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.

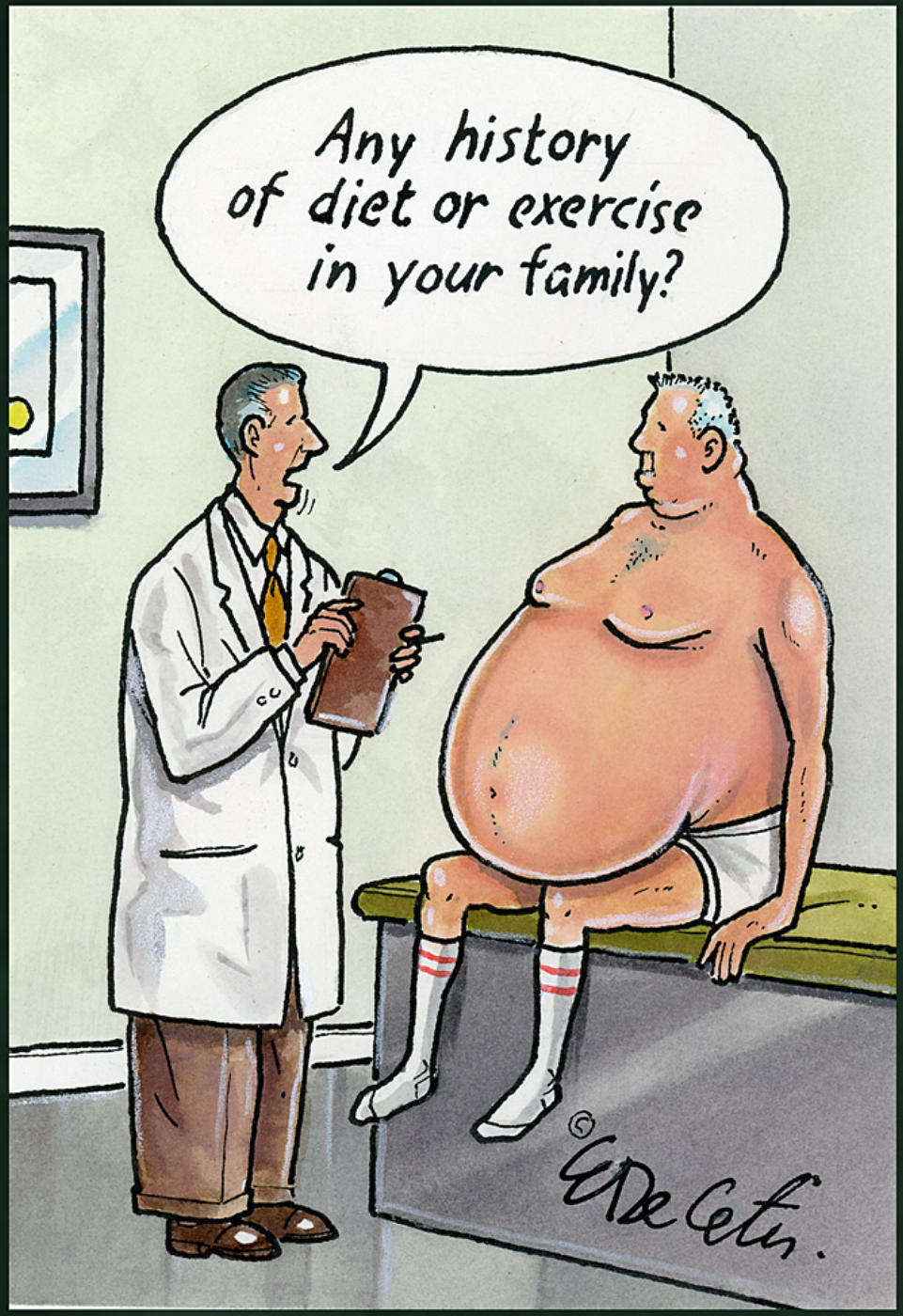
F0 F1 F2 F3

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 gropus
    - 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?

PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B (2011) 278, 1626–1632
doi:10.1098/rspb.2010.1890
Published online 24 November 2010

Canaries in the coal mine: a cross-species analysis of the plurality of obesity epidemics

Yann C. Klimentidis[1], T. Mark Beasley[1], Hui-Yi Lin[4], Giulianna Murati[5], Gregory E. Glass[6], Marcus Guyton[1], Wendy Newton[7], Matthew Jorgensen[8], Steven B. Heymsfield[9], Joseph Kemnitz[7], Lynn Fairbanks[10] and David B. Allison[1,2,3,*]

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.

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- **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.**

Obesity Research & Clinical Practice (2016) 10, 243–255

ELSEVIER

ORIGINAL ARTICLE

Secular differences in the association between caloric intake, macronutrient intake, and physical activity with obesity

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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/m^2 higher in 2006 that in 1988 in the mutually adjusted model ($P < 0.05$).

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- **Physical activity is INCREASING, not DECREASING**

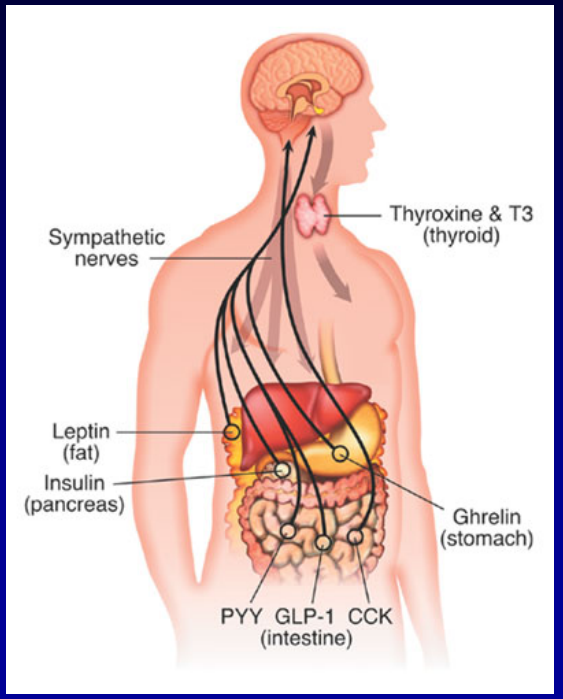
- **Energy balance model is insufficient to explain rise in BMI between 1988 and 2006**

How does obesity occur ?

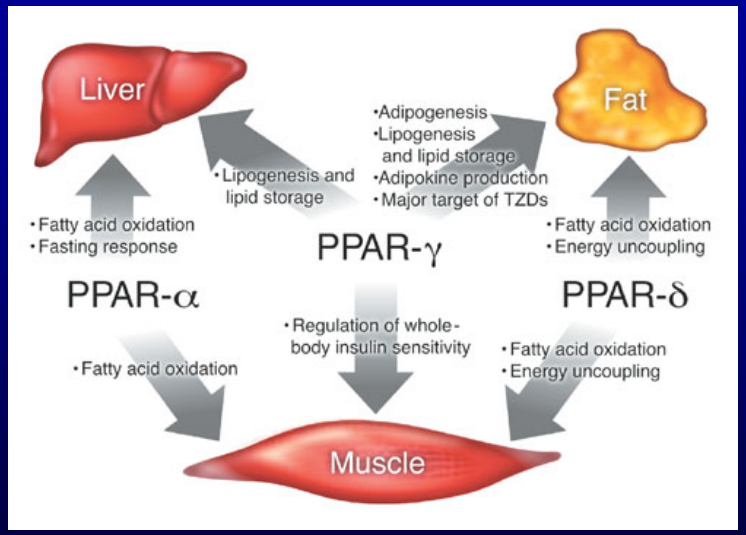
- Prevailing wisdom – "couch potato syndrome"
  - Positive energy balance, i.e., too much food, too little exercise
- Are there other factors in obesity ?
  - Stress (elevated glucocorticoids)
  - Inadequate sleep (stress?)
  - "Thrifty" genes which evolved to make the most of scarce calories
  - Viruses,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



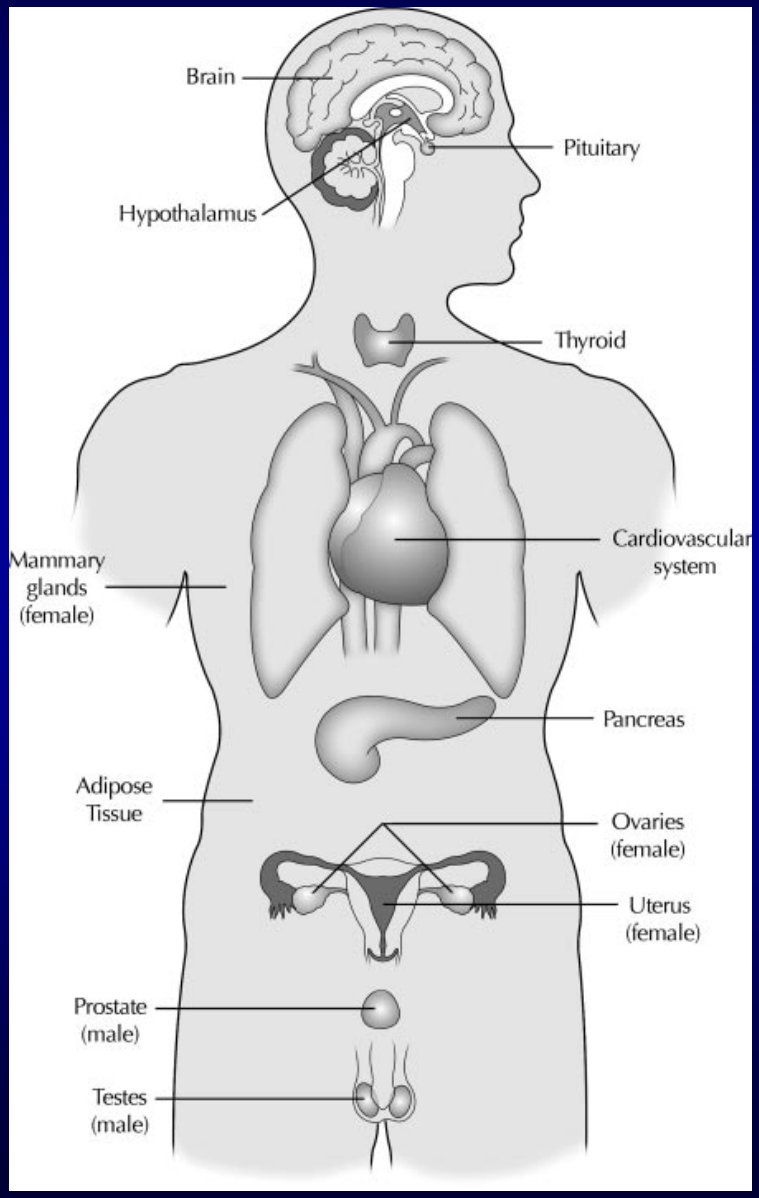
- Hormonal control of fat cell development and lipid balance
  - Regulated through nuclear hormone receptors RXR, PPARγ
  - PPARγ – master regulator of fat cell development
    - Increased fat cell differentiation
    - Increased storage in existing cells
    - Increased insulin sensitivity



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 (food, water)
  - dietary components (pesticides)
  - food packaging
  - personal care products
  - cleaning materials

NIEHS
National Institute of Environmental Health Sciences

Endocrine Disrupting Chemicals (EDCs)

HERBICIDES

2,4,-D
2,4,5,-T
Alachlor
Amitro
Atrazine
Linuron
Metribuzin
Nitrofen
Trifluralin

FUNGICIDES

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

METALS

INSECTICIDES

Aldicarb
beta-HCH
Carbaryl
Chlordane
Chlordecone
DBCP
Dicofol
Dieldrin
DDT and metabolites
Endosulfan
Heptachlor / H-epoxide
Lindane (gamma-HCH)
Malathion
Methomyl
Methoxychlor
Oxychlordane
Parathion
Synthetic pyrethroids
Transnonachlor
Toxaphene

INDUSTRIAL CHEMICALS

Bisphenol - A
Polycarbonates
Butylhydroxyanisole
Cadmium
Chloro- & Bromo-diphenyl
Dioxins
Furans
Lead
Manganese
Methyl mercury
Nonylphenol
Octylphenol
PBDEs
PCBs
Pentachlorophenol
Penta- to Nonylphenols
Perchlorate
PFOA
p-tert-Pentylphenol
Phthalates
Styrene

Testosterone synthesis inhibitor
Estrogen receptor agonist
Thyroid hormone disruptor
Androgen receptor antagonist

NIEHS
National Institute of Environmental Health Sciences

Endocrine Disrupting Chemicals (EDCs)

Pesticides

Alachlor
Amitro
Nitrofen
Trifluralin

Herbicides

Fenarimol
Hexachlorobenzene

Fungicides

Tributyltin
Vinclozolin
Zineb

Solvents

METALS

Flame Retardants

Carbaryl
Chlordane

Industrial byproducts

Dieldrin
DDT and metabolites
Endosulfan

Surface protectors

Methomyl
Methoxychlor

Sunscreens

Transnonachlor
Toxaphene

Plastics

Butylhydroxyanisole
Cadmium

Plasticizers

Lead
Manganese
Methyl mercury

Cosmetics

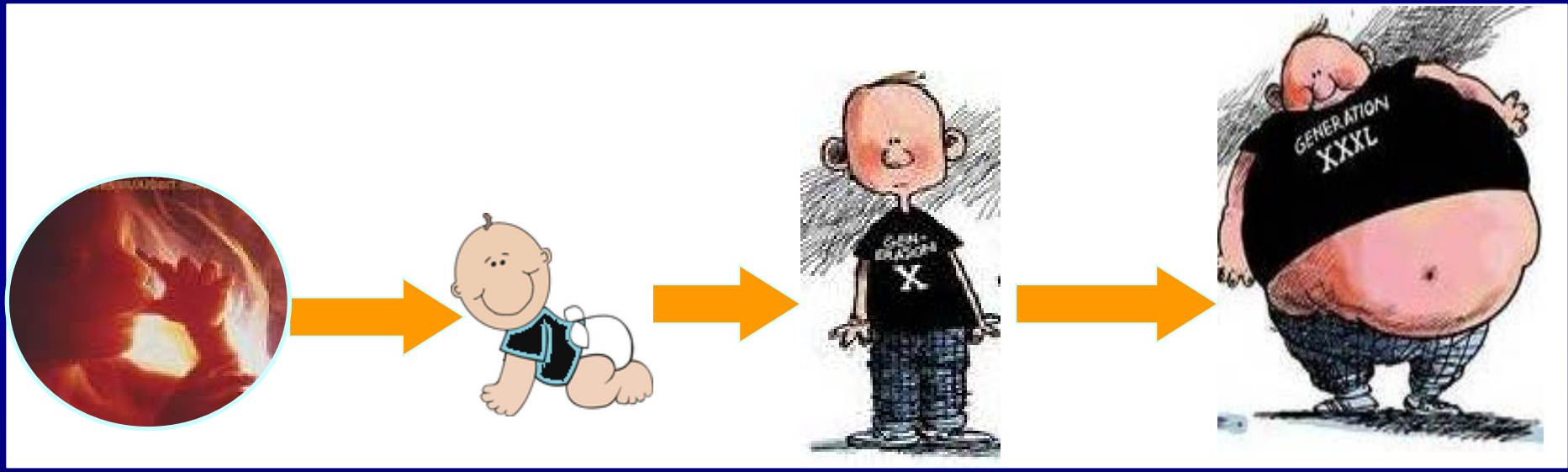
PCBs
Pentachlorophenol

Over 1,000 EDCs

p-tert-Pentylphenol
Phthalates
Styrene

Testosterone synthesis inhibitor
Estrogen receptor agonist
Thyroid hormone disruptor
Androgen receptor antagonist

Endocrine Disrupting Chemicals (EDCs)



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

Endocrine Disrupting Chemicals (EDCs)

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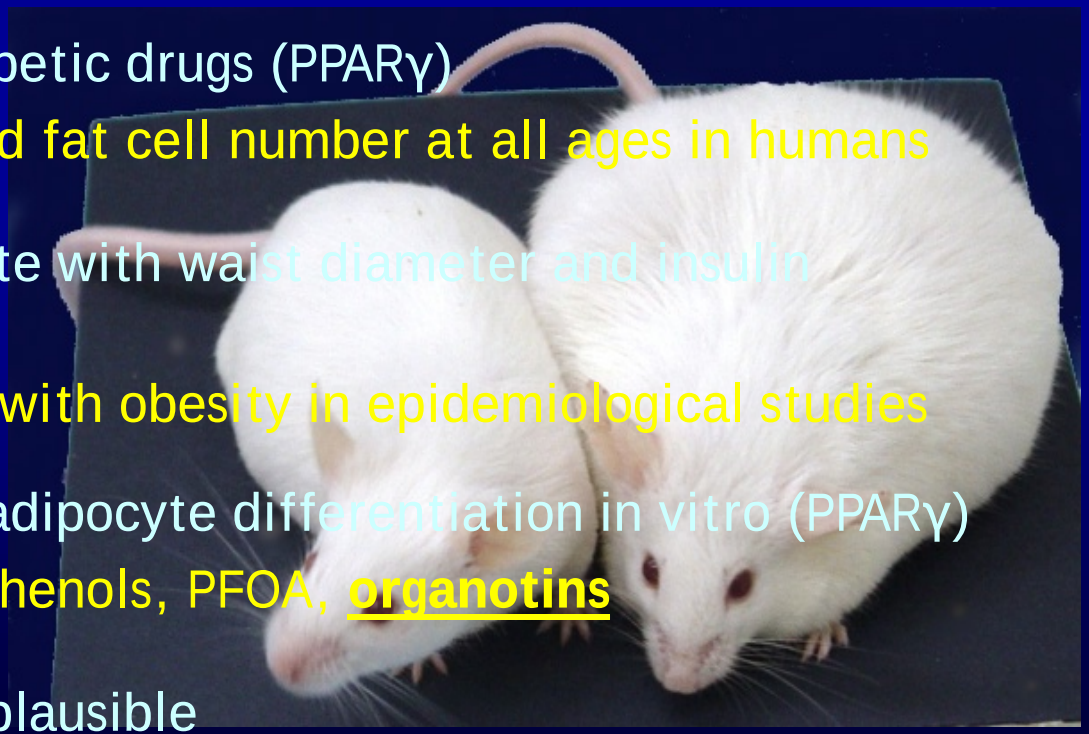
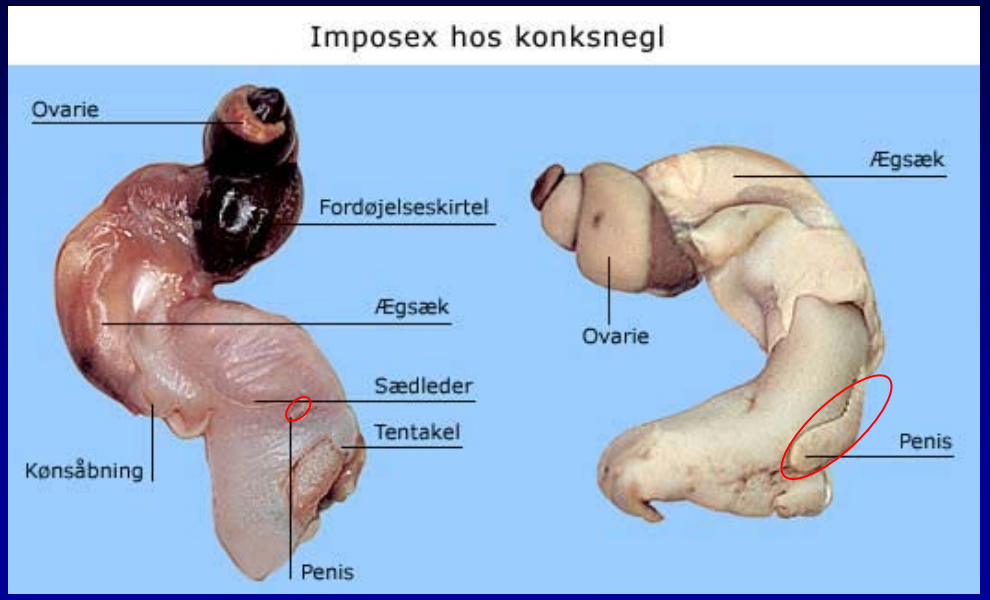
EDCs and the obesogen hypothesis

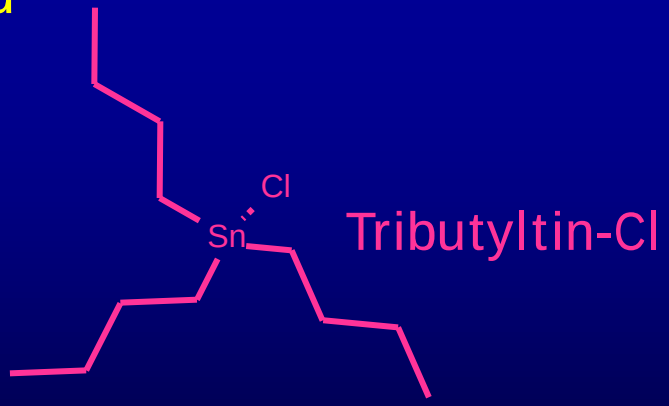
- ***Obesogens*** - chemicals that inappropriately stimulate adipogenesis and fat storage, disturb adipose tissue homeostasis, or alter control of appetite/satiety to lead to weight gain and obesity
- Pre- and postnatal exposure to EDCs such as environmental estrogens (ER) increases weight
  - DES, genistein,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

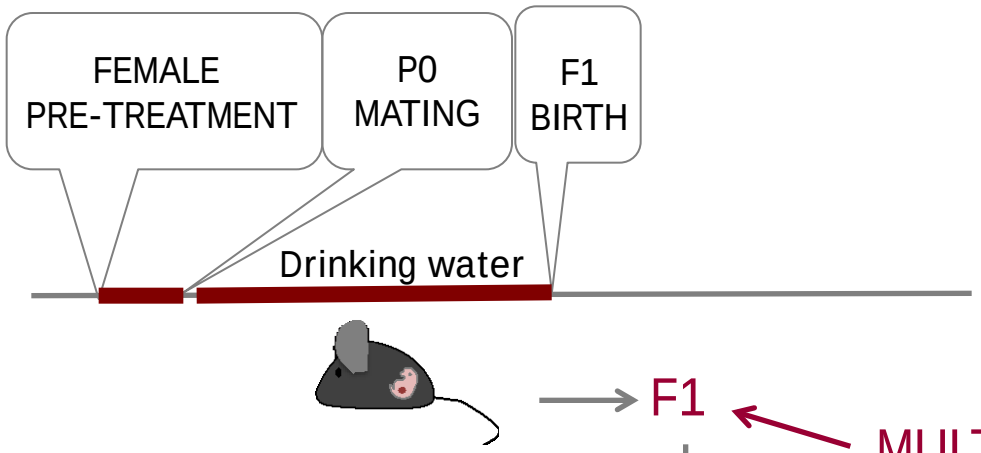
- Organotins -> imposex mollusks
- Sex reverses genetically female flounder and zebrafish -> males
- Which hormone receptors might be organotin targets?
- We found that tributyltin (TBT)
  - Binds and activates at ppb (low nM) two nuclear receptors, RXR and PPARγ critical for adipogenesis
  - TBT induced 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

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

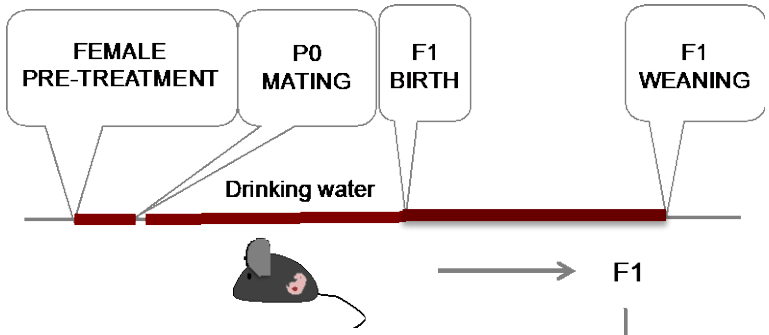
F2 → Sacrificed / Mating → F3 → Sacrificed

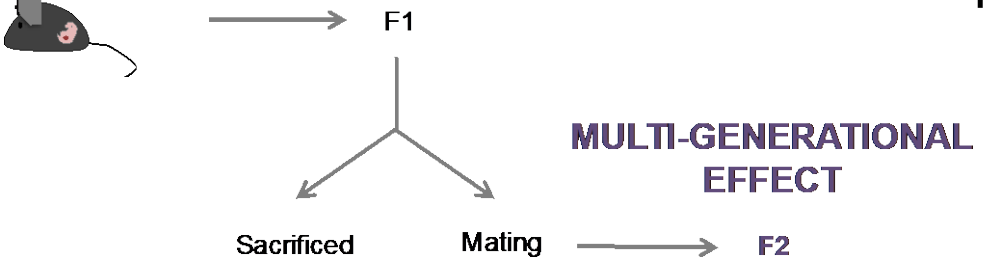
Results

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

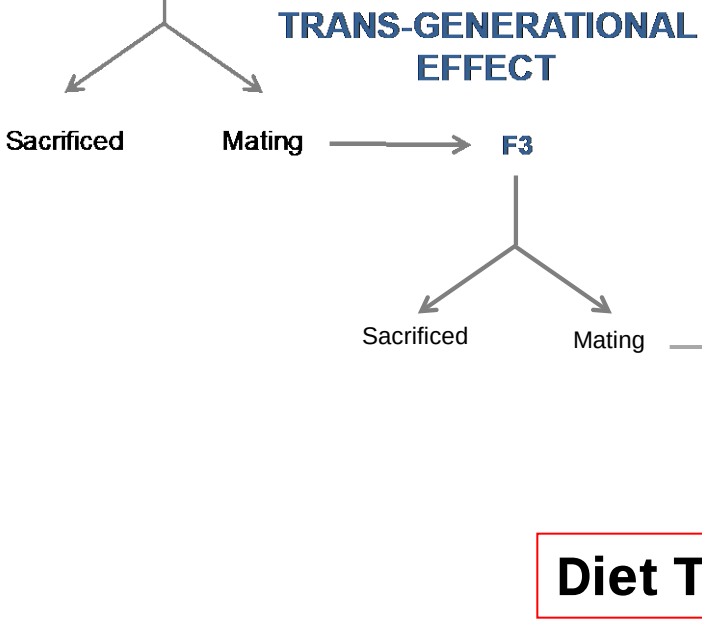
Chamorro-Garcia et al., Environ Health Perspect, 2013

How does TBT exposure elicit transgenerational effects?





MULTI-GENERATIONAL EFFECT



TRANS-GENERATIONAL EFFECT

Diet Test

TREATMENTS

DMSO

TBT 5 nM (~50x < NOAEL)

TBT 50 nM (~5x < NOAEL)

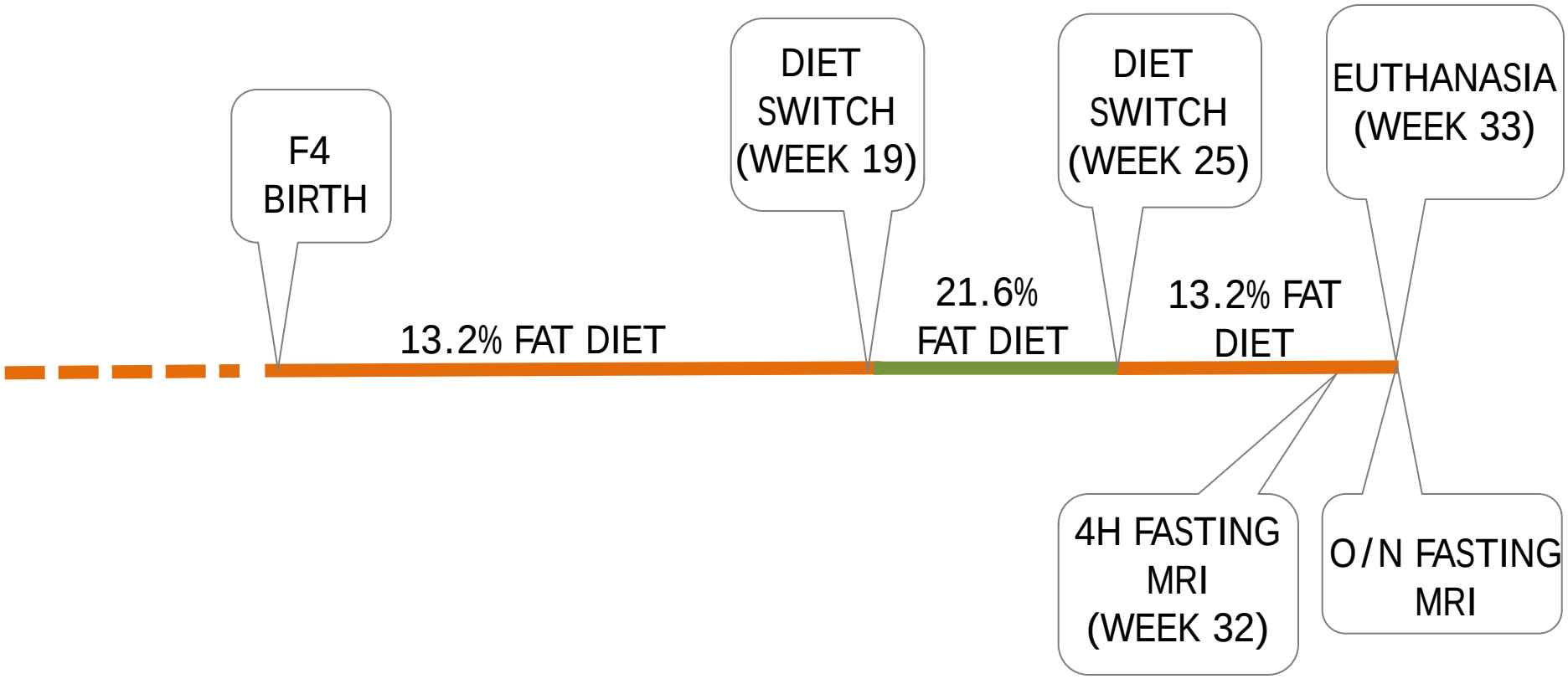
ENDPOINTS

8 weeks old - MSCs

- Transcriptomics
- Methylomics
- Lineage commitment
- Body weight
- Body composition
- Serum analysis
- Epididymal sperm

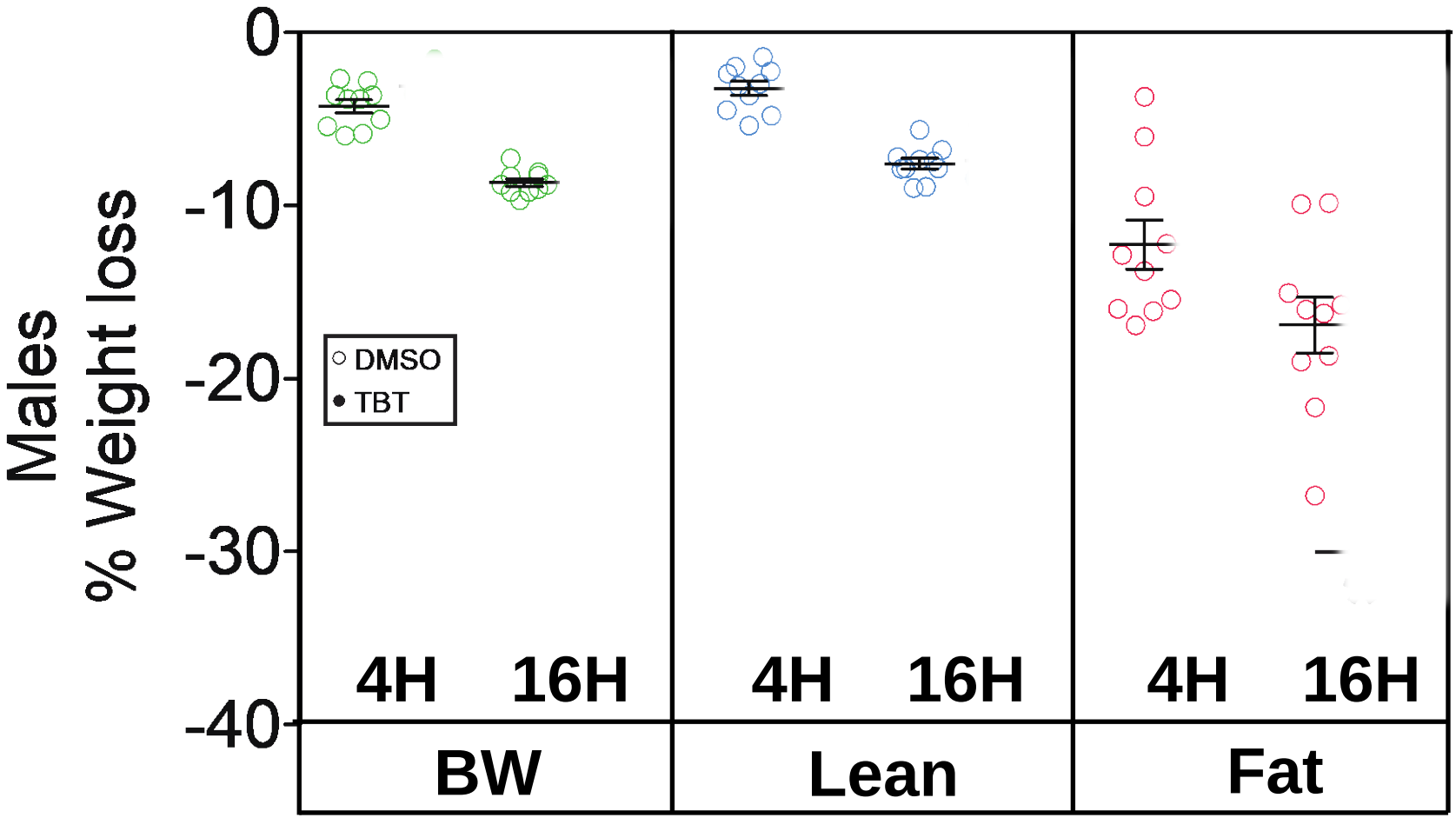
Chamorro-Garcia et al., Nature Communications,2017

Effects of diet on F4 animals



Chamorro-Garcia et al., Nature Communications,2017

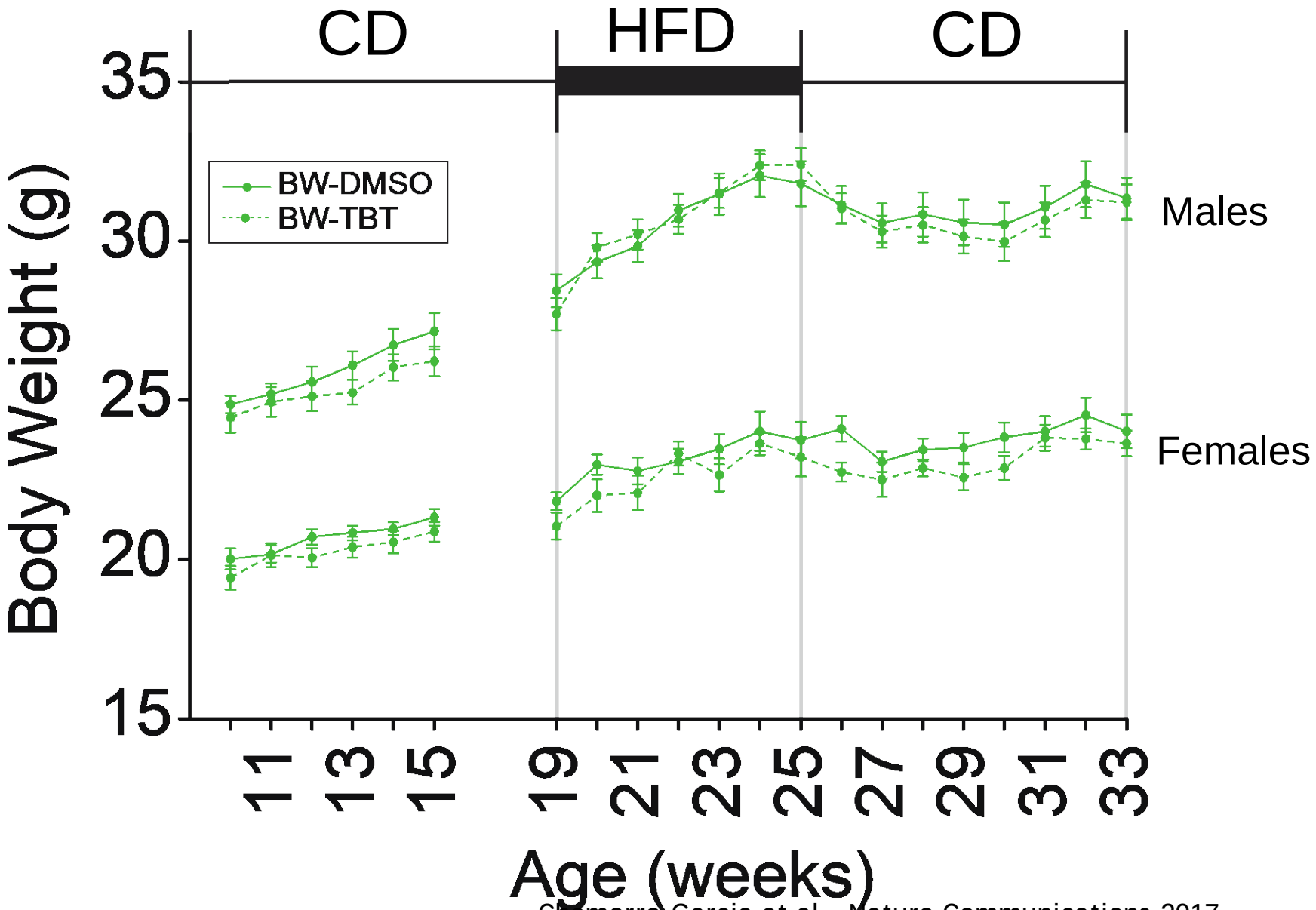
F4 TBT males are resistant to fasting-induced fat loss



TBT animals do not mobilize fat comparably to controls

Chamorro-Garcia et al., Nature Communications,2017

Body weight is not changed by TBT exposure



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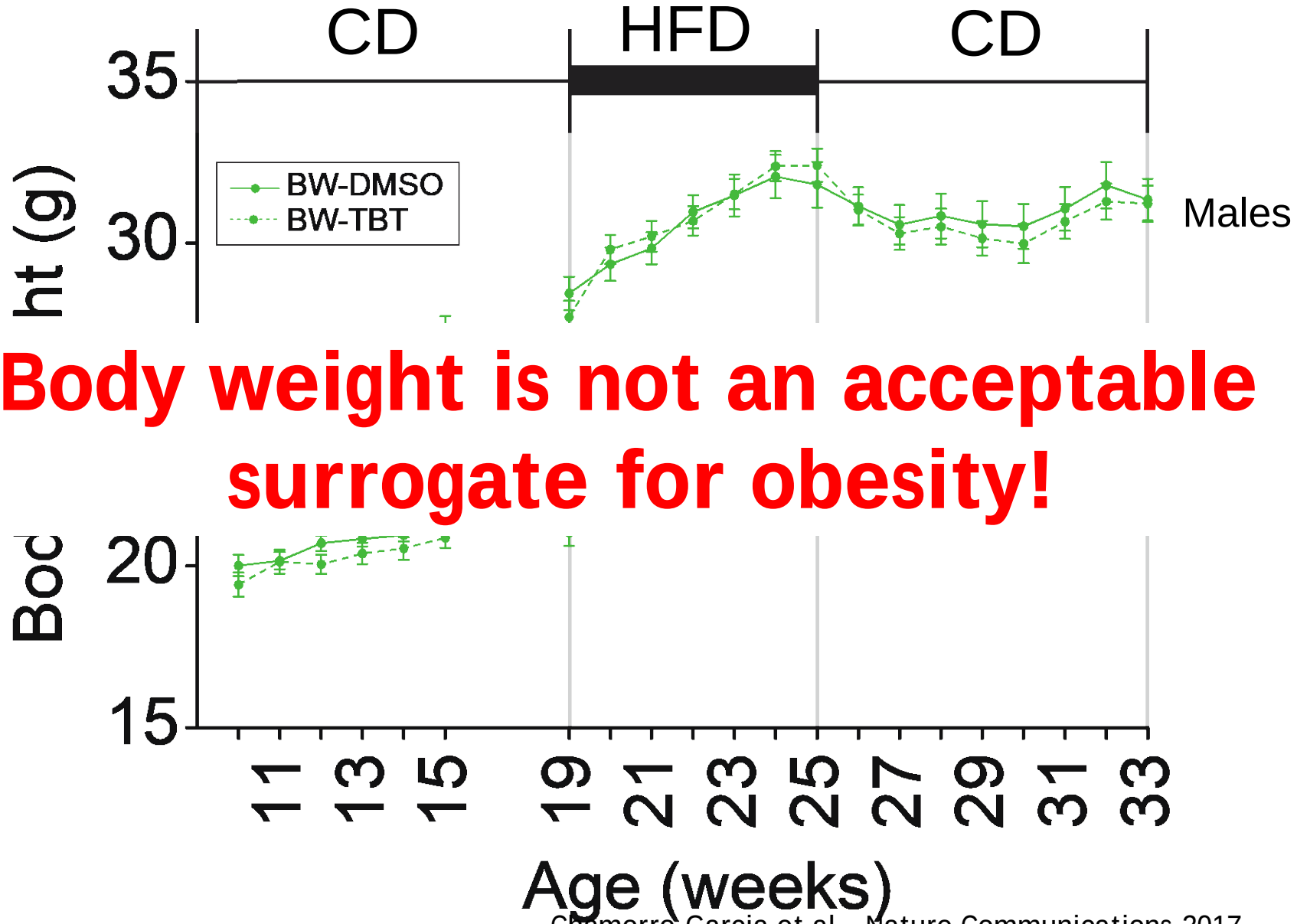
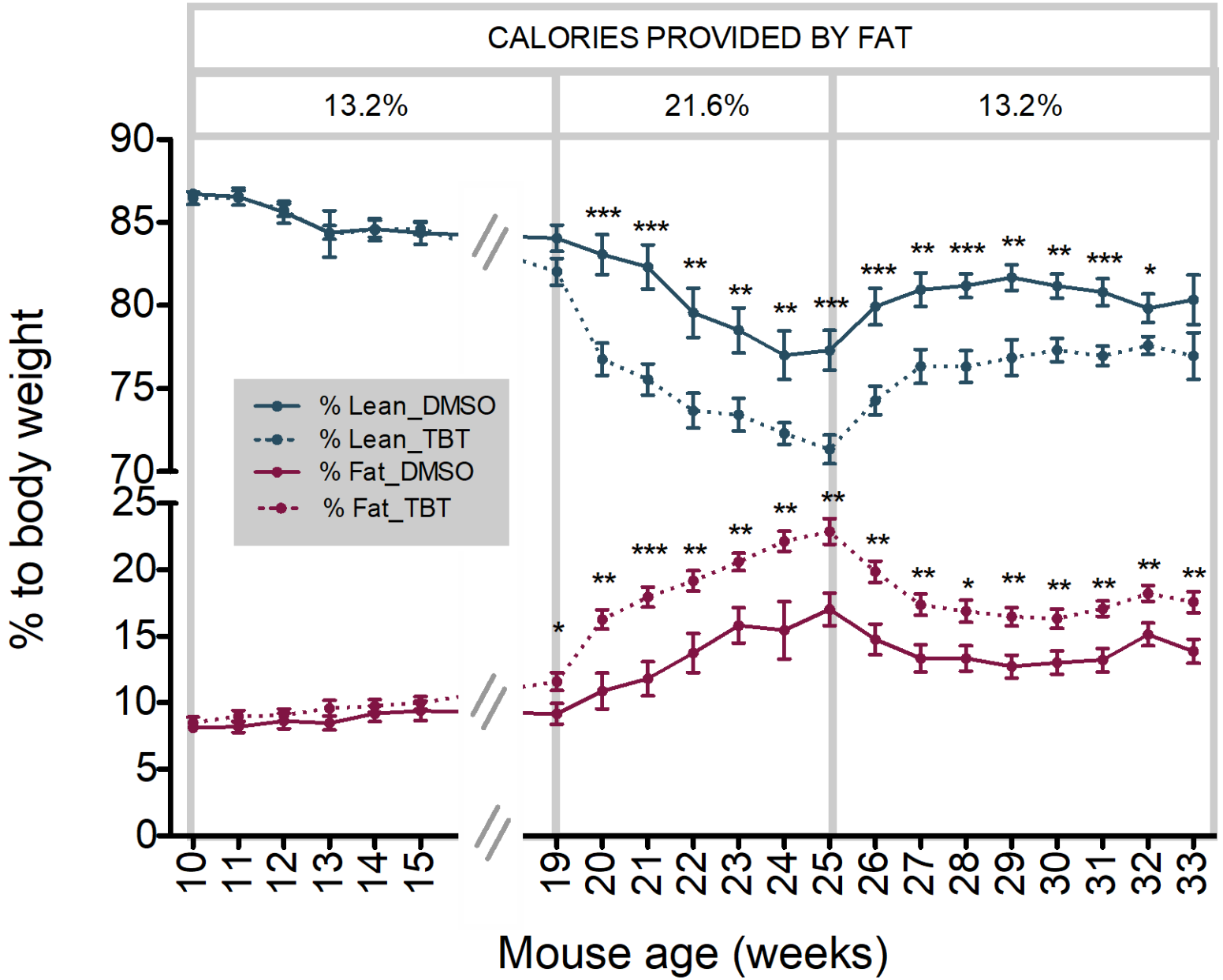


Body weight is not an acceptable surrogate for obesity!

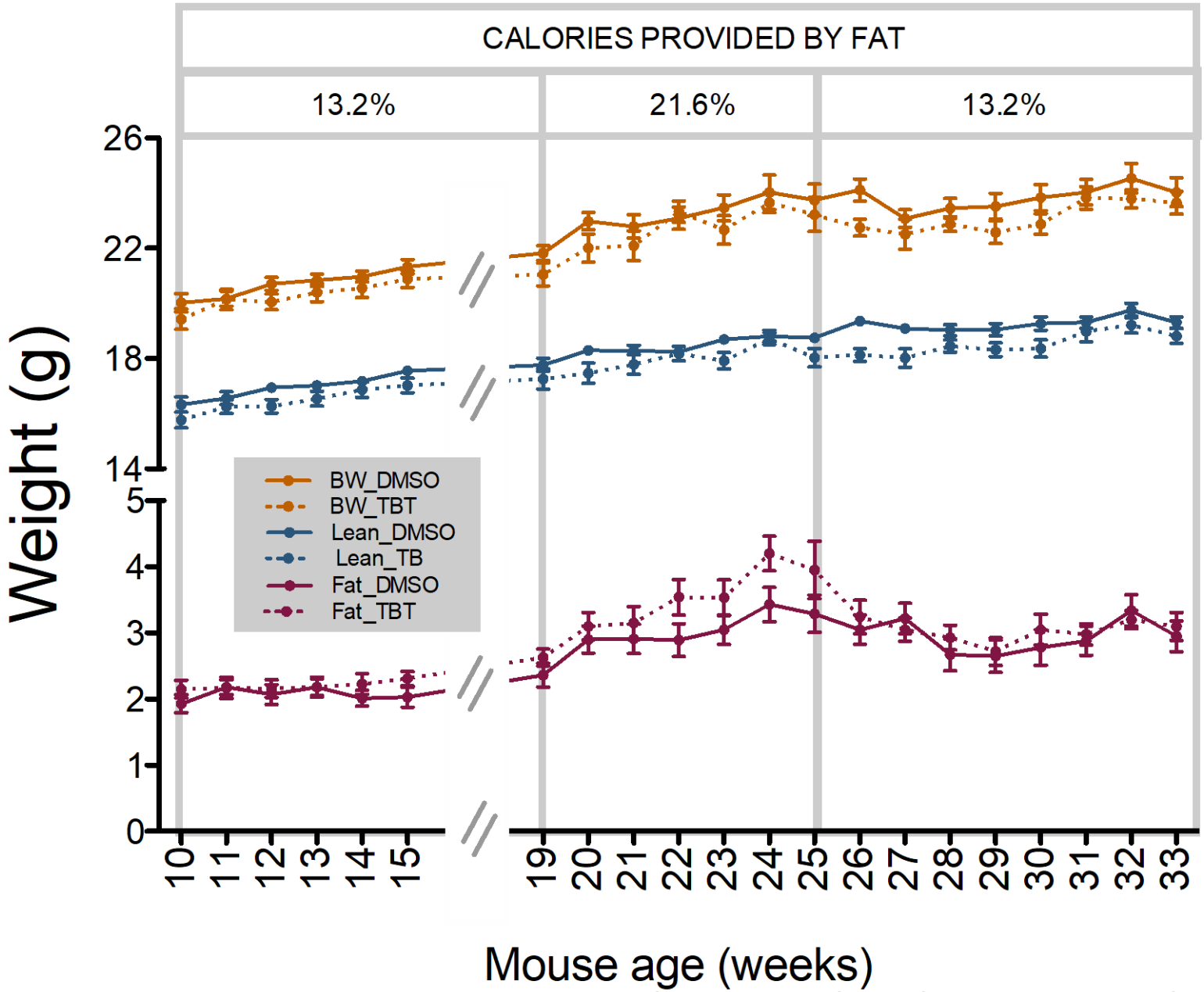
Chamorro-Garcia et al., Nature Communications,2017

Higher fat diet causes obesity in **F4** males

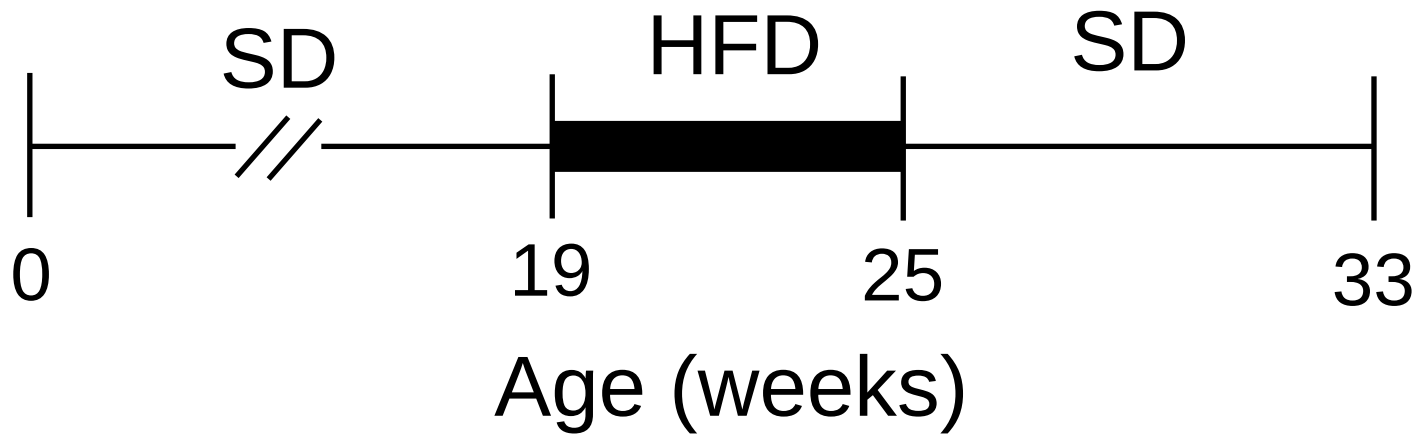


Chamorro-Garcia et al., Nature Communications,2017

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

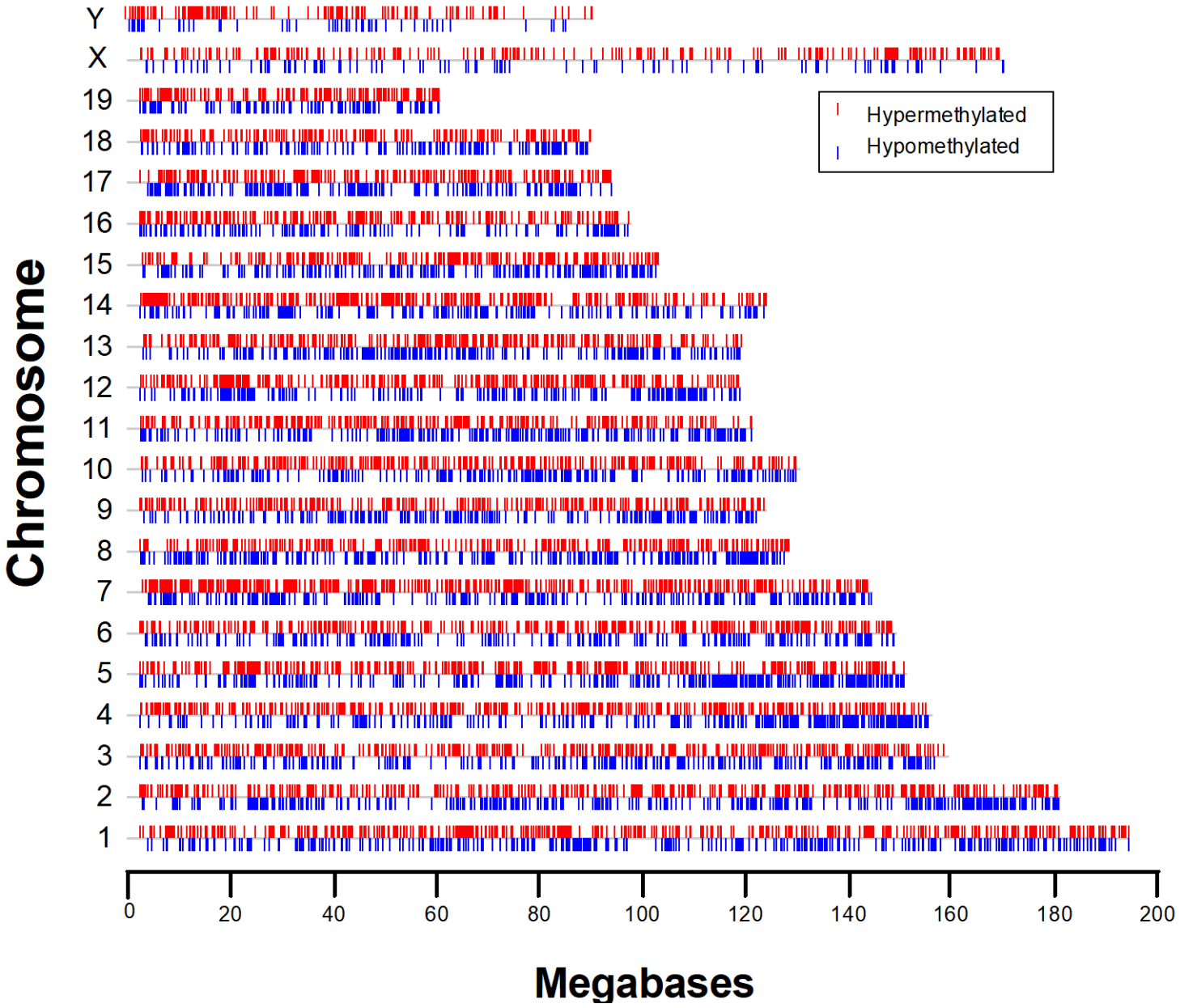
- DNA methylation analyses
- Gene expression analyses

MSCs (8 week old)

Liver (33 week old)

Fat tissue

Differentially Methylated Regions in F4 Male WAT



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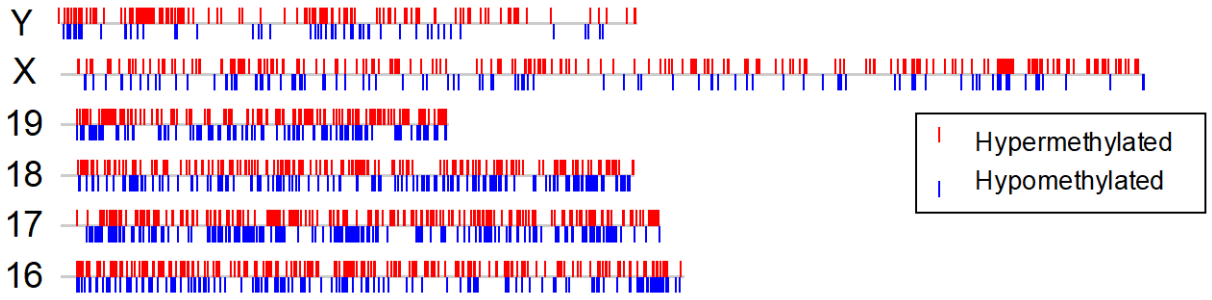
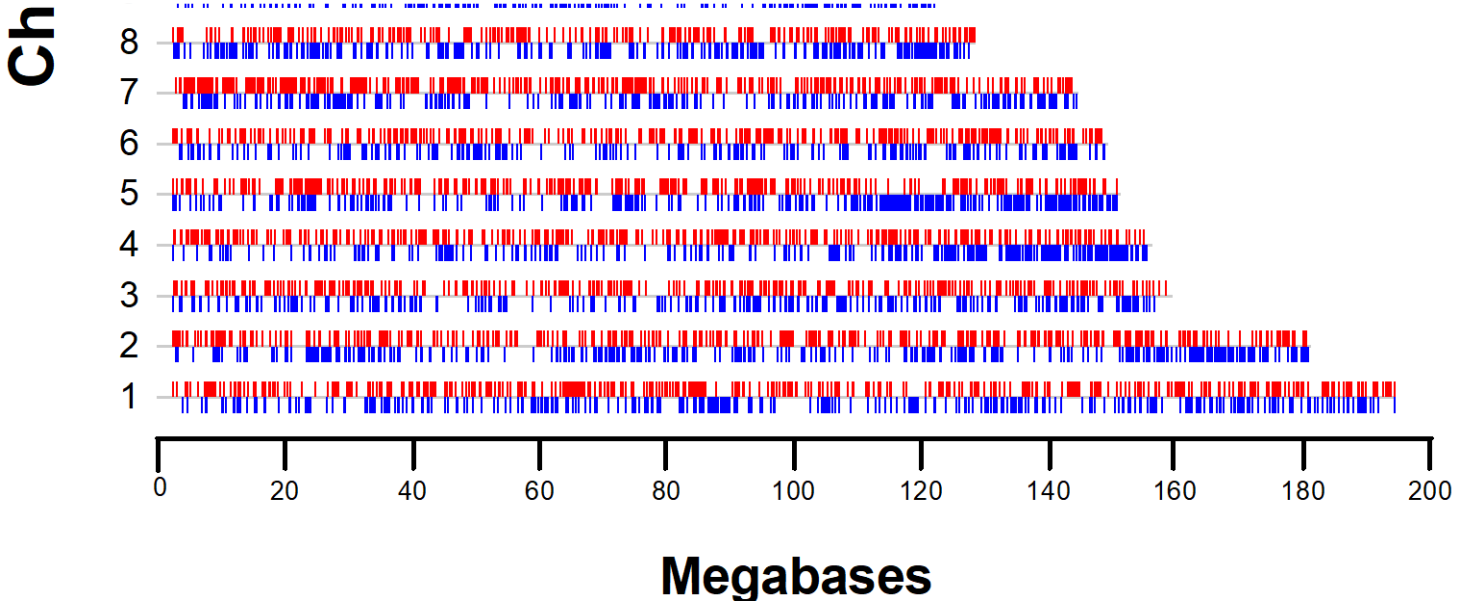
Differentially Methylated Regions in F4 Male WAT

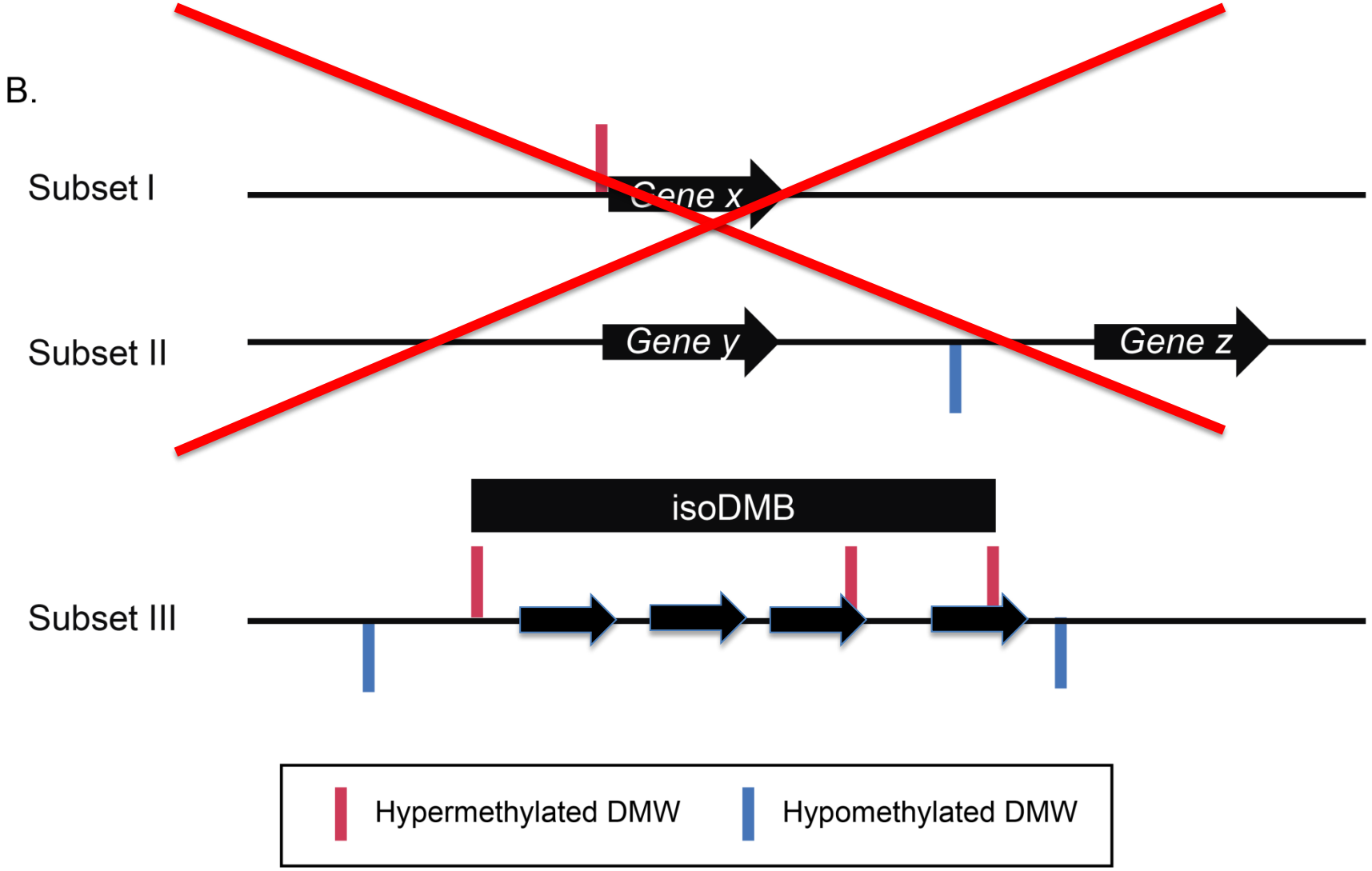


How do we get DMRs in F4 generation when germline reprogramming erases existing methylation every generation?



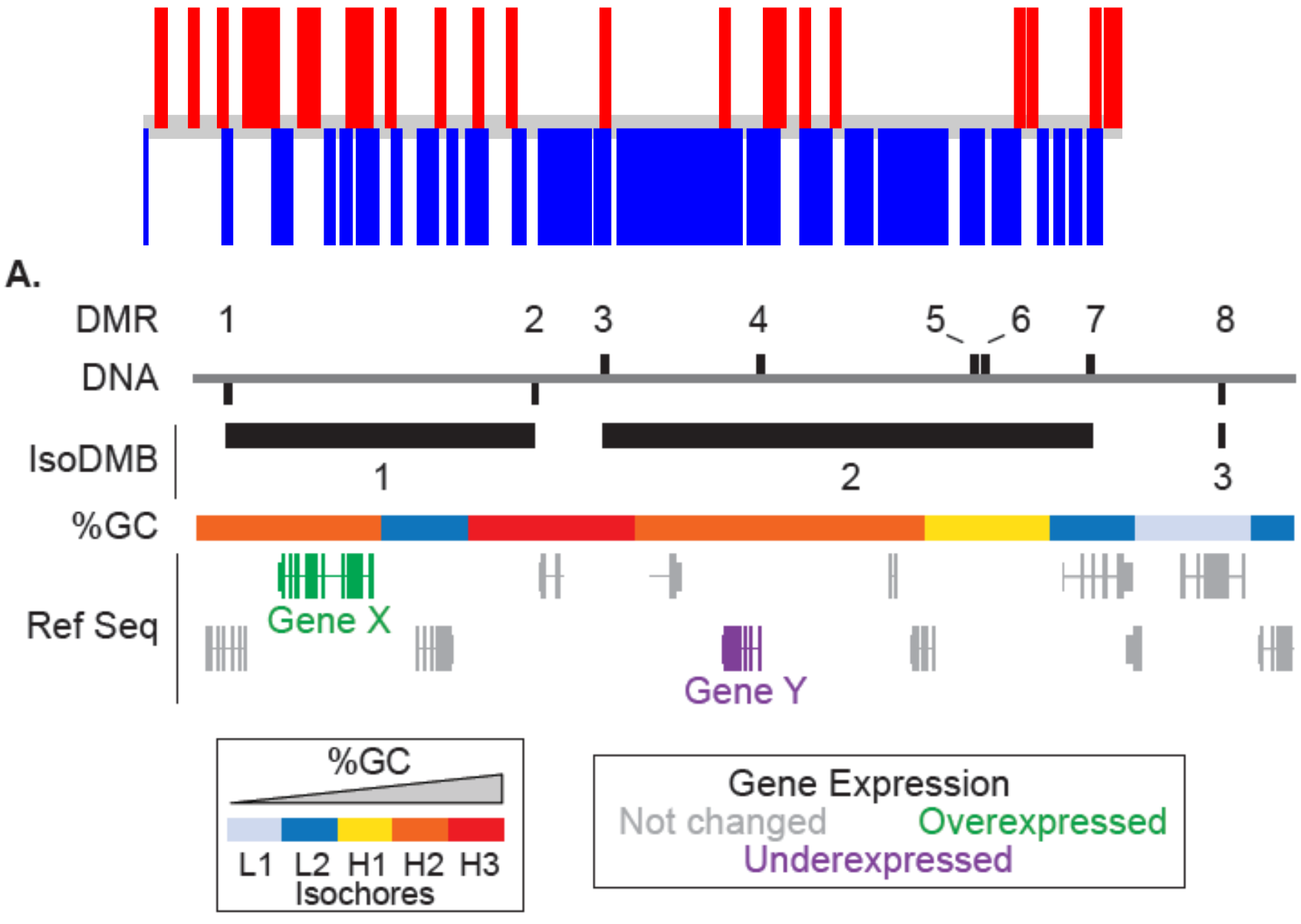
Chamorro-Garcia et al., Nature Communications,2017

Methylation and Differential Gene Expression



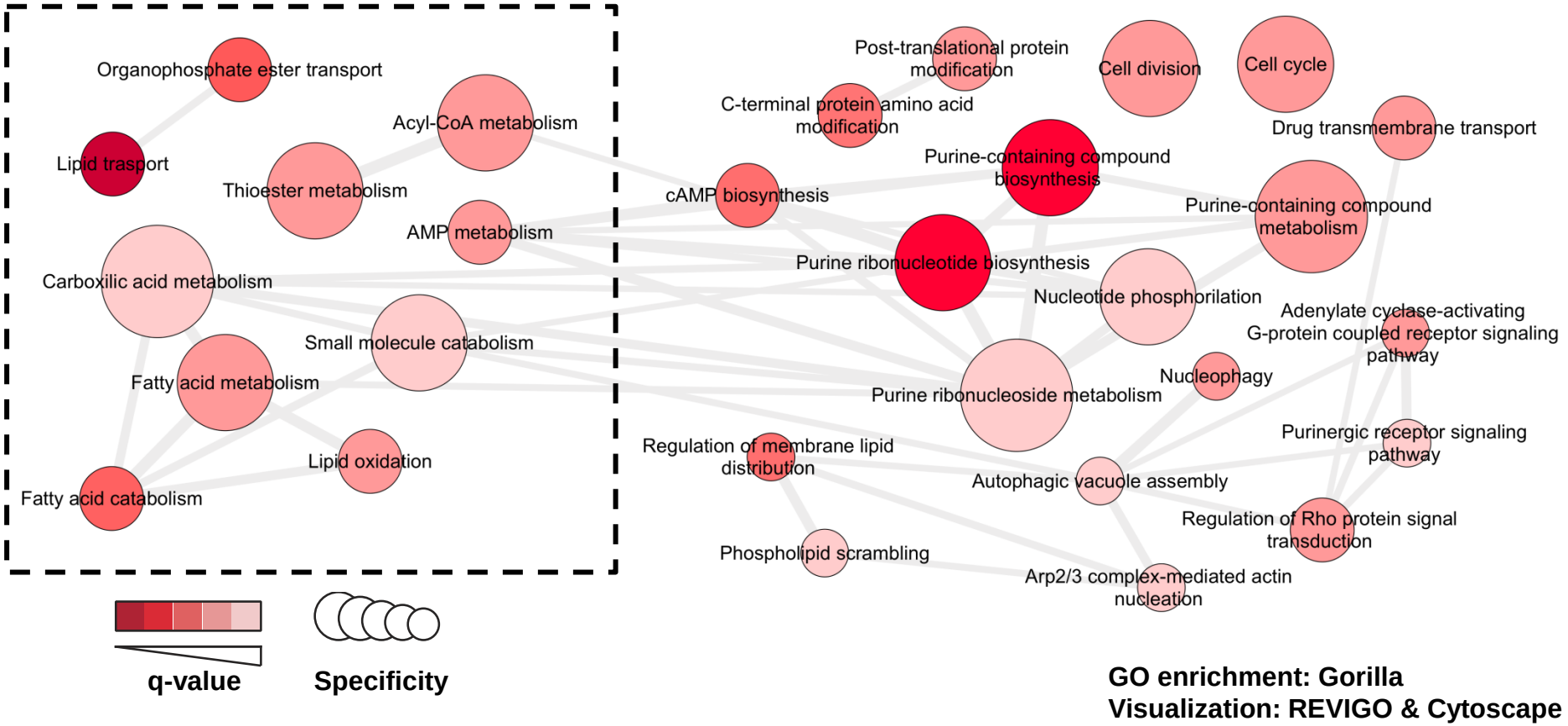
Chamorro-Garcia et al., Nature Communications,2017

isoDMBs are abundant in the genome



GO enrichment of Differentially Expresssed Genes in isoDMBs

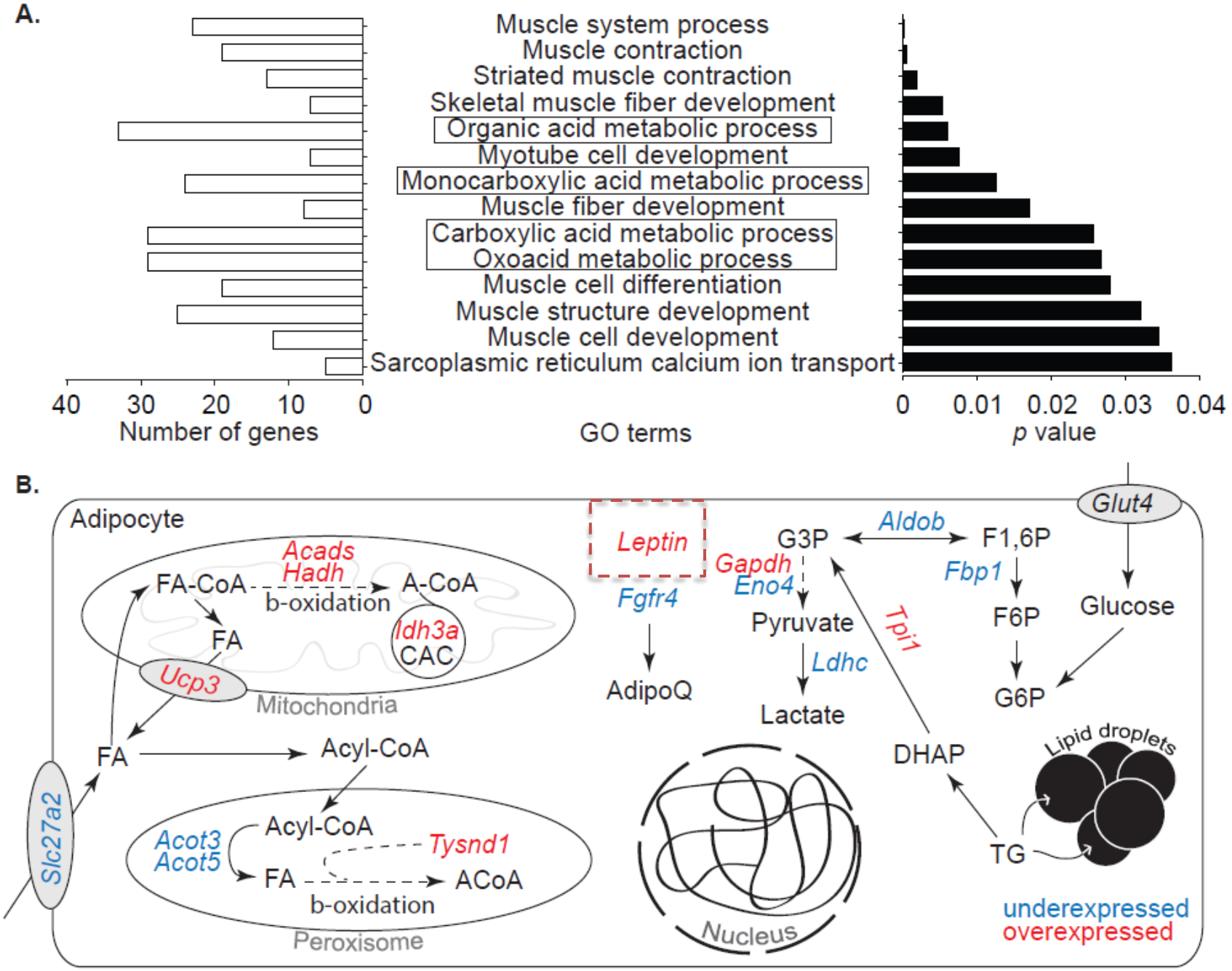
Lipid and fatty acid metabolism n=~100



GO enrichment: Gorilla
Visualization: REVIGO & Cytoscape

- 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



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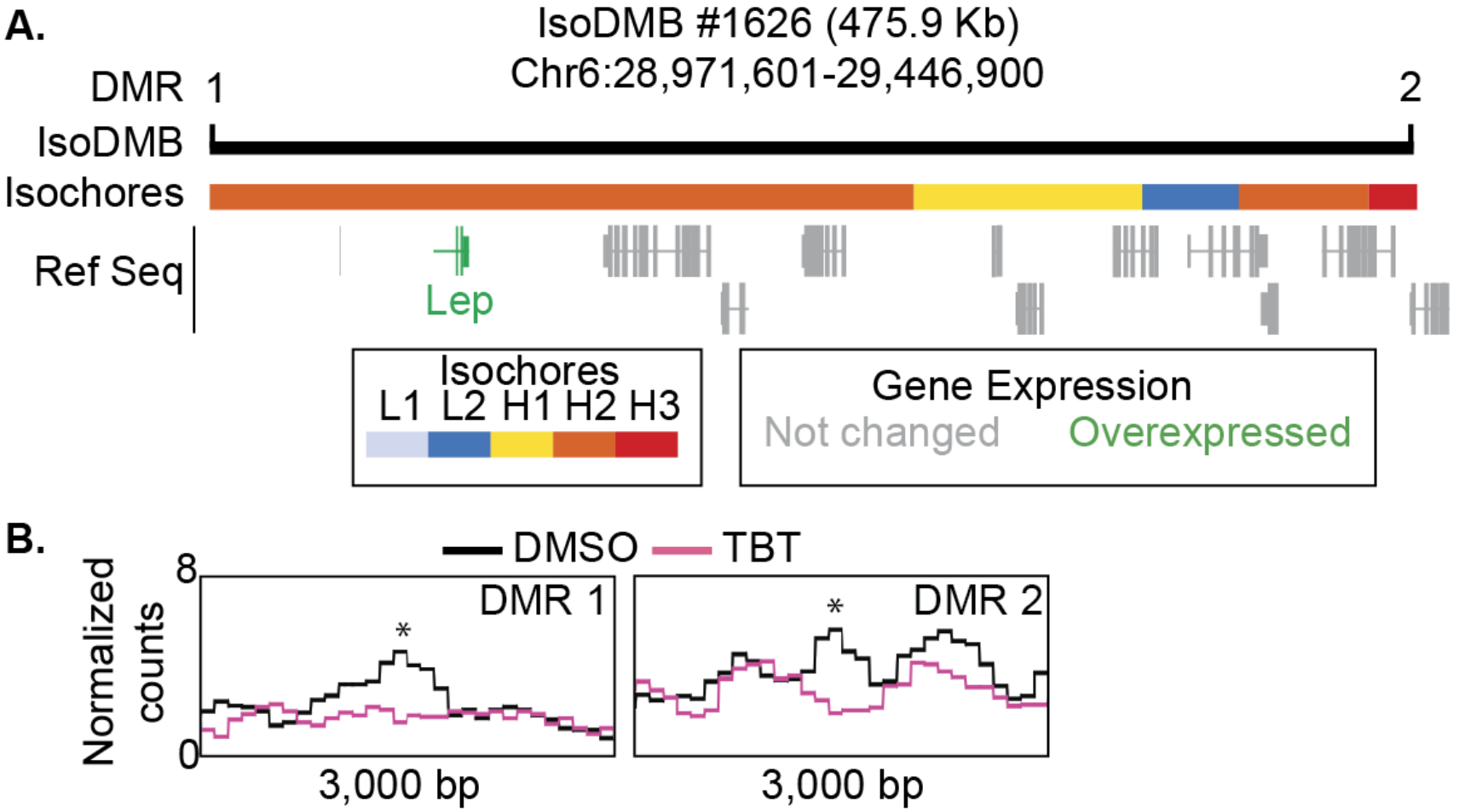
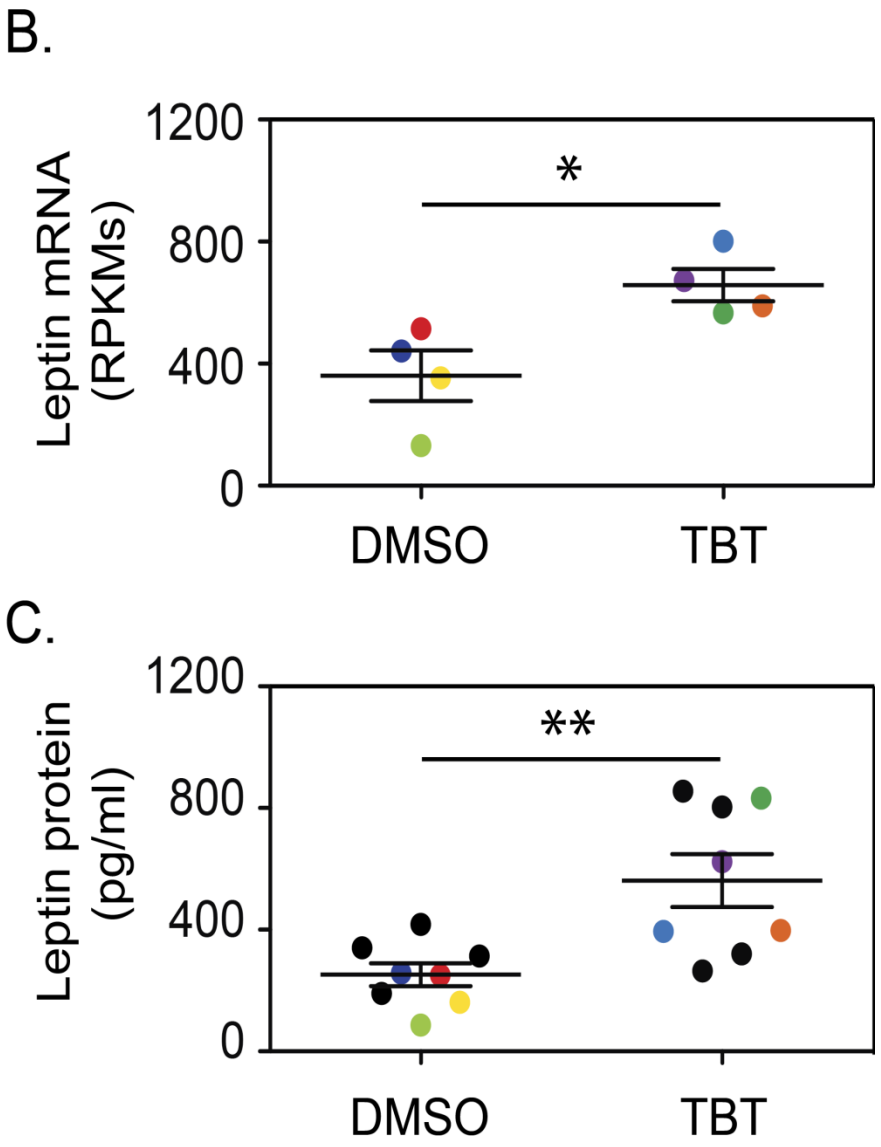
Leptin is Within a Hypo-methylated isoDMB



Chamorro-Garcia et al., Nature Communications, 2017

F4 Over-expression of Leptin mRNA and Protein
(only in males)

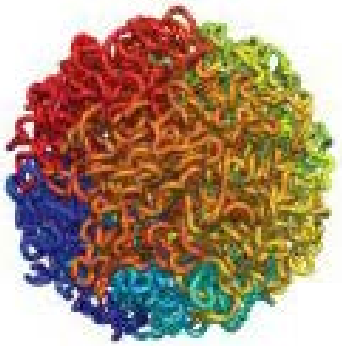


Obesity +
elevated
leptin levels
=
leptin resistance

Chamorro-Garcia et al., Nature Communications, 2017

TBT Exposure Alters Chromatin Structure Favoring Expression of Obesity-promoting Genes

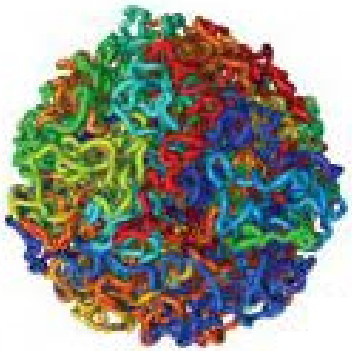
Veh



Permits/ facilitates

DNA methylation

TBT



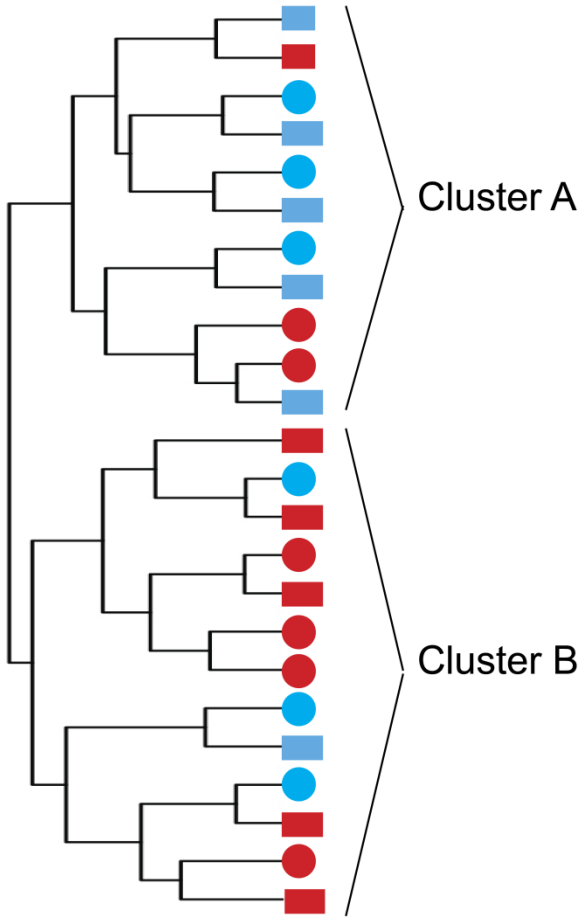
Alters access to programing enzymes

Altered DNA methylation, differential expression of leptin and important metabolic 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

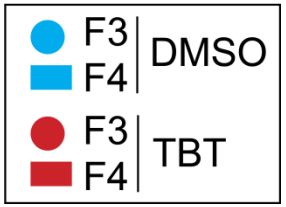
ATAC-seq of F3/F4 sperm reveals TBT-altered local chromatin accessibility

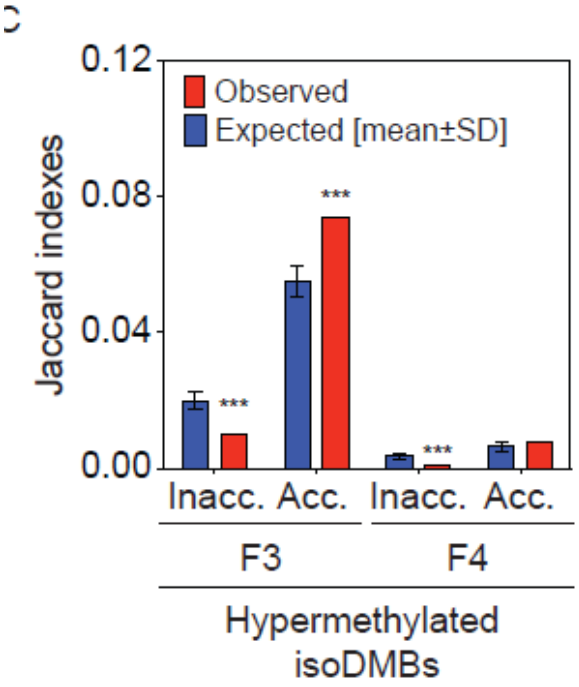


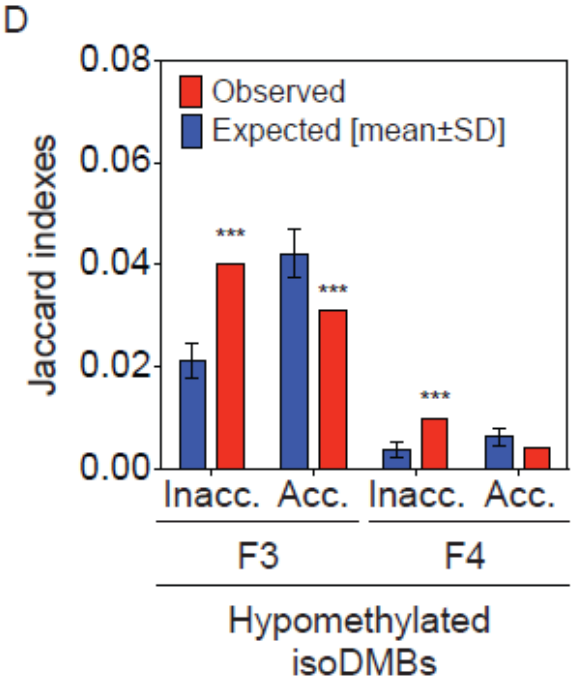
Cluster A *vs* B

Chi-square = 4.2

p value = 0.04





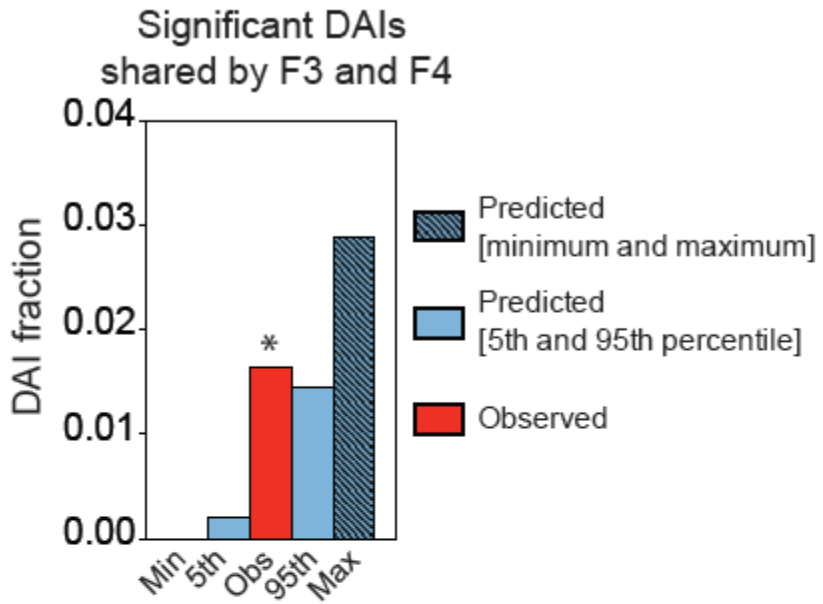


- Hypomethylated isoDMBs associated with inaccessible sperm chromatin and vice versa
- Is accessibility in sperm related to methylation status in somatic cells?

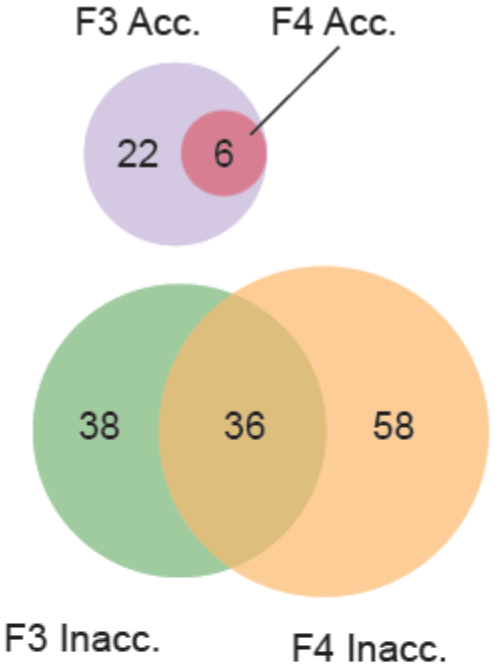
Chamorro-Garcia et al., Nature Communications, 2017

Inaccessible regions in F3/F4 sperm that are Hypomethylated in WAT contain key metabolic genes

A



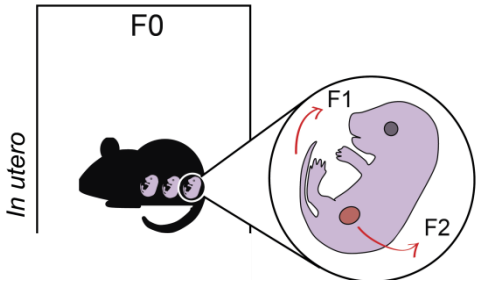
B

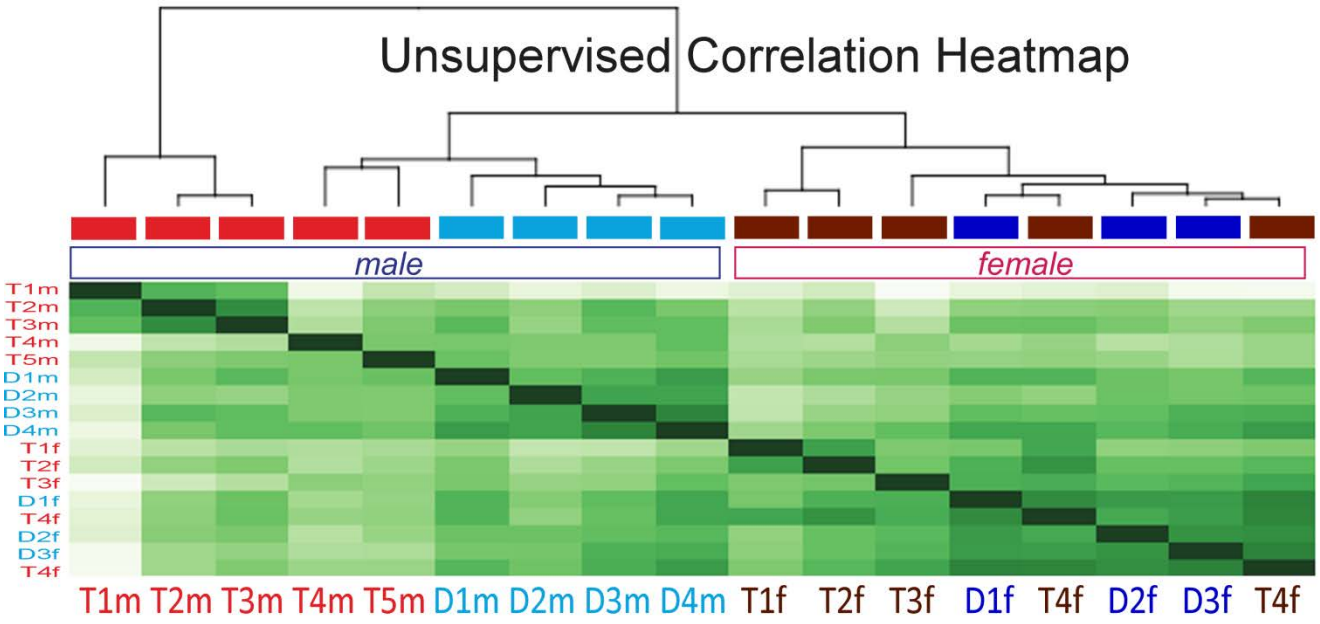


C

Inacc. in F3		GO term	Inacc. in F4	
# genes	*p* value		*p* value	# genes
818	0.000035	Cellular metabolic process	0.00036	134
675	0.000095	Cellular macromolecule metabolic process	0.00064	114
811	0.0013	Primary metabolic process	0.00068	92
850	0.0014	Organic substance metabolic process	0.0043	136
496	0.0015	Organic cyclic compound metabolic process	0.033	82
472	0.0035	Heterocycle metabolic process	0.014	81
883	0.0037	Metabolic process	0.0048	141
483	0.018	Regulation of primary metabolic process	0.00022	92

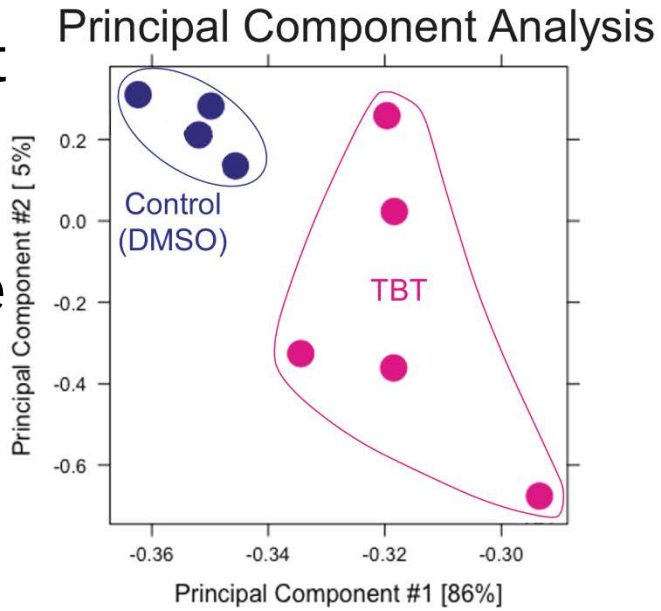
Chromatin Accessibility is Already altered in F1 PGCs

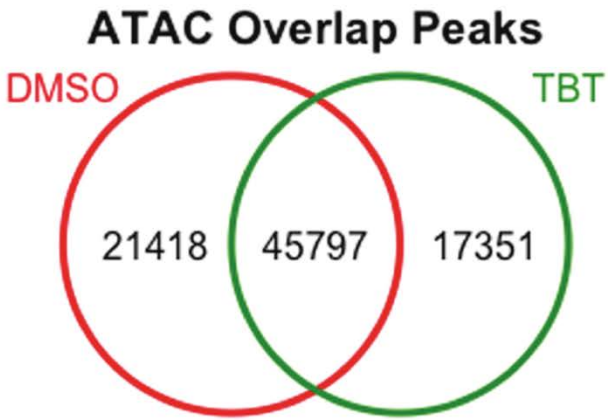




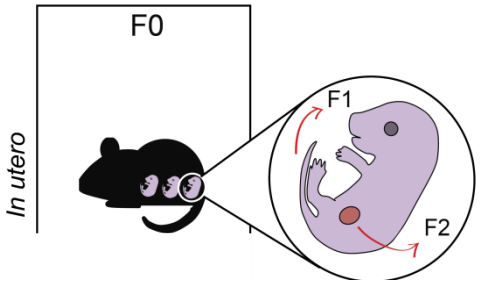
- Pool gonads by litter and sex

- 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 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
  - 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
  - PPARγ-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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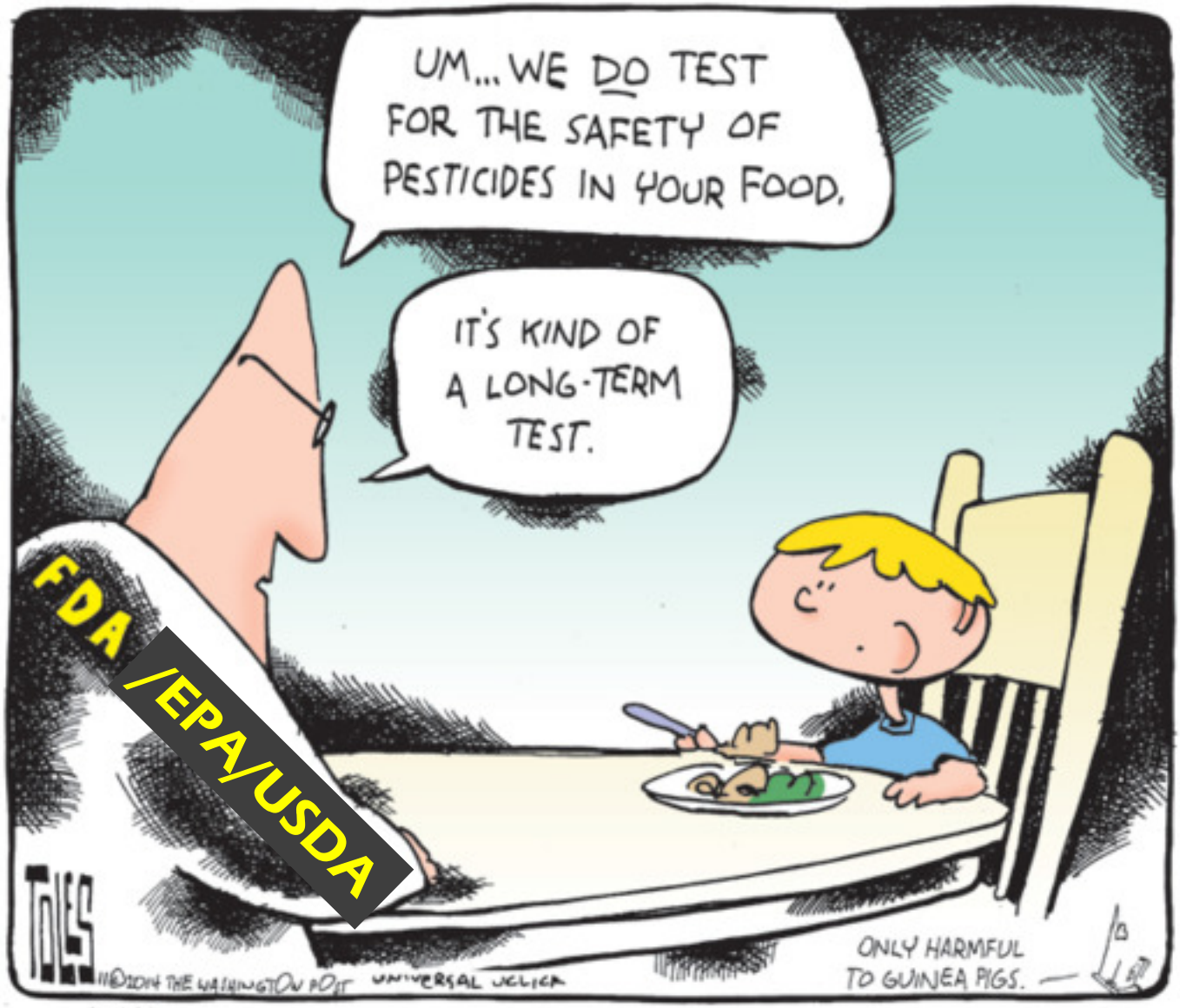
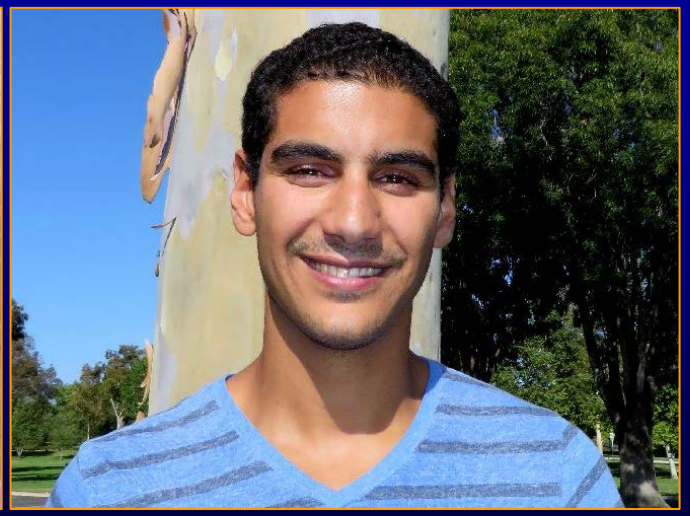
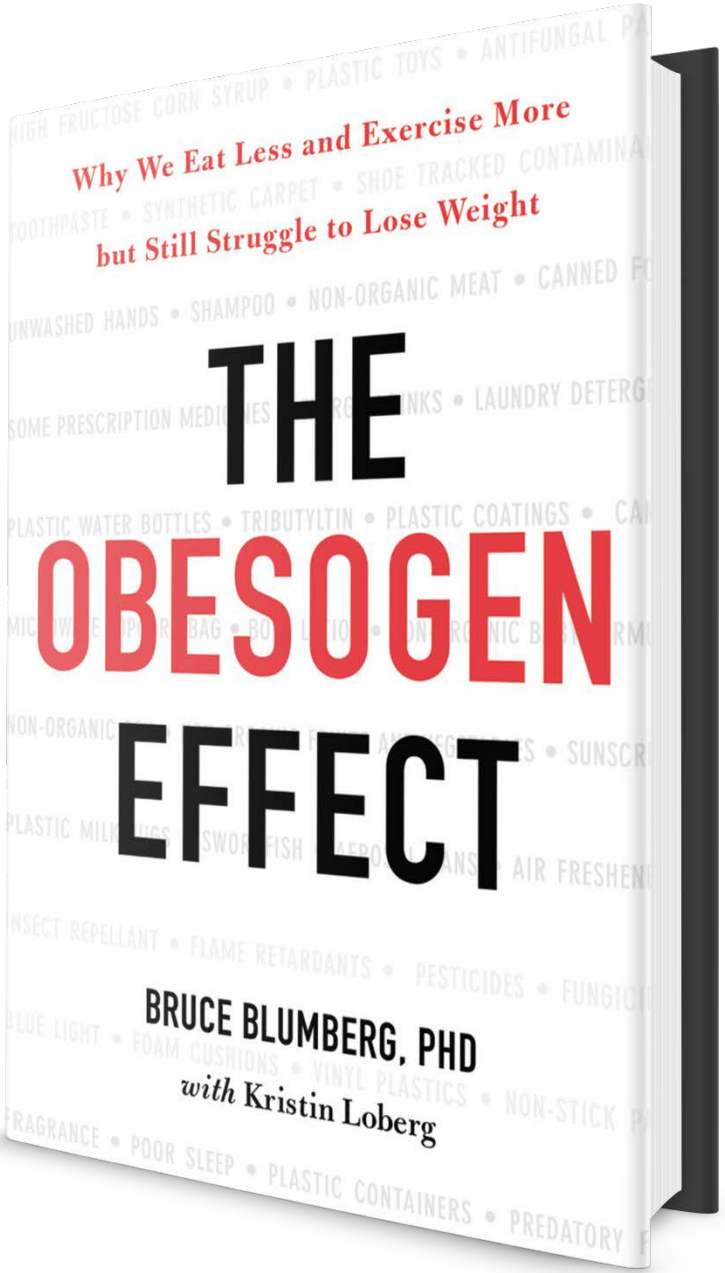
Testing for effects of chemical exposure



- 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
- MGH/Harvard Medical School
  - Toshi Shioda
- 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



https://www.theobesogeneffect.com/