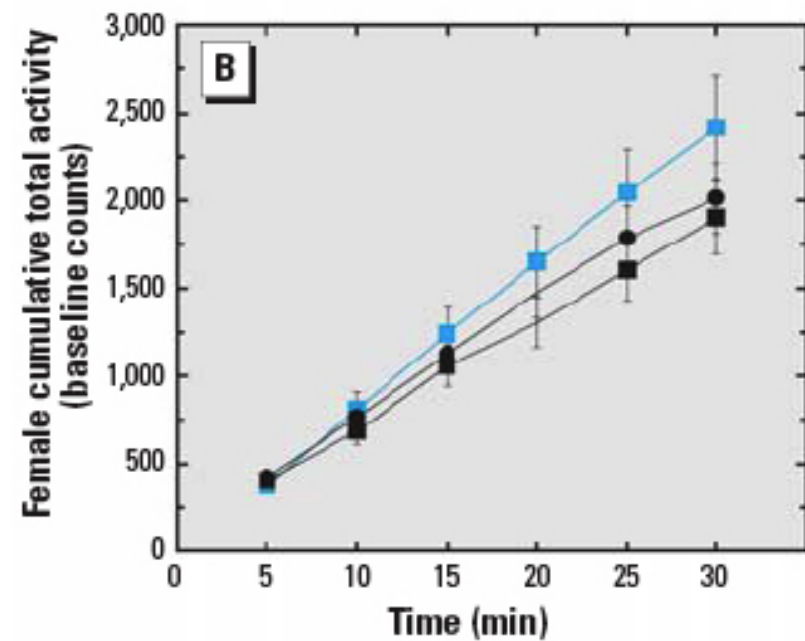
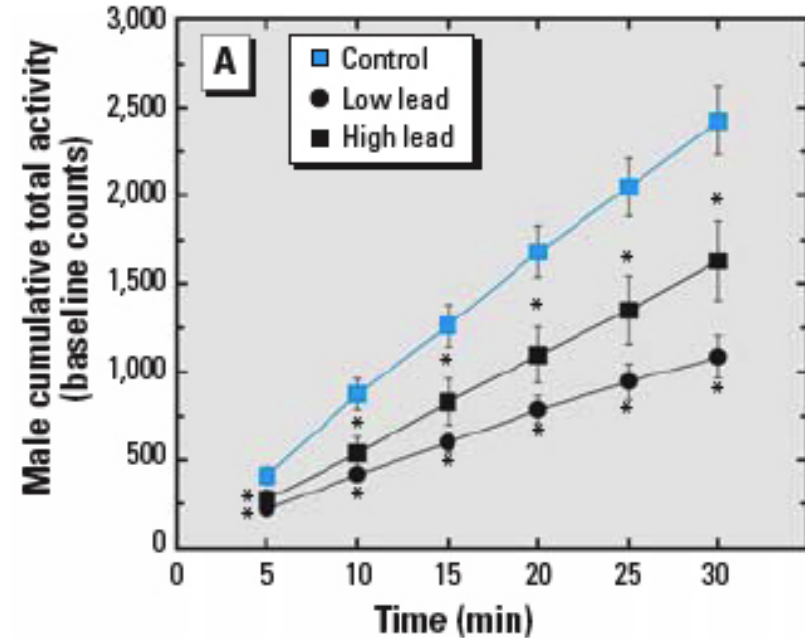
Effect noted in:  
Male rats only  
Greater at low dose

Leasure JL, et al, Environmental Health Perspectives, 116: 355-361 (2008)

# Why?

- Marked decrease in spontaneous locomotor activity for male rats.

Leasure JL, et al, Environmental Health Perspectives, 116: 355-361 (2008)



# Perfluoroalkyl Acids (PFAAs)

Environmental Health Perspectives May 2007 115:A251-256

## 1. PFAAs

- Surfactants/components in > 200 applications
  - Water/stain repellents

## 2. Perfluorooctanoic acid (PFOA)

- Teflon etc

## 3. Perfluorooctanoyl sulfonate (PFOS)

- Scotchguard, Stainmaster
- Long  $t_{1/2}$  in humans (4-7 yrs!)

## ■ Sources

- Food
  - Wrappers – eg. Microwave popcorn!
- Cook-ware
- Soil/dust
  - DuPont sites in NC
- Water
- Breast milk



# Gestational Exposure to PFOA (Teflon etc.) and Body Composition at 80 Weeks

Environmental Health Perspectives May 2007 115:A251-256



# A Role for Perfluorinated Chemicals (PCAs) in Human Obesity and Metabolic Disease?



## ■ Ubiquitous

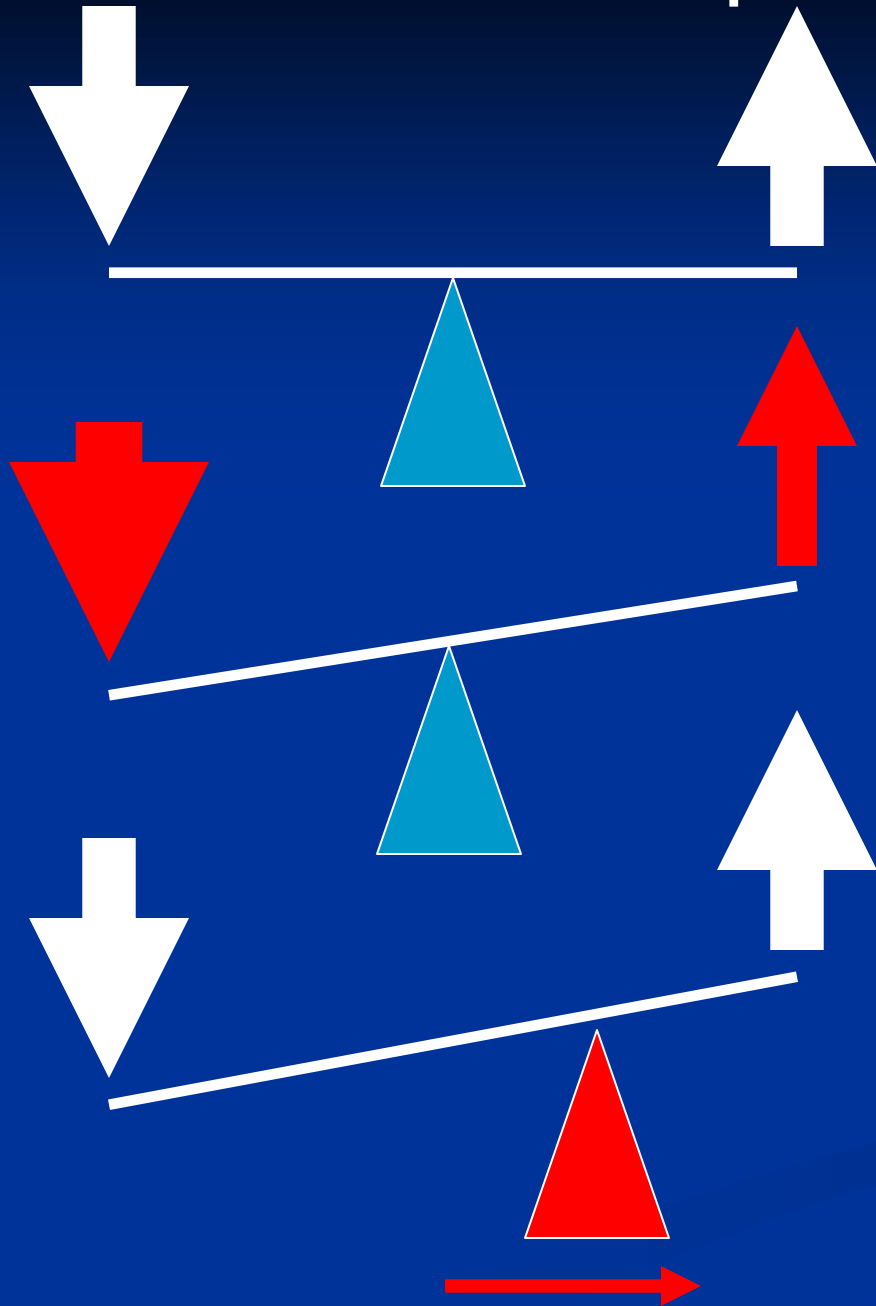
- > 90% adults have PCAs
  - PFOS @ 30.4 ppb
  - PFOA @ 5.2 ppb
- Cord blood
  - $\approx$  10% maternal serum levels
  - 100% with PFOS
  - 99% with PFOA
- Breast milk
  - $\approx$  1% maternal serum levels
  - 200 ngm/day to infant

## ■ Correlations

- “Dose dependent” decrease in birth weight and cord blood levels
  - - 69 gms for PFOS
  - - 104 gms for PFOA

E intake

E expenditure



- Neutral/negative

- $E_{in} \approx E_{exp}$ .

- Weight neutral

- Positive

- $E_{in} > E_{exp}$ .

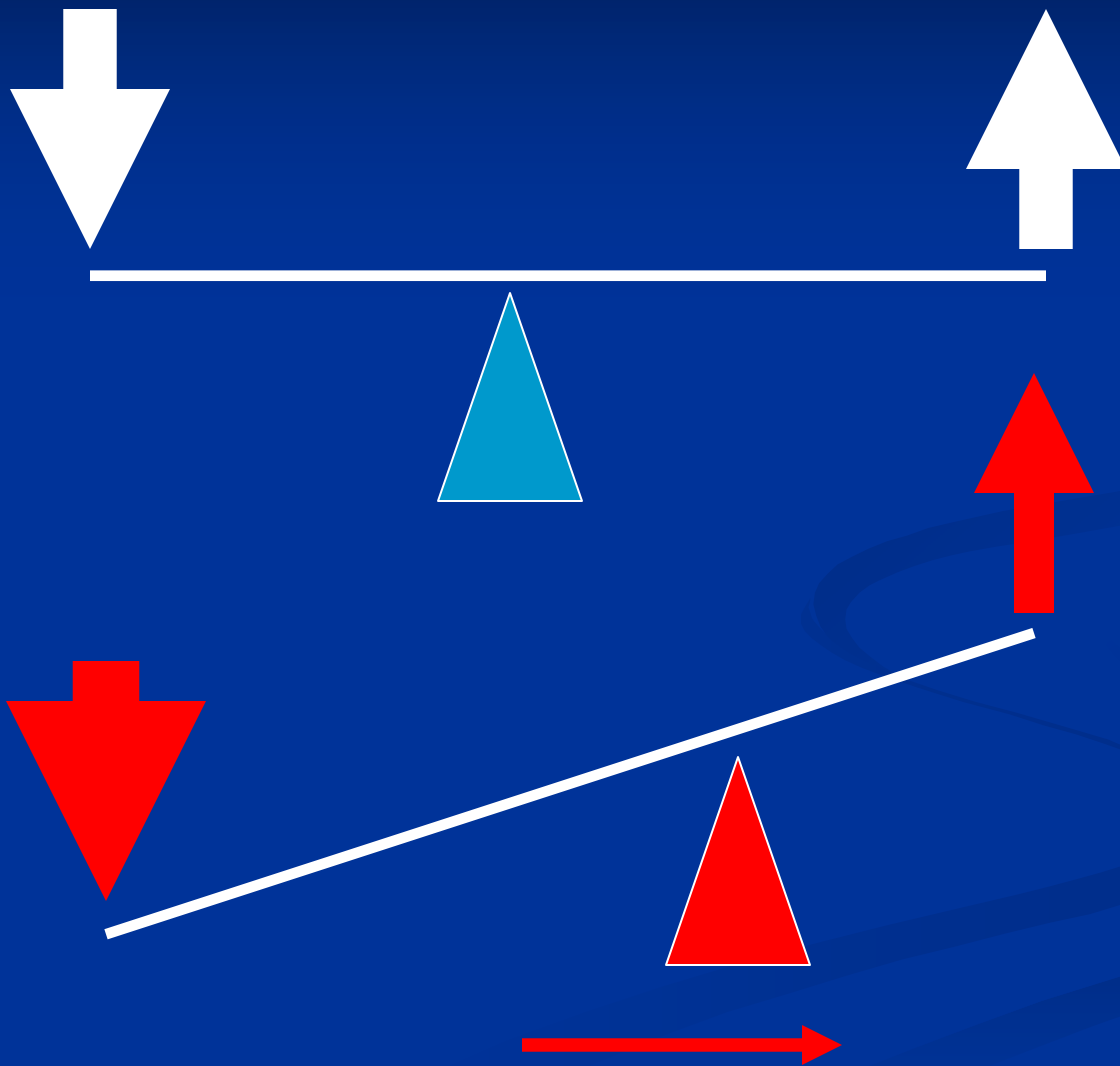
- Obesity

- Positive

- $E_{in} \approx E_{exp}$ .

- Obesity due to programming

# A perfect storm?



# Recommendations to minimize risk

- Avoid polycarbonate food packaging (bisphenolA)
  - Hard, clear - labeled as “PC” or #7,
  - # 1, 2, 5 aren't sources of bisphenolA
  - If polycarbonate is used:
    - Avoid washing at high temperatures
    - Avoid harsh detergents
    - Avoid scratching
    - Discard if cloudy or crazed
- Avoid polycarbonate baby bottles
  - Leaching accelerates after 50-100 washes
  - Select glass or polypropylene (softer more opaque)



# Recommendations to minimize risk

- Never heat food in plastic containers
  - Microwave in ceramic or glass containers
  - Don't let cling wrap touch food – use paper towel
- Avoid micro waving in grease or water resistant paper packaging (perfluoroalkyl acids)
  - Microwave popcorn, pot pies etc.
- Avoid food in plastic wrap
  - Pliable cling wrap contains phthalates as plasticizer
  - Especially avoid meats and cheeses in cling wrap
    - Buy @ deli and have wrapped in paper (PFAAs??)
  - Cut away portion in contact with wrap

# Recommendations to minimize risk

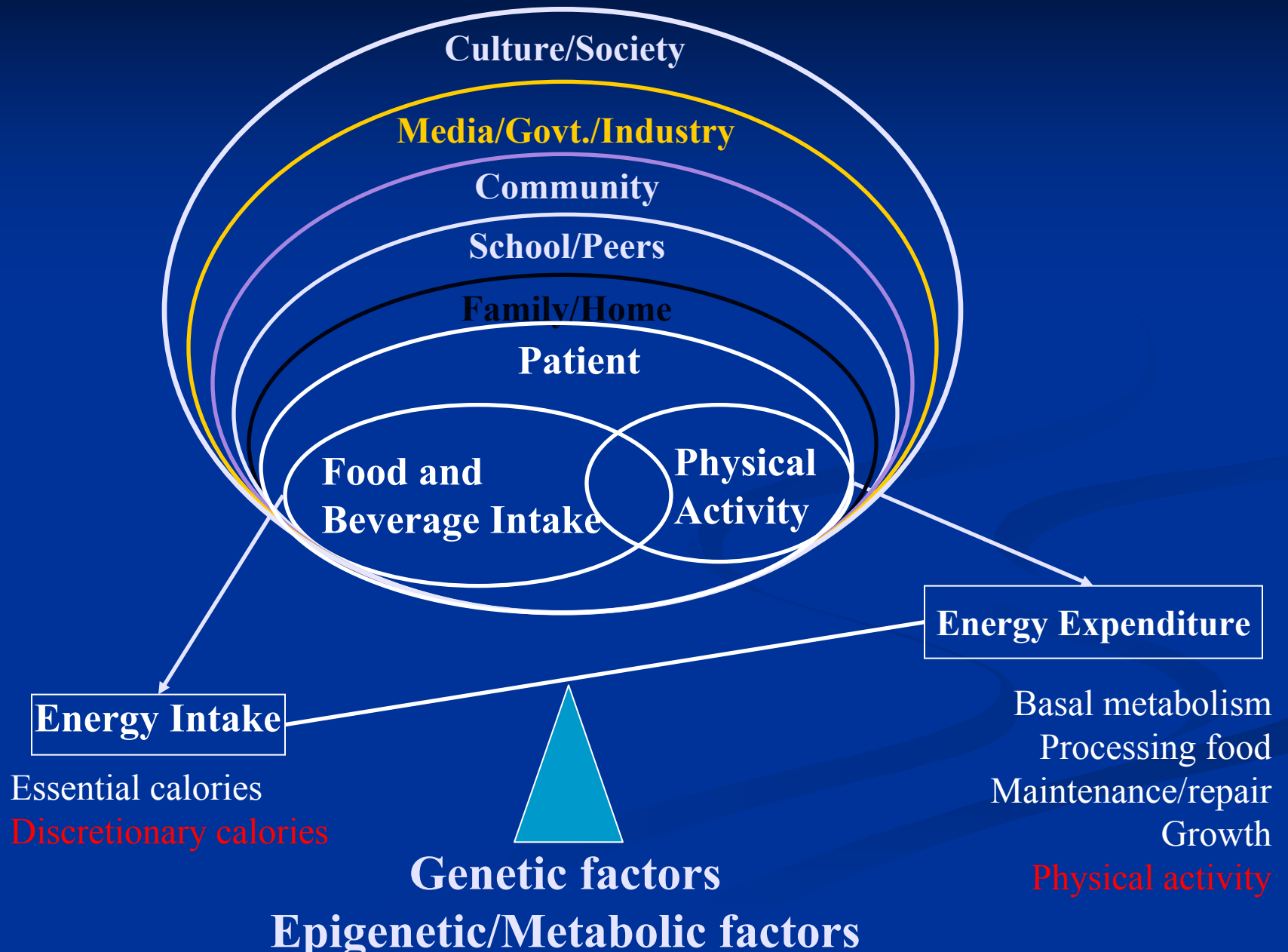
- Avoid canned foods (BPA from lining)
  - Single soda per day ↑ odds of obesity
  - Glass, frozen or fresh
- Avoid ready to feed and concentrated formula in cans (BPA)
- Choose organic food options (OP pesticides)
- Avoid smoking
  - Nicotine replacement contraindicated in pregnancy
- Use filtered tap water
  - Activated carbon filter
  - Don't reuse bottled water bottles

# Recommendations to minimize risk

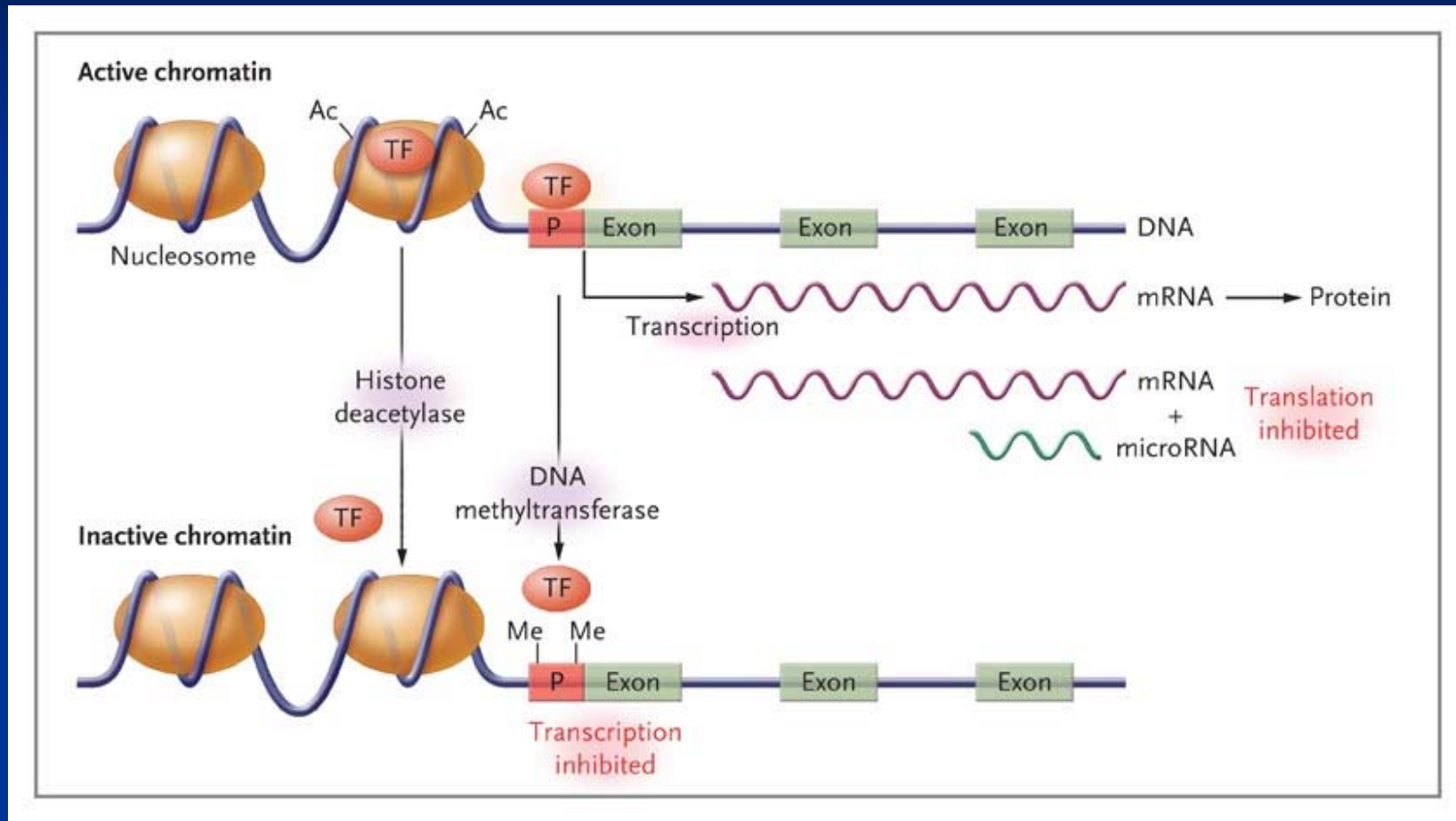
- Look for PVC-free products (organotins, phthalates)
  - Consider high quality wooden toys
    - Plan Toys, Turner Toys, Haba, Selecta, Holztiger
- Limit infant care products and unless medically necessary avoid lotions and powders
  - Look for “phthalate free” products
- Avoid scented products and cosmetics
  - Cologne, lotion, personal care products
- Avoid scented household products (phthalates)
  - Glade plug ins etc. ??
- Minimize inhalation and hand-mouth ingestion
  - Vacuuming
  - Routine filter maintenance

# Ecological Systems Theory Model

Davison KK, Birch LL *Obes Rev* 2001;2:159-71



# Regulation of Gene Expression through Epigenetic Processes



Gluckman P et al. N Engl J Med  
2008;359:61-73



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