Persistent Organic Pollutants and Diabetes:

What we know and what we don't know

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Current paradigm of type 2 diabetes

Calories intake >consumption
Excess energy intake & sedentary life style

Gene

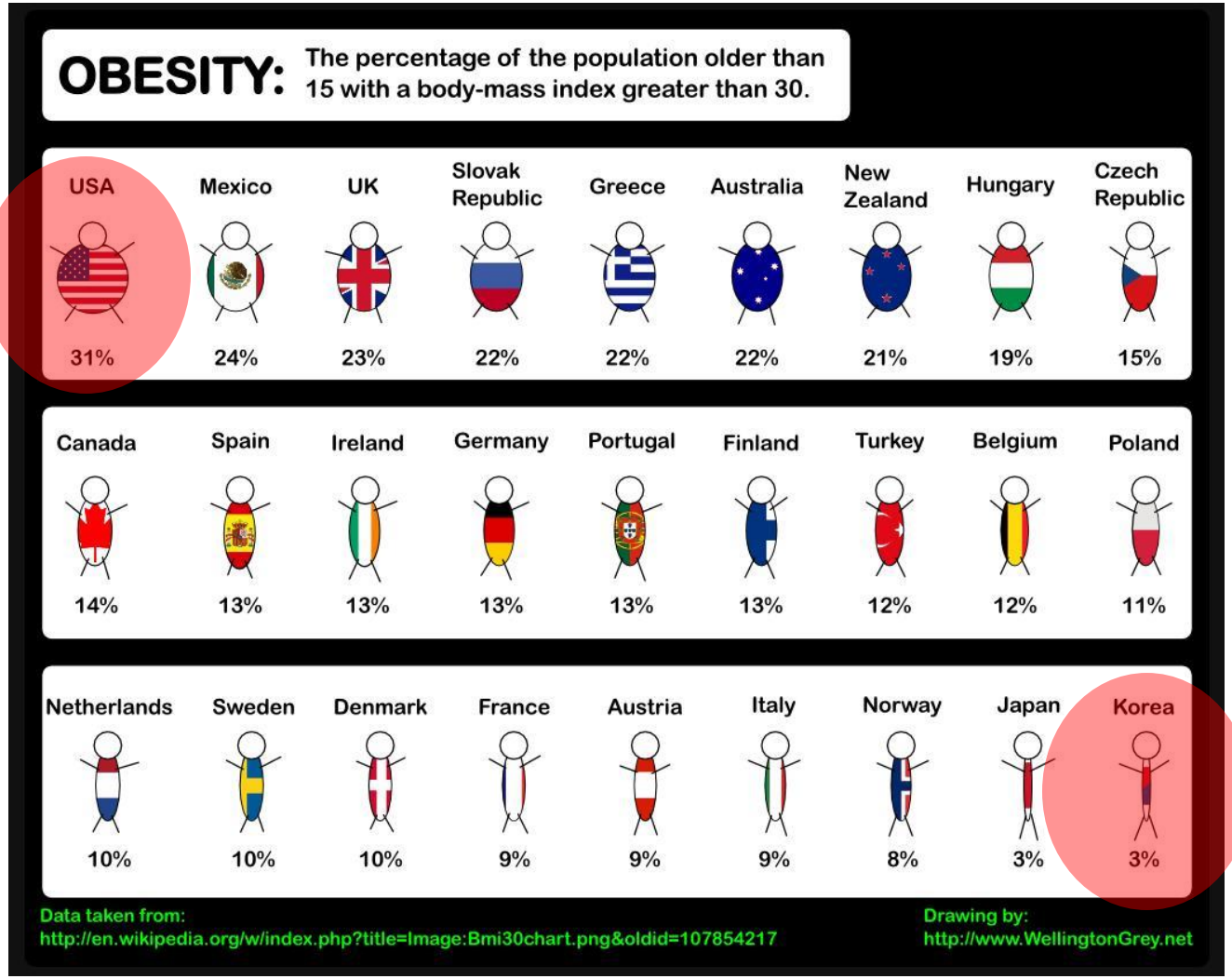
Insulin resistance

Metabolic syndrome: cluster of CVD risk factors

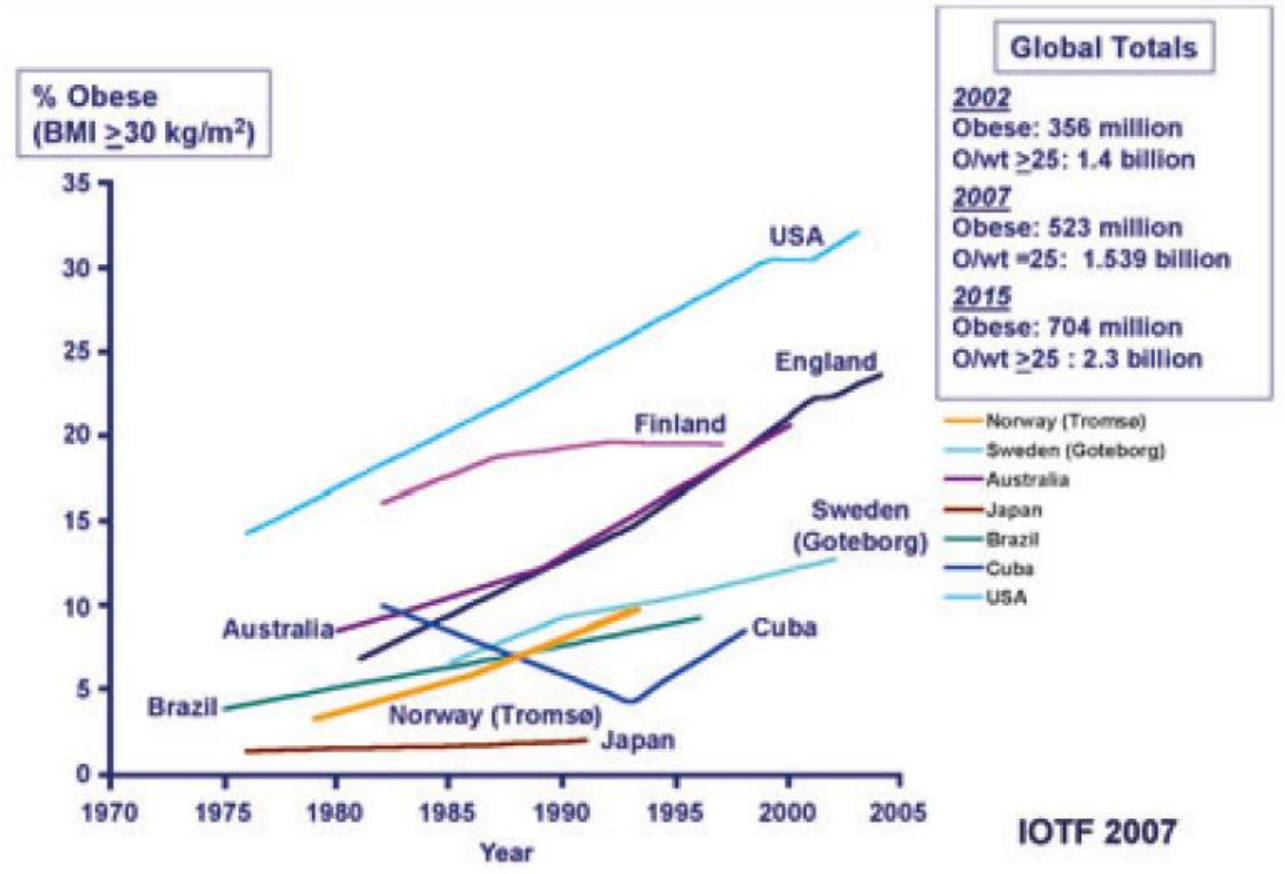
Pancreatic β cell insufficiency

Type 2 diabetes

Comparison of obesity prevalence



Comparison of increasing trend of obesity



James WPT. J Int Med 2008

Comparison of prevalence of type 2 diabetes

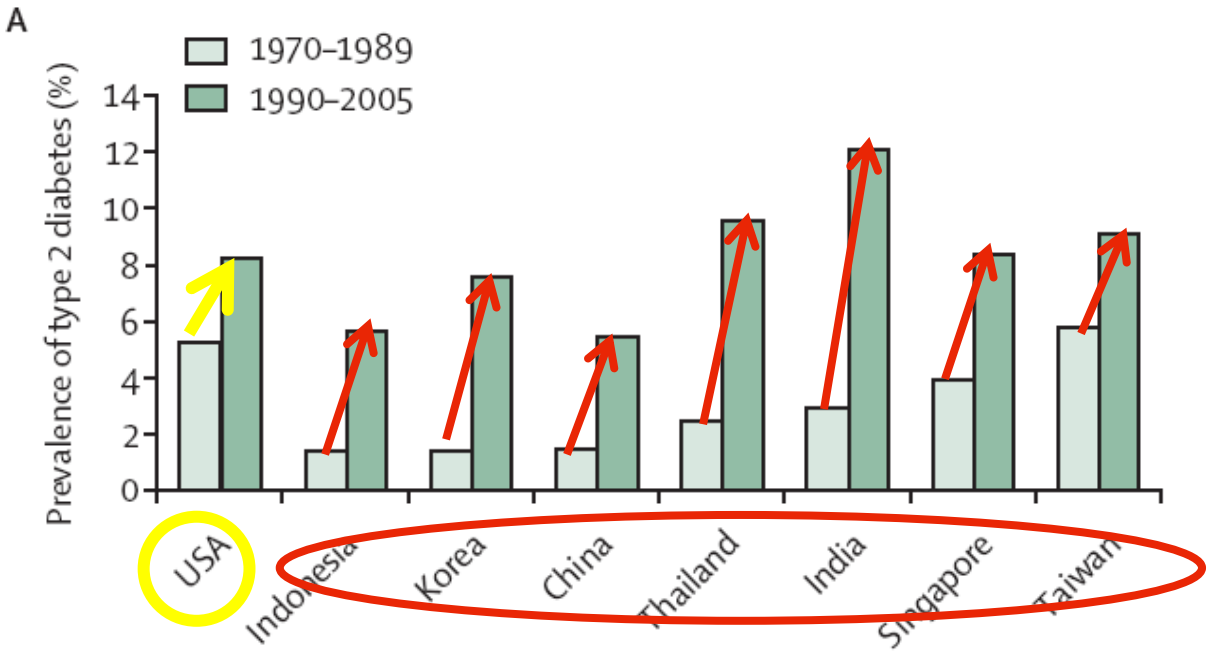


Figure 1: Comparison of prevalence rates of diabetes in selected countries between 1970–1989 and 1990–2005

Why??

- **Rapid changes in lifestyle**
- **Strong genetic susceptibility**

  -Prominent central obesity
  -Early beta-cell failure

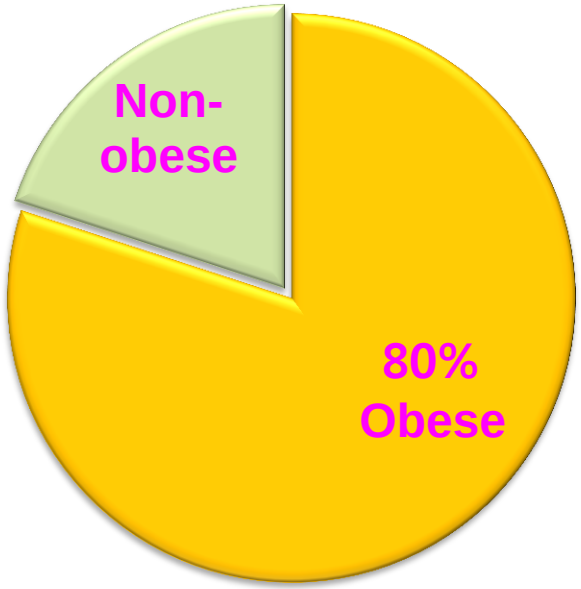
Really??

Yoon KH et al. Lancet 2006;368:1681-8

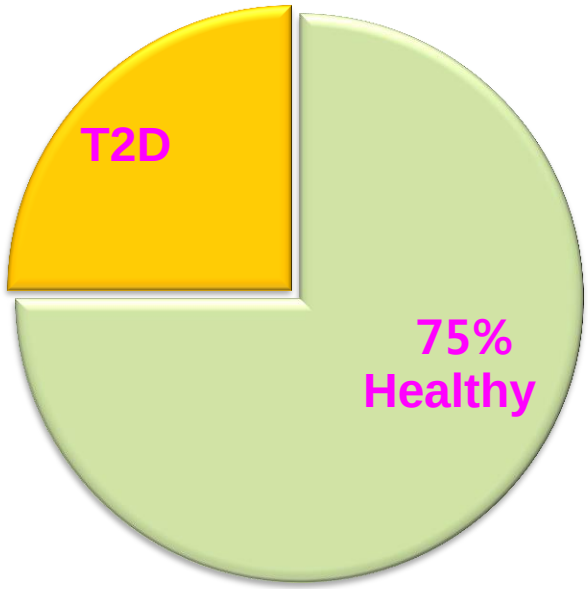
75% of obese persons never develop type 2 diabetes

According to the statistics of the U.S.

Patients with T2D

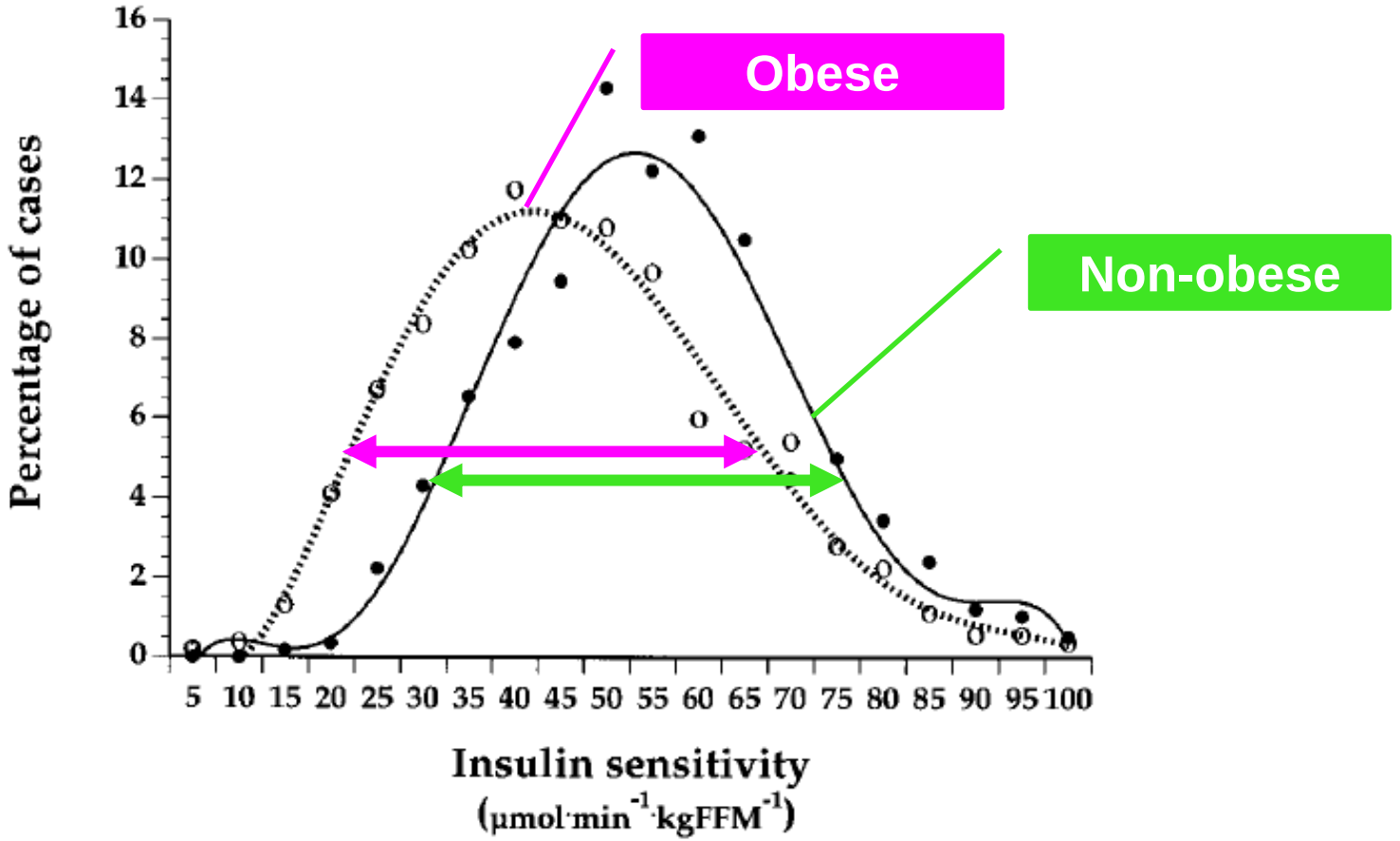


Persons with obesity



Gregg EW, et al. Diabetes Care 2004;27:2806-12

Variability of insulin resistance in obese persons



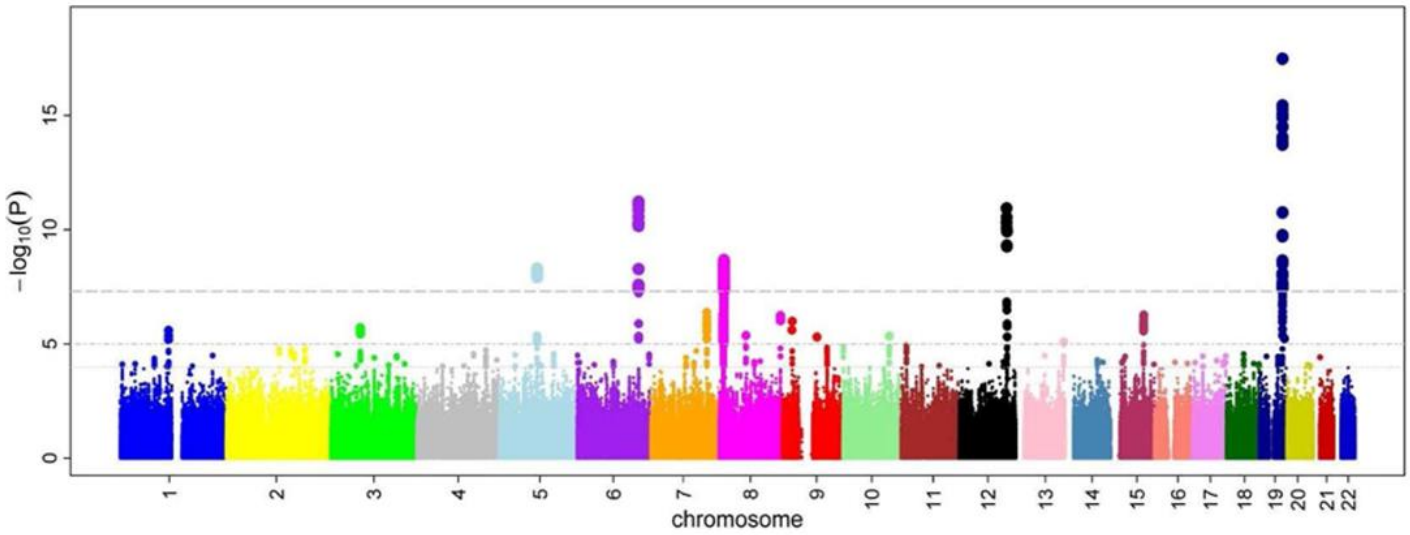
Ferrannini E, et al. J Clin Invest 1997;100:1166-73

Obesity is not a sufficient cause of insulin resistance and type 2 diabetes

Then, how about genes??

Data from genome-wide associations studies (GWAS) are far from clear

In sum, they might explain "statistically" only about 10% of the phenotypic variability

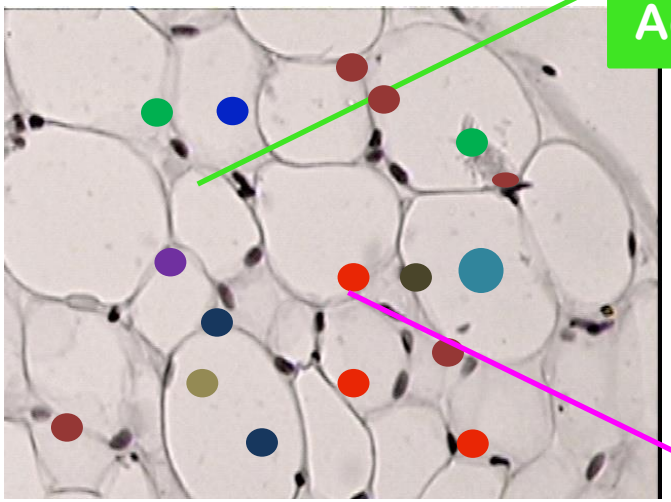


Billings LK, et al. Ann N Y Acad Sci 2010;1212:59-77

Therefore,
there should be something else,
neither obesity nor genes

In a modern chemical-contaminated society…

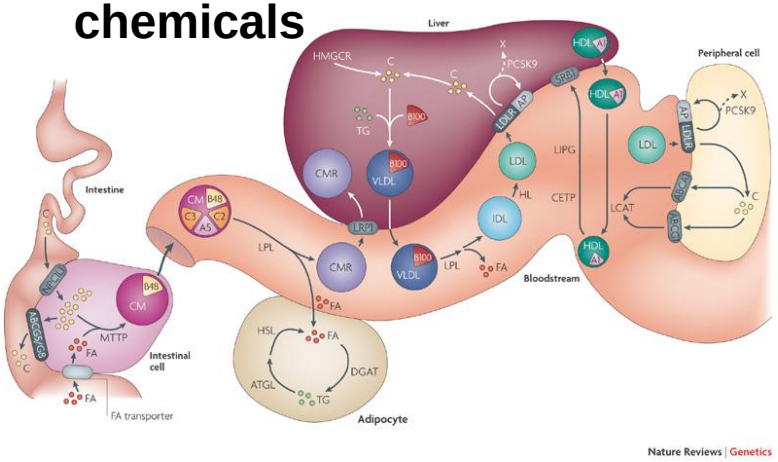
Adipose tissue is not a pure organ anymore



Adipocyte

Lipophilic chemicals

In addition, all lipids in human body are contaminated with lipophilic chemicals



I hypothesized that these kinds of chemicals like persistent organic pollutants (POPs) may be a key in the pathogenesis of type 2 diabetes

Not all of a sudden…

I was not a researcher in the field of environmental pollutants……

I had never heard of POPs before Nov 2005……

All hypotheses on POPs started with serum γ-glutamyltransferase

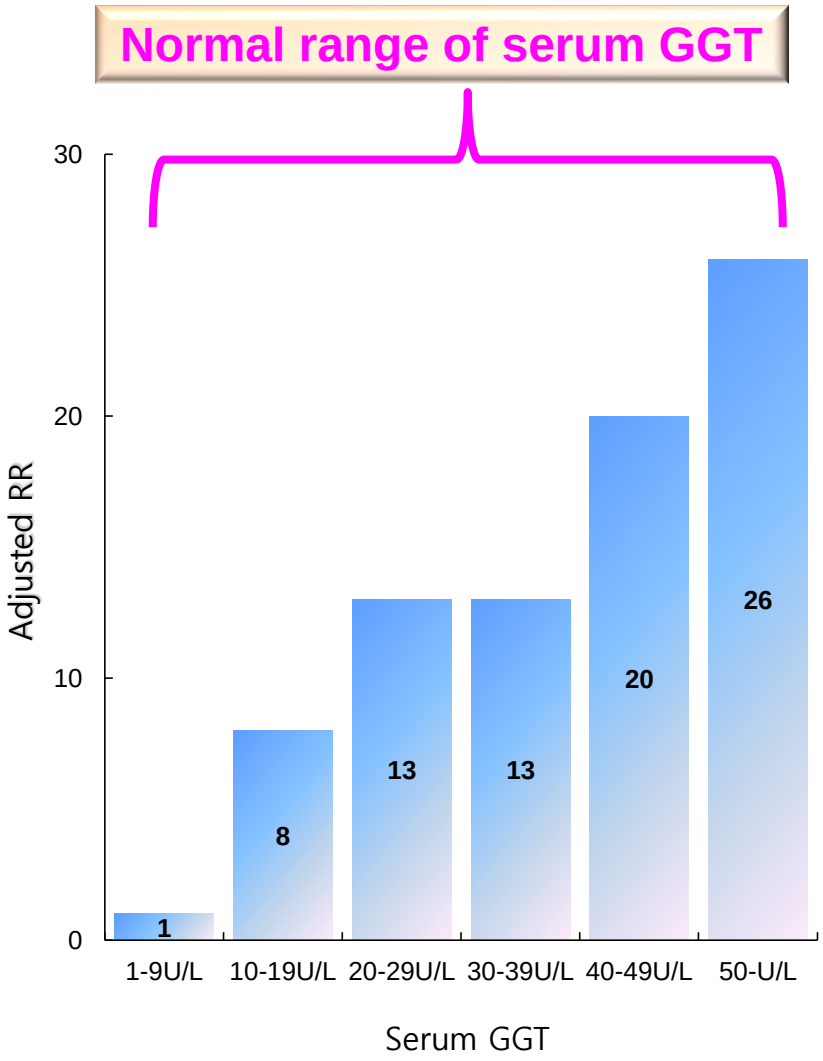
- **What's serum gamma glutamyltransferase (GGT)?**

  -Very well-known liver enzyme

  - Conventionally, used as a marker of alcohol consumption or hepatobiliary diseases

  - Easy and cheap measurement

Serum GGT strongly predicted type 2 diabetes



~~Conventional interpretation~~

~~-Alcohol consumption~~
~~-Liver dysfunction~~

Lee DH, et al. Diabetologia. 2003;46:359-64

Interaction between serum GGT and obesity on the risk of type 2 diabetes

**Well-known associations between obesity and diabetes was not clearly observed among persons with low normal serum GGT,
obesity predicted diabetes only when they had certain levels of serum GGT**

..Suggesting that something related to physiological functions of serum GGT may play a more fundamental role in the development of type 2 diabetes …

Lee DH, et al. Diabetologia. 2003;46:359-64

Association of Serum Carotenoids and Tocopherols with γ-Glutamyltransferase: The

Association between serum γ-glutamyltransferase and dietary factors: the Coronary Artery Risk Development in Young Adults (CARDIA) Study[1–3]

Background: Our pr
γ-glutamyltransferas
oxidative stress, sup
studies. To further e
to oxidative stress, v
tween serum caroten
antioxidant propertie
Methods: Study part
men and women 17–
carotenoids and toco
and 7, and serum GC
Results: Circulating

Letter to the Editor

Arterioscler Thromb Vasc Biol 2008;28:e26-8

Is Serum Gamma-Glutamyltransferase a Biomarker of Xenobiotics, Which Are Conjugated by Glutathione?

96 to

Is serum gamma-glutamyltransferase a marker of exposure to various environmental pollutants?

Free Radic Res 2009;43:533-7

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Korea.

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Abstract

It was previously hypothesized that serum gamma-glutamyltransferase (GGT) within its reference range predicts various clinical outcomes as a sensitive marker of oxidative stress in humans. This study further hypothesizes that serum GGT can mark exposure to various environmental pollutants, based both on recent epidemiological findings and on well-established biochemical features of cellular GGT. Cellular GGT is a prerequisite for metabolism of GSH conjugates that detoxify xenobiotics to mercapturic acid. Under this concept, serum GGT may increase with increasing exposure to environmental pollutants which need to be conjugated to GSH. Supporting this concept, it was recently reported that serum GGT within its reference range was linearly associated with important environmental pollutants, including lead, cadmium, dioxin and organochlorine pesticides. As a marker of the amount of conjugated xenobiotics, recent epidemiological findings about serum GGT imply the possibility of harmful effects of various environmental pollutants at background levels currently regarded as safe.

At that time, I was not sure if this hypothesis was correct or not

However, this hypothesis led me to look for low dose environmental chemicals as a fundamental cause of type 2 diabetes

What kinds of chemicals can explain the association between serum GGT and type 2 diabetes?

Considering previous findings on serum GGT,
they need to satisfy several conditions….

- may be exposed through food consumption, especially meat intake
- may be associated with adipose tissue
- may be lipophilic
- may be metabolized by glutathione conjugation
- may be reports on an increase of serum GGT in occupationally exposed workers
- If any, it may be diabetogenic in *in*-vitro experiments

THEN…the answer is….

What are POPs?

- **POPs include hundreds of different chemicals with common properties**

  - long term persistence in the environment
  - bioaccumulation in fatty tissues of living organisms
  - very long half lives (several years to decades)

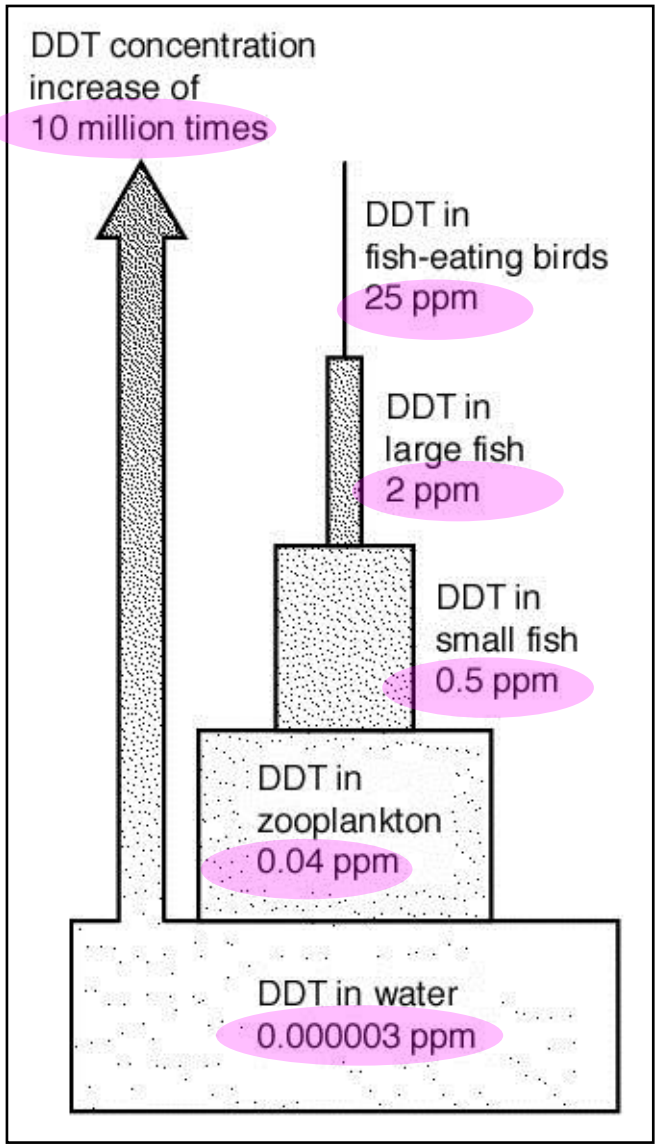
- **Typical examples of POPs**

  - Organochlorine pesticides (OC pesticides): DDT, Chlordane, Lindane..
  - Polychlorinated biphenyls (PCBs)
  - Dioxins
  - Many other POPs-like chemicals

- **The most problematic POPs were already banned several decades ago in most developed countries, but they are currently detected in almost all general populations**

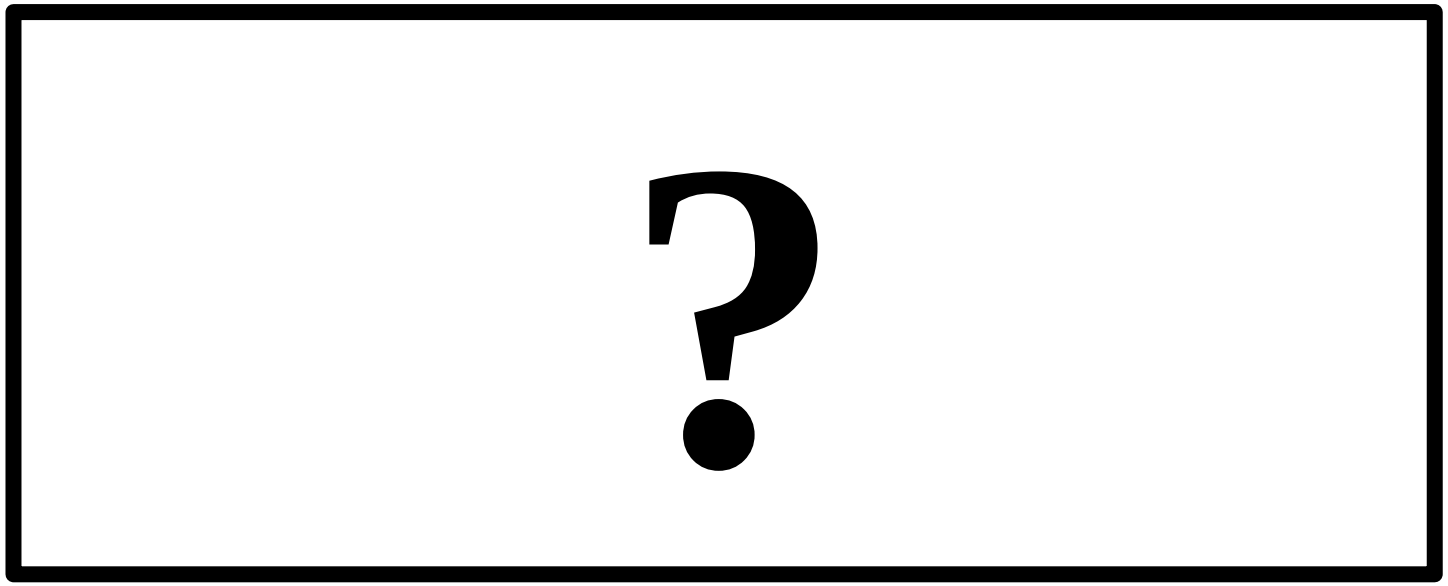
Completely

POPs have contaminated food chain on earth



At present, the main external exposure source in general population is fatty animal food.

What we currently know



NHANES 1999-2002: measurement of 50 POPs in the U.S. general population

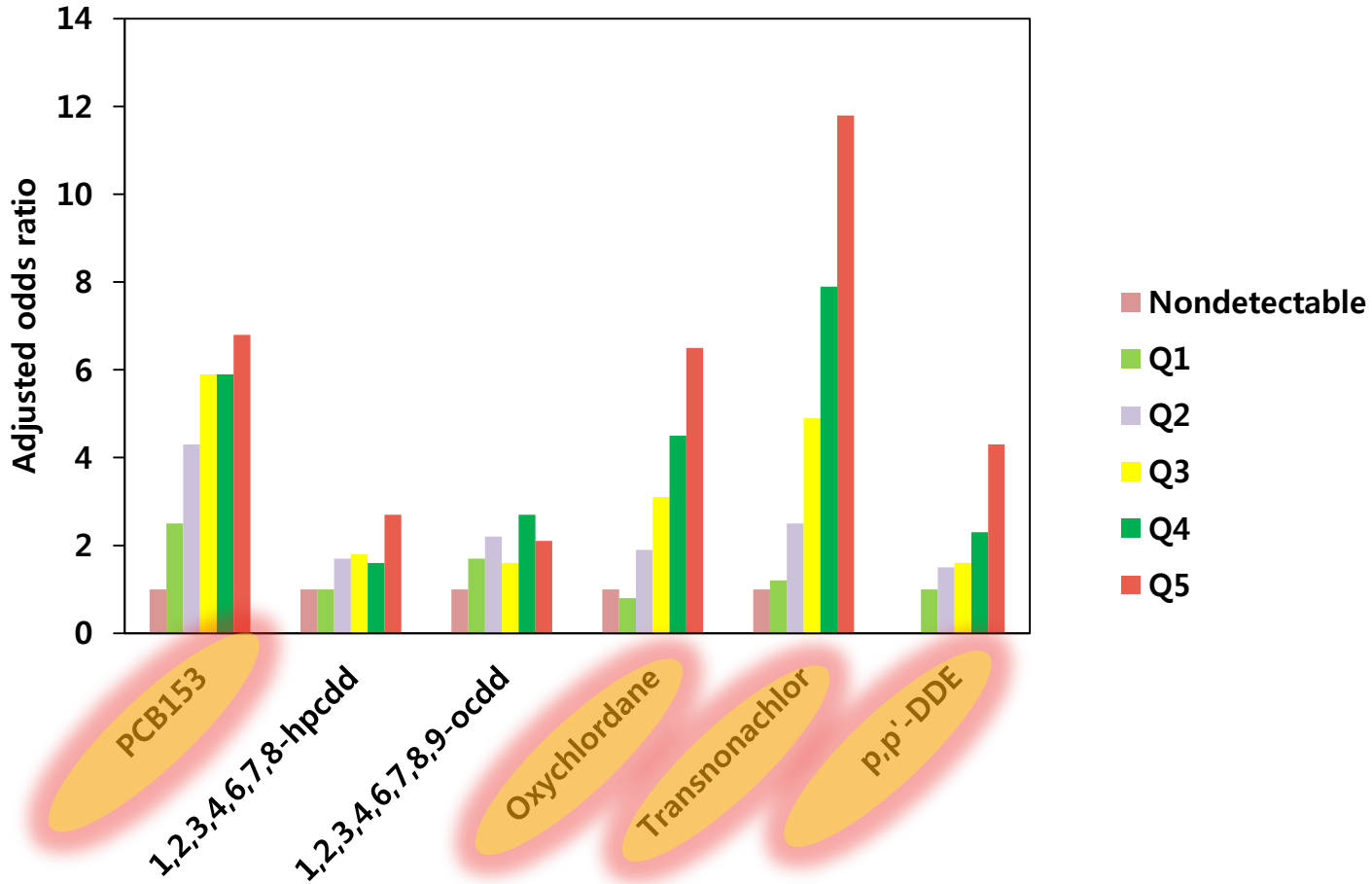
- Polychlorinated Dibenzo-p-dioxins (PCDDs)
- Polychlorinated Dibenzofurans (PCDFs)
- Dioxin-like PCBs
- Non-dioxin-like PCBs
- Organochlorine Pesticides

6 POPs which were detected among ≥ 80% of subjects

- 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153)
- 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin
- 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin
- oxychlordane
- p,p'-DDE
- trans-nonachlor

POPs and Type 2 diabetes: individual 6 POPs

- Cross-sectional study
- 2016 U.S. adult population (NHANES dataset)
- 6 POPs detectable in >80% of study subjects



† Adjusted for age, race, sex, poverty income ratio, body mass index, and waist circumference

Lee DH, et al. Diabetes Care 2006;29;1638-44

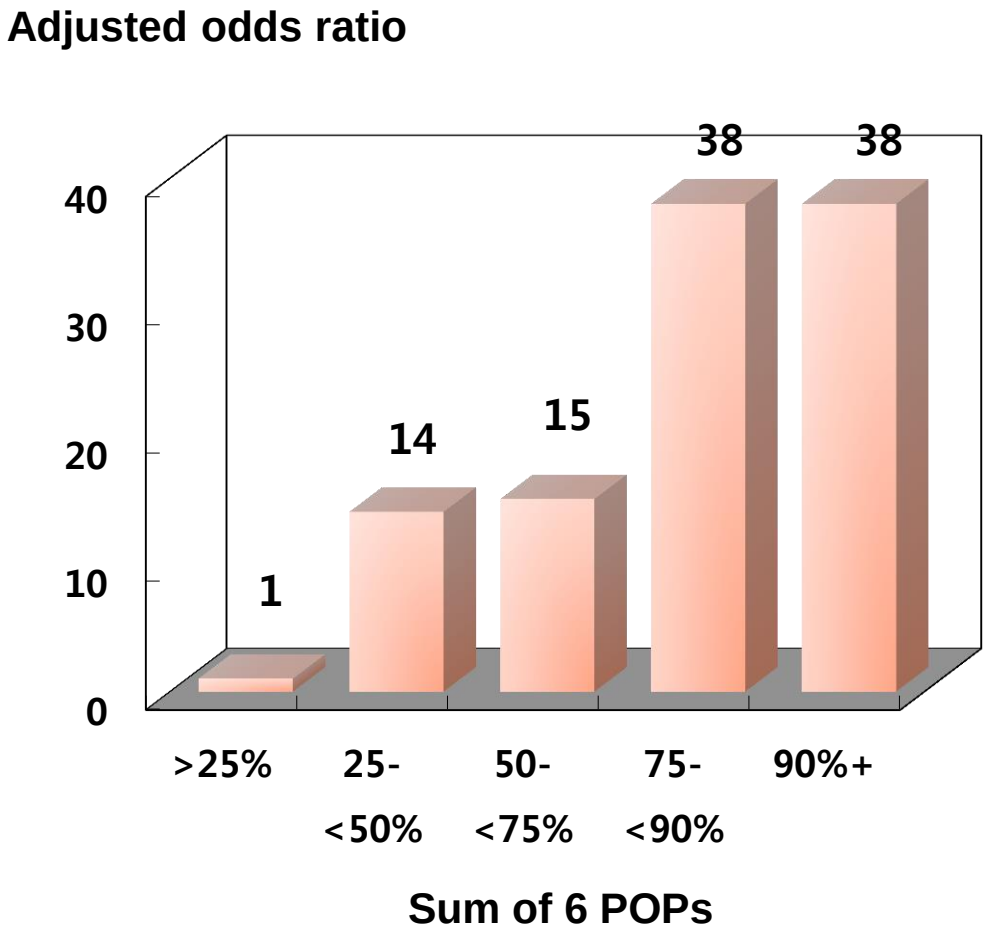
However, strong positive correlations among serum concentrations of POPs in general population

	PCB 153	1,2,3,4,6,7,8-hpcdd	1,2,3,4,6,7,8,9-ocdd	Oxy-chlordane	p,p'-DDE	Trans-nonachlor
PCB153	1	+ 0.41	+ 0.49	+ 0.72	+ 0.44	+ 0.71
1,2,3,4,6,7,8-hpcdd		1	+ 0.78	+ 0.47	+ 0.37	+ 0.45
1,2,3,4,6,7,8,9-ocdd			1	+ 0.53	+ 0.40	+ 0.53
Oxy-Chlordane				1	+ 0.50	+ 0.92
p,p'-DDE					1	+ 0.53
Trans-nonachlor						1

- Meaning of epidemiological finding on individual POP is questionable..
- Even though I am talking about one specific POP, it reflects results on POPs mixtures, not that specific POP.
- Also, as a reference group with low levels in a wide range of POPs was important, we tried summary measures of multiple POPs

Lee DH, et al. Diabetes Care 2006;29;1638-44

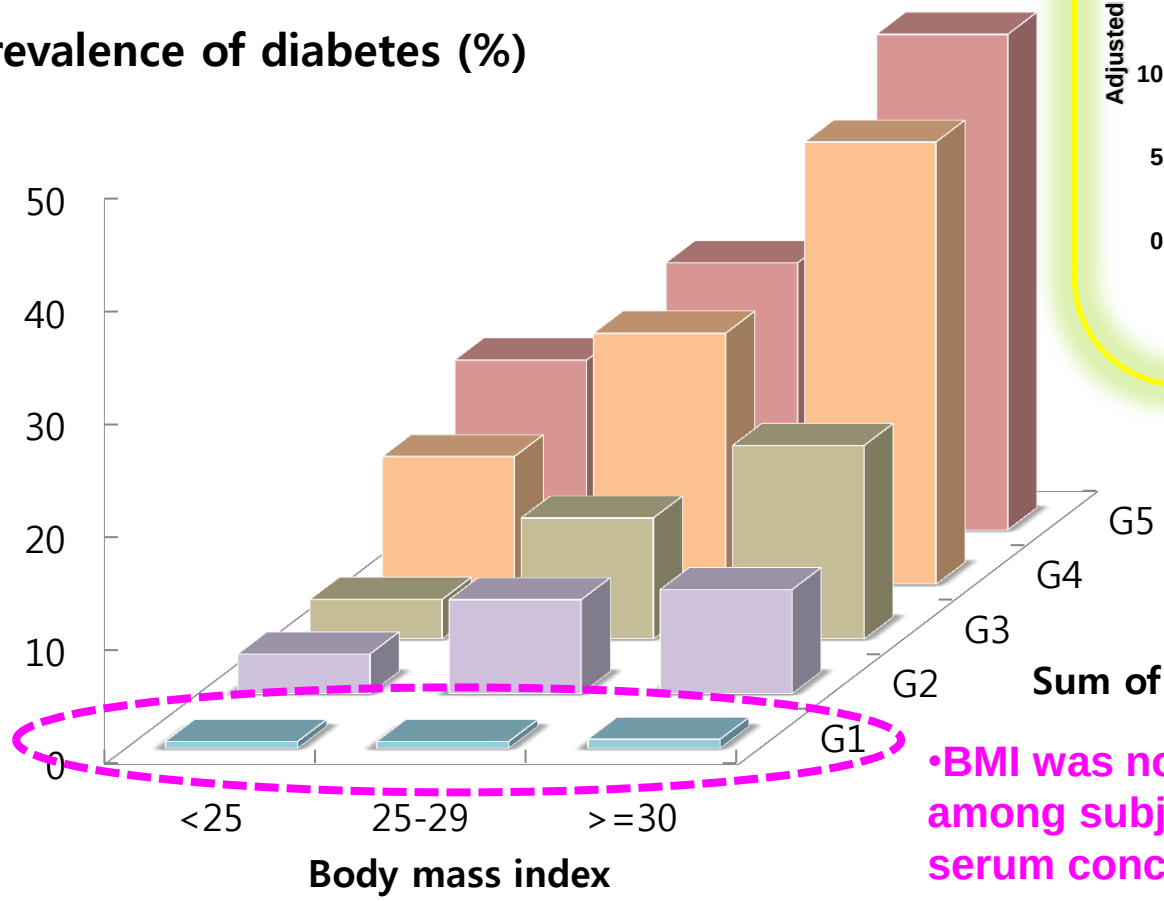
POPs and Type 2 diabetes: summary measure of POPs



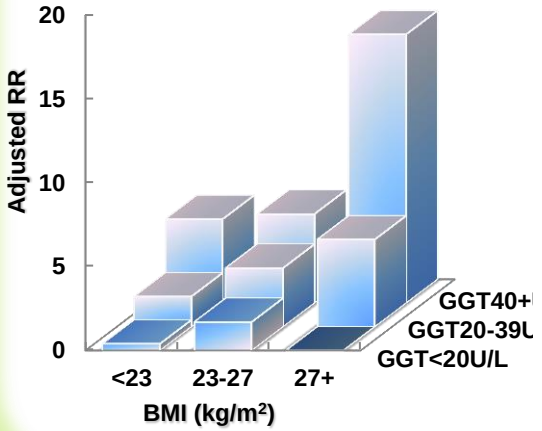
† Adjusted for age, race, sex, poverty income ratio, body mass index, and waist circumference

Lee DH, et al. Diabetes Care 2006;29;1638-44

Interaction between POPs and obesity



Interaction between serum GGT and obesity



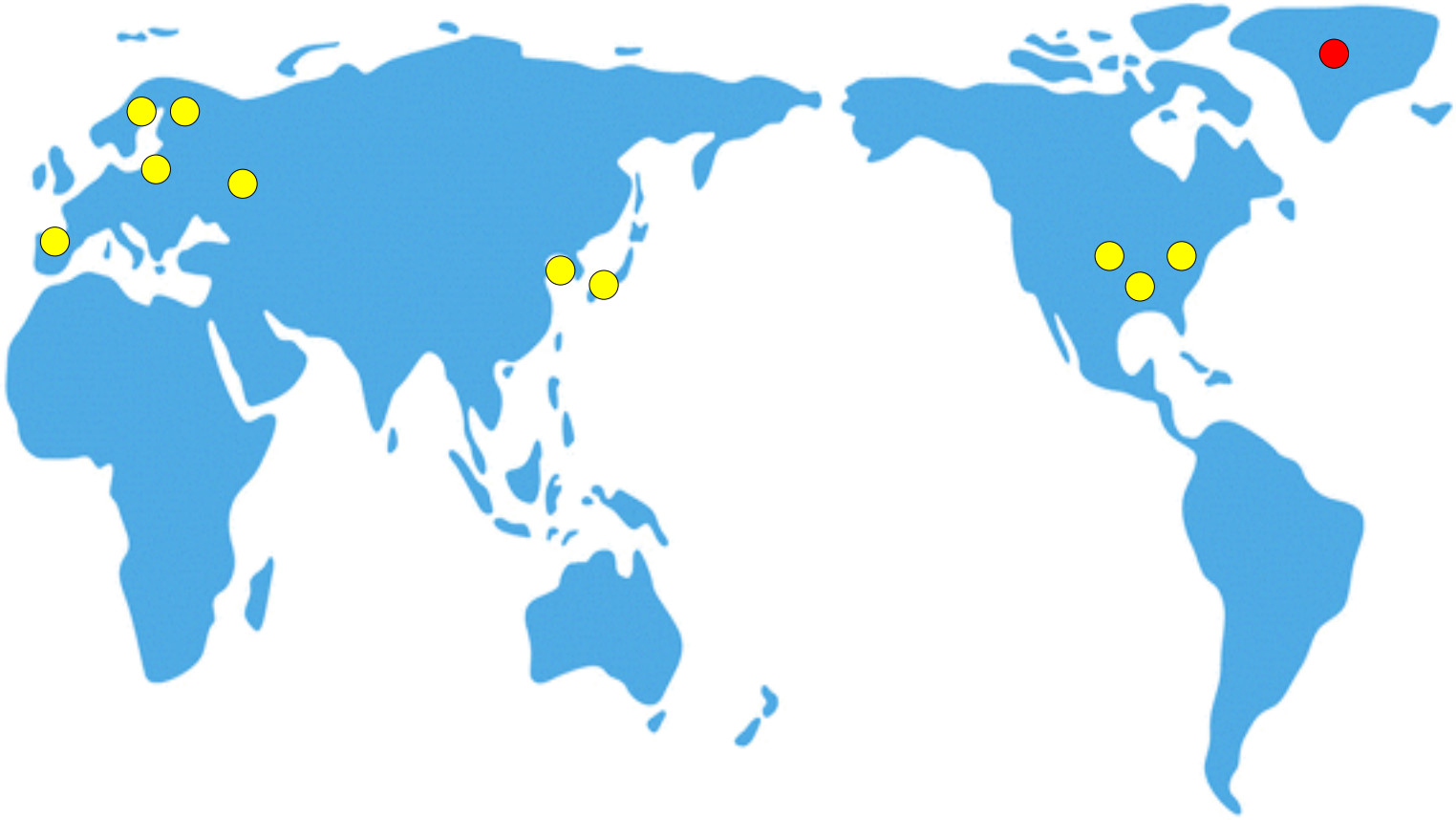
•BMI was not associated with T2D among subjects with very low serum concentration of POPs

† Adjusted for age, race, sex, poverty income ratio, body mass index, and waist circumference

Lee DH, et al. Diabetes Care 2006;29;1638-44

Replication in other cross-sectional studies, except one

No association
in Greenland Inuit
(high body burden of POPs)

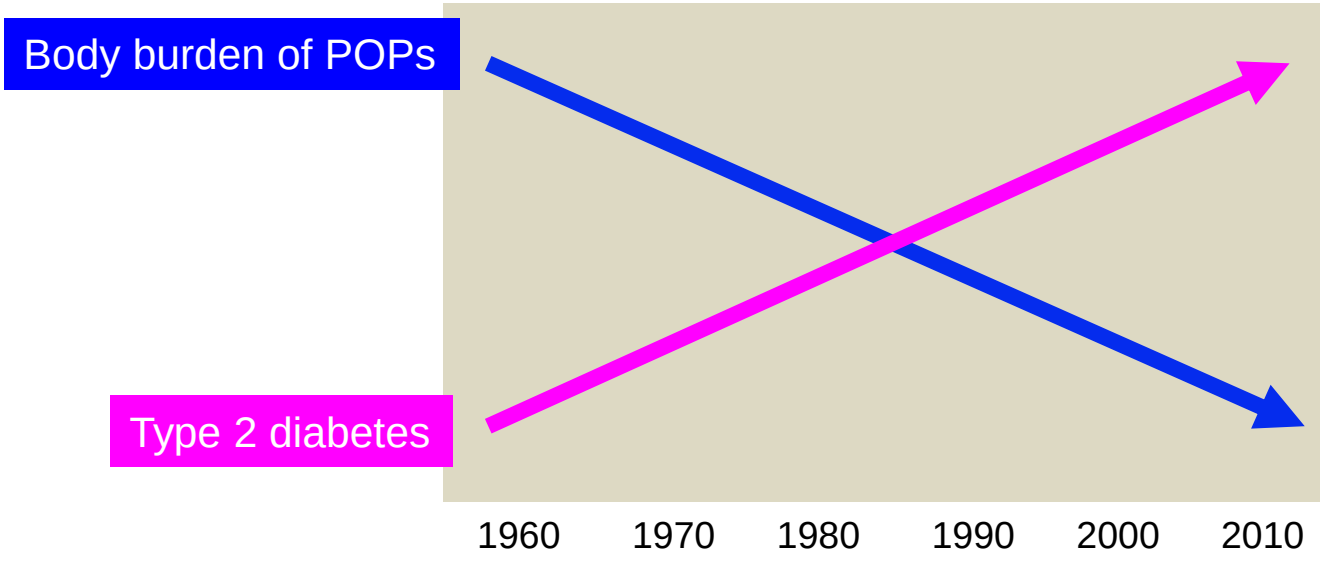


Critique

Q : Mismatch of time trends????

Body burden of chlorinated POPs in human has been decreasing since banning, but type 2 diabetes is currently epidemic

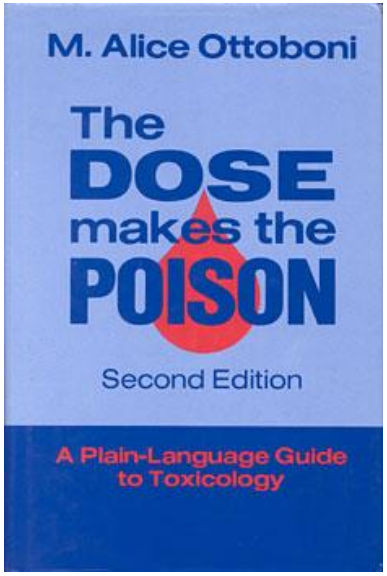
How this kind of discrepancy of time trend is possible, if chlorinated POPs are really important in the pathogenesis of type 2 diabetes?



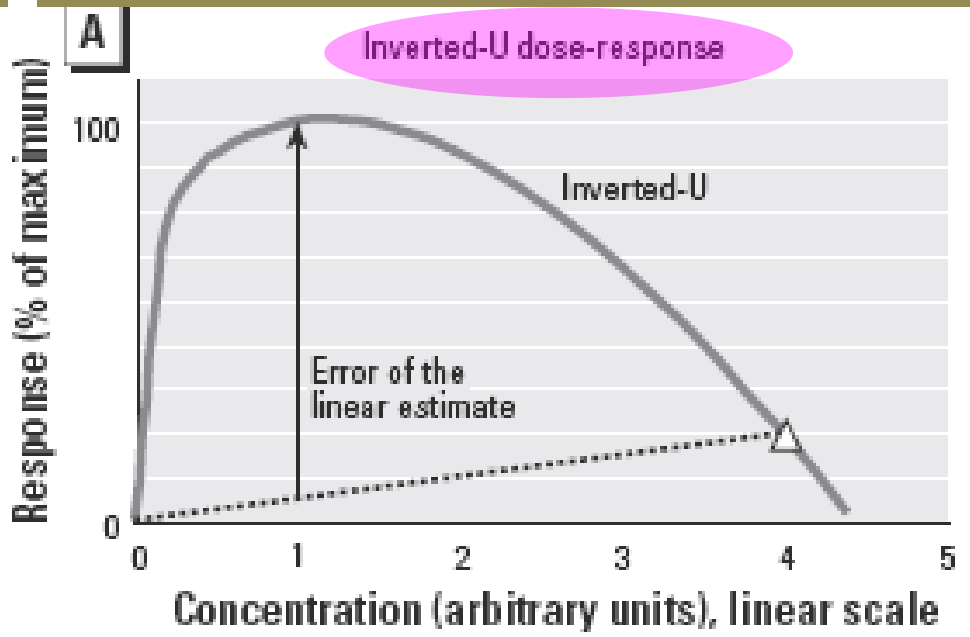
First explanation: non-monotonic dose response relations

Even though there is a strong dogma of "the dose makes the poison" in traditional toxicology,
In some mechanisms, low dose can be more harmful than high dose

Traditional toxicology
linear dose-response relations



A recent hot debate:
non-monotonic dose-response relations

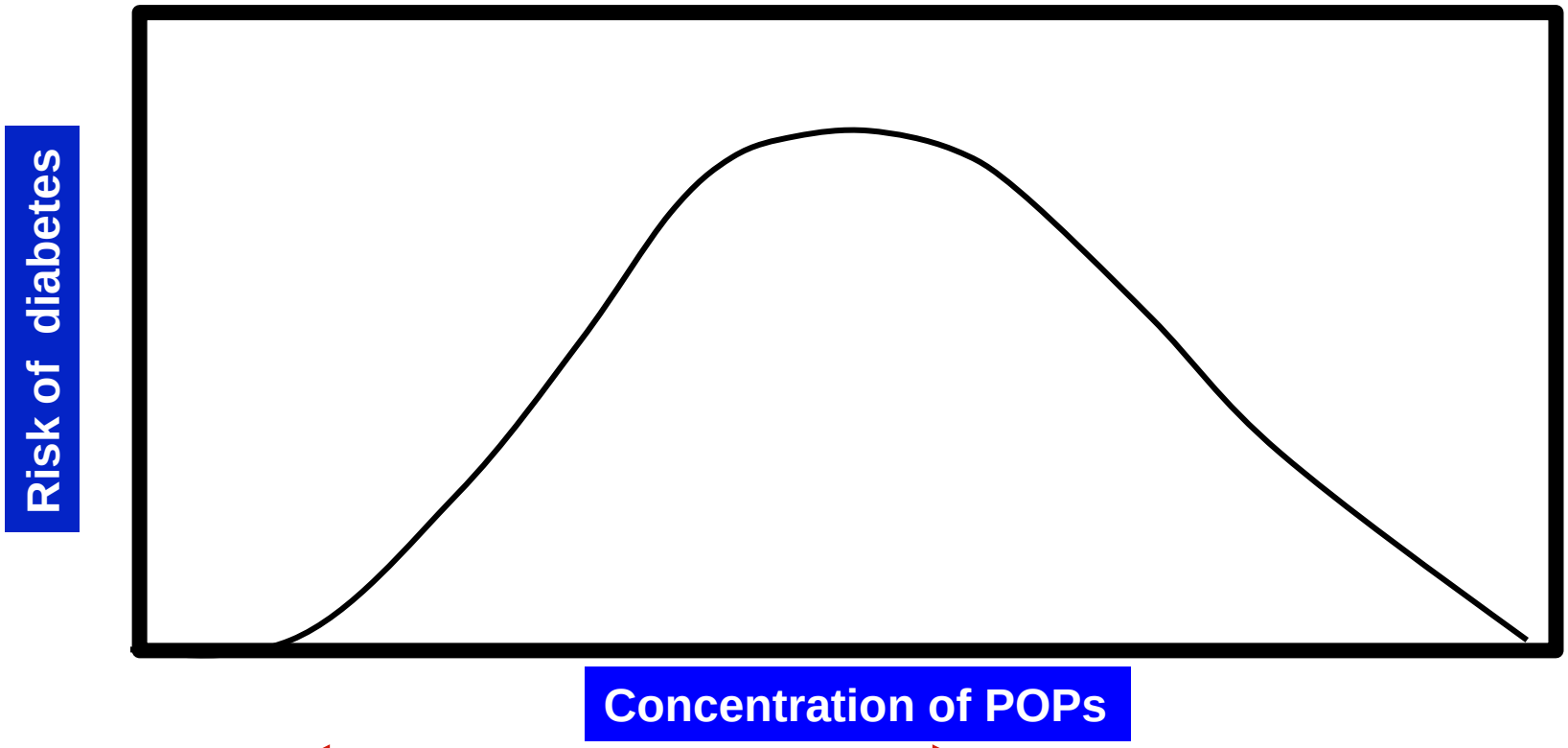


<NOAEL

Vom Saal F et al. Environ Health Perspect 2003;111:994-1006

First explanation: why no association in Greenland Inuit?

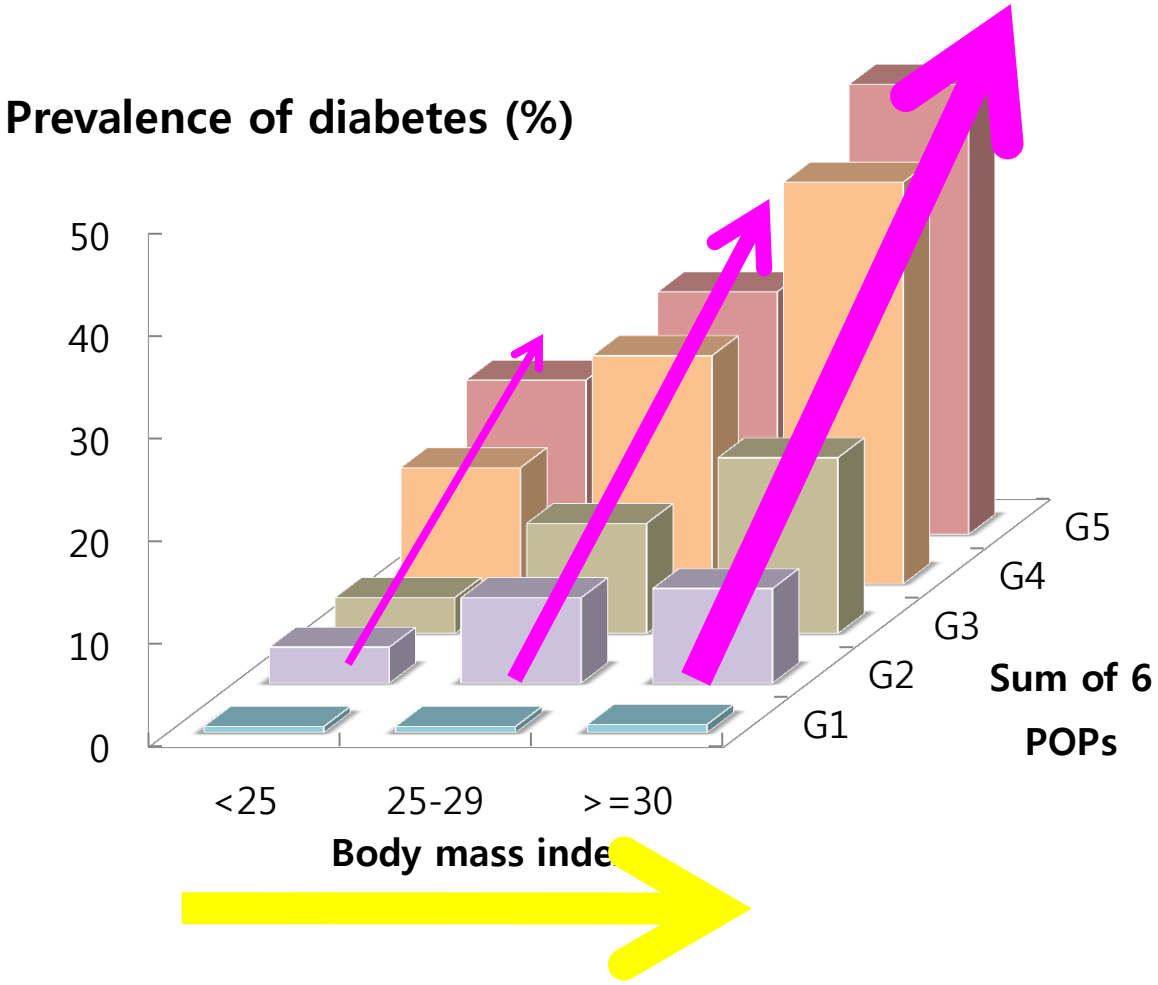
Under the inverted U-shaped association, we can expect different study results depending on POPs distribution of populations



U.S.: low body burden
A strong dose-response relation

Greenland Inuit : high body burden
Population level: Diabetes↑
Individual level: No association

Second explanation: Interaction with obesity??



When there are certain levels of POPs, obesity certainly make the situation worse

Lee DH, et al. Diabetes Care 2006;29;1638-44

Evidence from recent prospective studies

- **CARDIA study (U.S.): a nested case-control study**

  - 18 year follow-up
  - Study subjects: 120 young adults aged 20~32
  - Focusing on OC pesticides and PCBs

- **PIVUS study (Sweden)**

  - 5 year follow-up
  - Study subjects: 1,000 elderly aged 70
  - Focusing on OC pesticides and PCBs

Prospective studies demonstrated..

Healthy persons
with "low POPs mixture"
at baseline

Healthy persons
with "high POPs mixture"
at baseline

Follow up

- Diabetes
- Insulin resistance
- Dyslipidemia
- Obesity

"Obesogen hypothesis"

-Chemicals that inappropriately stimulate adipogenesis and fat storage through endocrine disrupting mechanisms

-A variety of chemicals can be classified as obesogens

Lee DH et al. Environ Health Perspect 2010;118:1235-42
Lee DH et al. PLoS One 2011;6:e15977
Lee DH et al. Diabetes Care 2011;34:178-84

Evidence from an experimental study on POPs mixture

Persistent Organic Pollutant Exposure Leads to Insulin Resistance Syndrome

Jérôme Ruzzin,[1] Rasmus Petersen,[2,3] Emmanuelle Meugnier,[4] Lise Madsen,[1,3] Erik-Jan Lock,[1] Haldis Lillefosse,[1] Tao Ma,[2,3] Sandra Pesenti,[4] Si Brask Sonne,[3] Troels Torben Marstrand,[5] Marian Kjellevold Malde,[1] Zhen-Yu Du,[1] Carine Chavey,[6] Lluis Fajas,[6] Anne-Katrine Lundebye,[1] Christian Lehn Brand,[7] Hubert Vidal,[4] Karsten Kristiansen,[3] and Livar Frøyland[1]

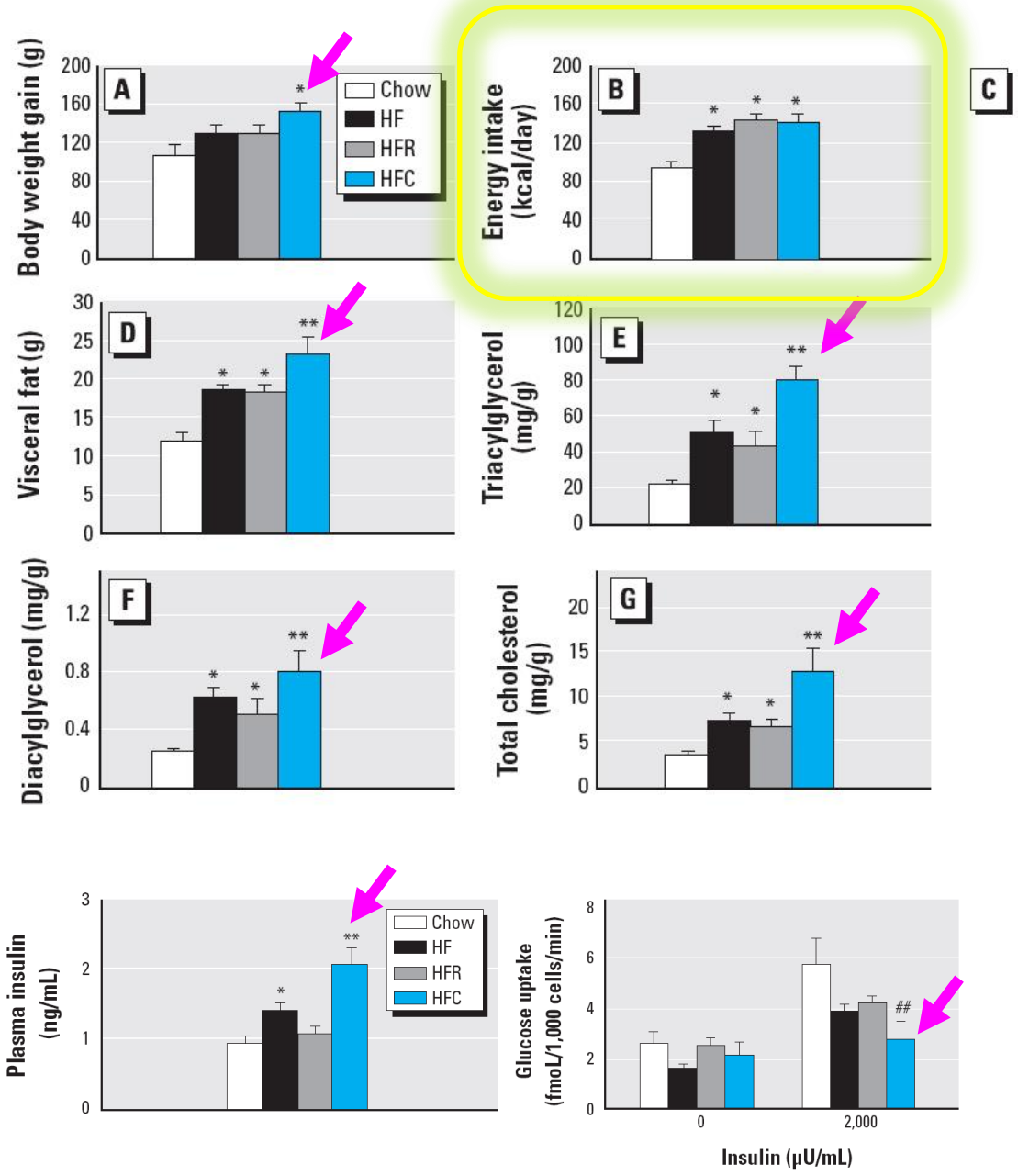
[1]National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway; [2]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark; [3]Department of Biology, University of Copenhagen, Copenhagen, Denmark; [4]INSERM U-870, INRA (Institut national de la recherche agronomique) U-1235, Lyon 1 University, Institut National des Sciences Appliquées de Lyon and and Hospices Civils de Lyon, Oullins, France; [5]The Bioinformatics Centre, Department of Biology and Biotech Research and Innovation Centre, University of Copenhagen, Copenhagen, Denmark; [6]Institut de Recherche en Cancérologie de Montpellier, INSERM U896 Metabolism and Cancer Laboratory, Montpellier, France; [7]Department of Insulin Pharmacology, Histology and Delivery, Novo Nordisk A/S, Maaloev, Denmark

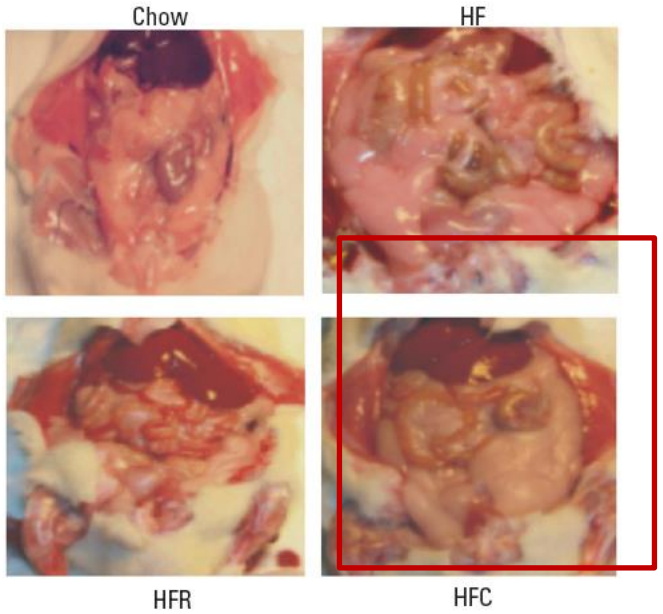
Sprague-Dawley rats for 4 weeks

- **High fat diet containing crude fish oil(HFC) : contaminated with mixed POPs**
- **Standard diet, 17% fat-derived calories**
- **High fat diet : 65% fat-derived calories**
- **High fat diet containing refined fish oil(HFR) : artificially removed POPs from fish oil**

NOTE: Body burden of POPs in rats were similar with that in human aged 40~50

Ruzzin J et al. Environ Health Perspectives 2010;118:465-71





POPs –contaminated fish oil group developed

- **Visceral obesity**
- **Dyslipidemia**
- **Steatohepatitis**
- **Insulin –resistance**

Ruzzin J et al. Environ Health Perspectives 2010;118:465-71

Following two experiments by the same team

Experiment 1: POPs -contaminated salmon fillet for 8 wks

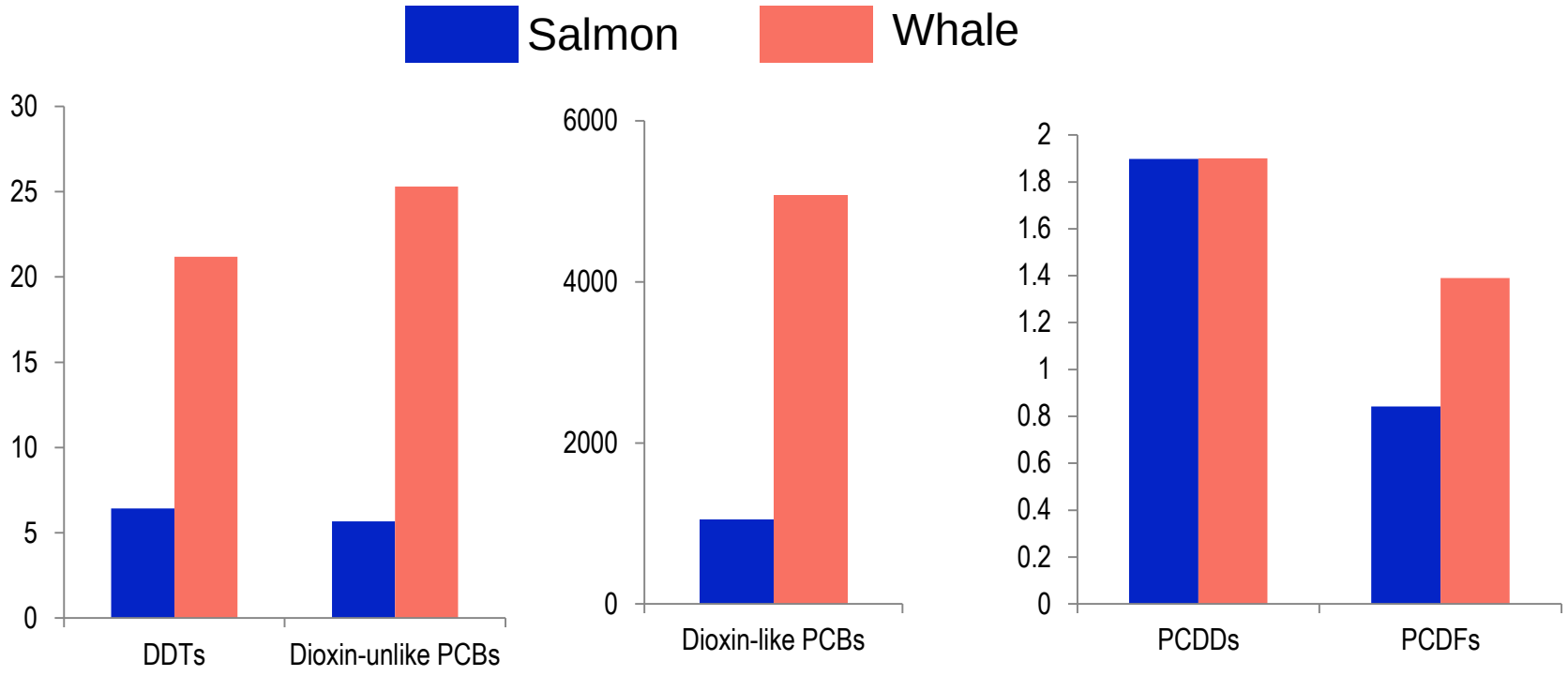
Experiment 2: POPs-contaminated whale meat for 8 wks

Salmon treated mice: harmful!!	**Whale treated mice: beneficial!!**
•Visceral obesity •Insulin resistance •Glucose intolerance •Hepatic steatosis •Triacylglycerol accumulation in muscle	•Reduced body weight •Increased insulin sensitivity •Improved glucose tolerance

Completely opposite results between Salmon and Whale!!

Ibrahim MM, et al. PLoS One 2011;6:e25170
Ibrahim MM, et al, Toxicology letter 2012;215:8-15

Again, these opposite results reflect low dose effects of POPs??



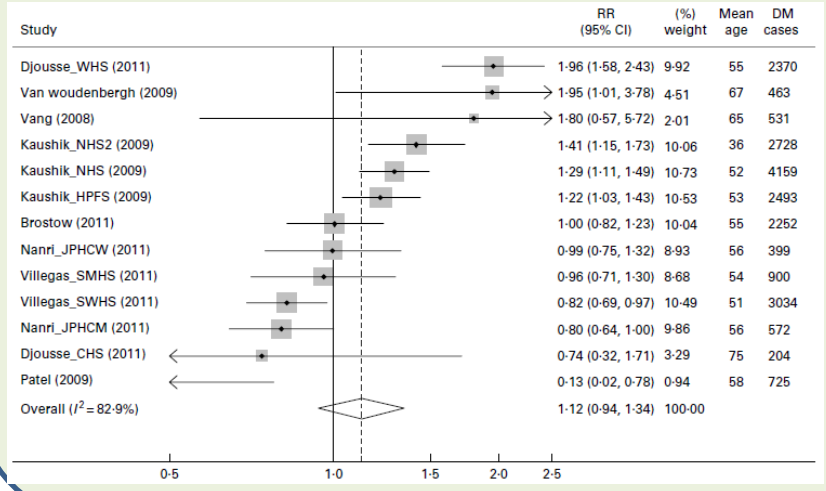
Organochlorine pesticides and PCBs, not PCDD, were 10~15 times higher in whale meat than salmon fillet

Ibrahim MM, et al. PLoS One 2011;6:e25170
Ibrahim MM, et al, Toxicology letter 2012;215:8-15

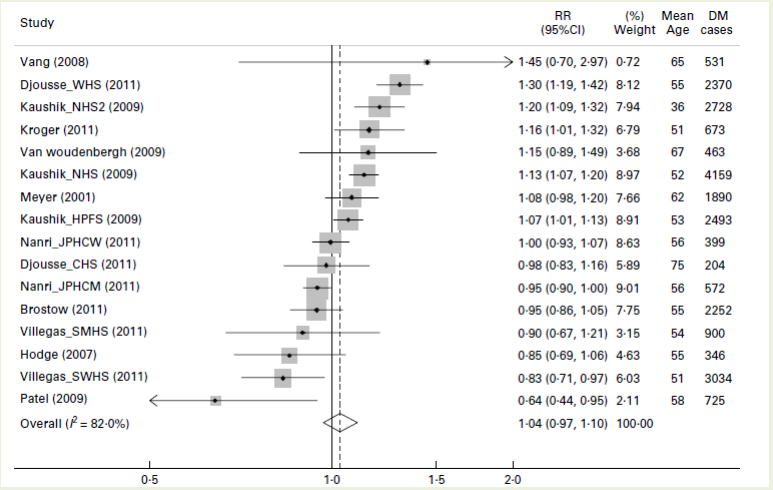
Omega-3 fatty acids consumption and type 2 diabetes

Recent meta-analysis on fish consumption or omega-3 fatty acid consumption showed very inconsistent results from positive to inverse associations suggesting mixture effects of "benefits from omega-3 fatty acid" and "harms from POPs contamination".

Meta-analysis: Fish or seafood consumption and T2D

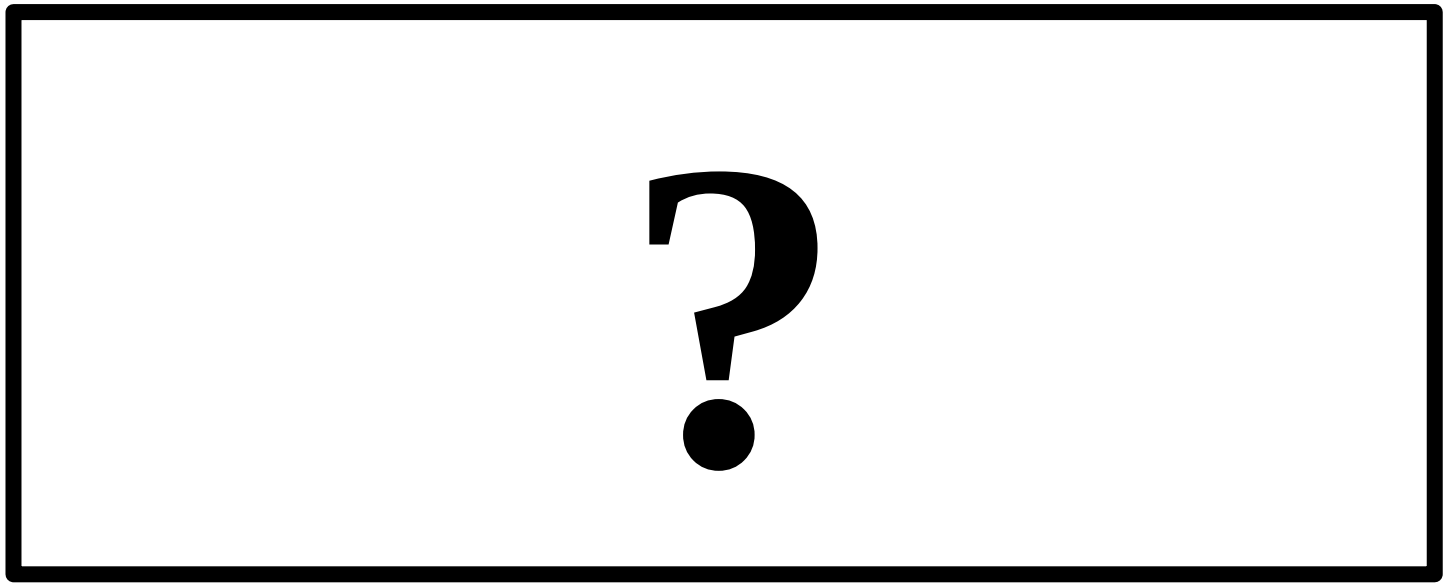


Meta-analysis: Omega-3 fatty acid consumption and T2D



Wu JHY, et al. Br J Nut 2012;107:S214-S227

What we exactly don't know



What we exactly don't know

1. Molecular mechanisms for inverted U-shaped associations

Tow possible mechanisms

1. **Endocrine disruption**

2. **Mitochondrial dysfunction**

Possible mechanisms:
1. endocrine disrupting mechanisms ??

Despite strong experimental evidence
I am skeptical on this mechanism,

Because…

all experimental studies are one chemical–based ones

Humans are living in a sea of chemical mixtures..

Estrogenic
Anti-estrogenic
Androgenic
Anti-androgenic
Thryroidogenic
Glucocorticoidgenic
Many others….

Can we estimate net results of all chemical mixtures in human?

Possible mechanisms:
2. mitochondrial dysfunction-related mechanisms ??

Chronic exposure to low dose POPs mixture caused mitochondrial dysfunction in POPs-contaminated fish oil-treated rats

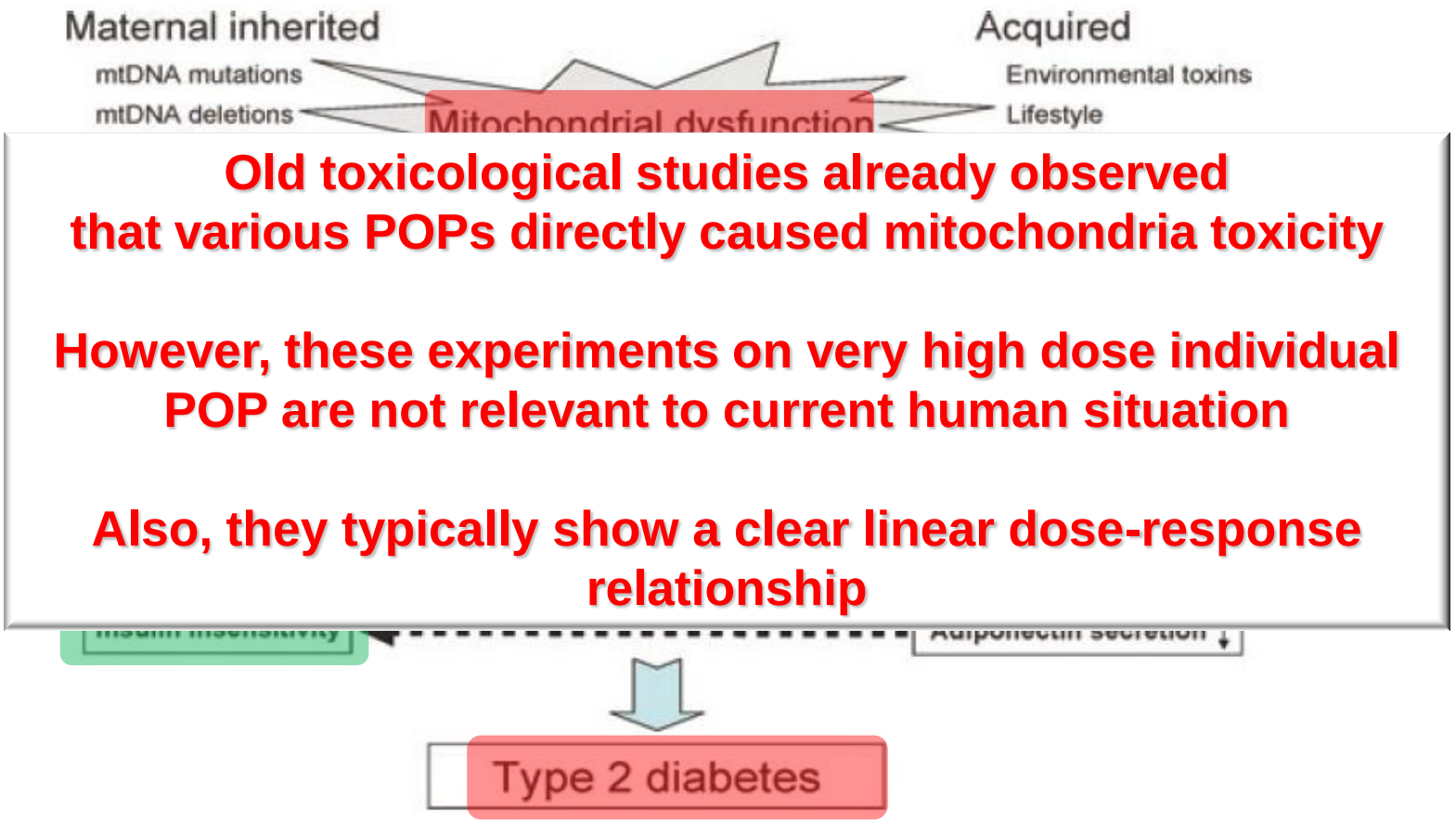
Table 1. Real-time PCR determination of mRNA expression of a set of relevant genes in the liver of rats fed HFR or HFC diets ($n = 9$ per group).

	HFR	HFC	*p*-Value
Genes related to mitochondrial function			
PGC1α ↓	0.73 ± 0.3	0.05 ± 0.02	0.043
PPARα (peroxisome proliferator-activated receptor α)	76 ± 7	75 ± 18	0.988
CS (citrate synthase) ↓	316 ± 19	214 ± 10	0.002
SDHA (succinate dehydrogenase) ↓	74 ± 2	63 ± 4	0.038
MCAD (medium chain acyl CoA dehydrogenase) ↓	332 ± 30	170 ± 18	0.003

High fat diet without POPs | **High fat diet with POPs**

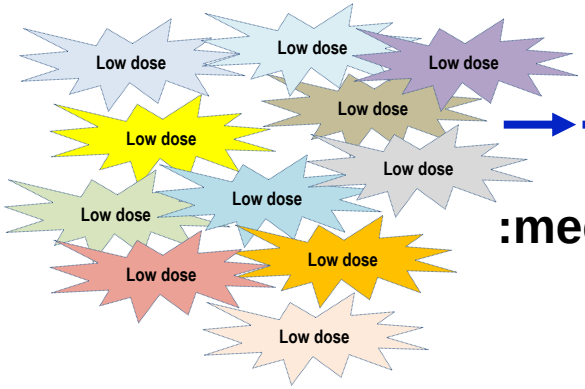
Ruzzin J et al. Environ Health Perspectives 2010;118:465-71

Mitochondrial dysfunction: a unifying mechanism of insulin resistance and type 2 diabetes



Wang CH et al. Ann N Y Acad Sci 2010;1201:157-65

However, there can be an indirect pathway leading to functional impairment of mitochondria



Chronic exposure to low dose POPs mixture

→ Chronic GSH depletion
:mechanistically closely related to serum GGT increase

Functional impairment of mitochondria

Indirect pathways can show an inverted U-shaped association because increased dose of POPs to a certain level can increase GSH levels and activate mitochondrial function ("mitohormesis")

What we exactly don't know

2. Effects of POPs on gut microbiota

Gut microbiota and obesity-related metabolic dysfunction

ARTICLE

doi:10.1038/nature12506

Richness of human gut microbiome correlates with metabolic markers

Emmanuelle Le Chatelier[1]*, Trine Nielsen[2]*, Junjie Qin[3]*, Edi Prifti[1]*, Falk Hildebrand[4,5], Gwen Falony[4,5], Mathieu Almeida[1], Manimozhiyan Arumugam[2,3,6], Jean-Michel Batto[1], Sean Kennedy[1], Pierre Leonard[1], Junhua Li[3,7], Kristoffer Burgdorf[2], Niels Grarup[2], Torben Jørgensen[8,9,10], Ivan Brandslund[11,12], Henrik Bjørn Nielsen[13], Agnieszka S. Juncker[13], Marcelo Bertalan[13], Florence Levenez[1], Nicolas Pons[1], Simon Rasmussen[13], Shinichi Sunagawa[6], Julien Tap[1,6], Sebastian Tims[14], Erwin G. Zoetendal[14], Søren Brunak[13], Karine Clément[15,16,17], Joël Doré[1,18], Michiel Kleerebezem[14], Karsten Kristiansen[19], Pierre Renault[18], Thomas Sicheritz-Ponten[13], Willem M. de Vos[14,20], Jean-Daniel Zucker[15,16,21], Jeroen Raes[4,5], Torben Hansen[2,22], MetaHIT consortium†, Peer Bork[6], Jun Wang[3,19,23,24,25], S. Dusko Ehrlich[1] & Oluf Pedersen[2,26,27,28]

We are facing a global metabolic health crisis provoked by an obesity epidemic. Here we report the human gut microbial composition in a population sample of 123 non-obese and 169 obese Danish individuals. We find two groups of individuals that differ by the number of gut microbial genes and thus gut bacterial richness. They contain known and previously unknown bacterial species at different proportions; individuals with a low bacterial richness (23% of the population) are characterized by more marked overall adiposity, insulin resistance and dyslipidaemia and a more pronounced inflammatory phenotype when compared with high bacterial richness individuals. The obese individuals among the lower bacterial richness group also gain more weight over time. Only a few bacterial species are sufficient to distinguish between individuals with high and low bacterial richness, and even between lean and obese participants. Our classifications based on variation in the gut microbiome identify subsets of individuals in the general white adult population who may be at increased risk of progressing to adiposity-associated co-morbidities.

Chatelier EL, et al. Nature 2013;500:541-6

Importantly, a main excretion route of POPs

- **Fecal excretion: main (90%)**

  1.Biliary excretion

  2.Passive exudation across large intestine

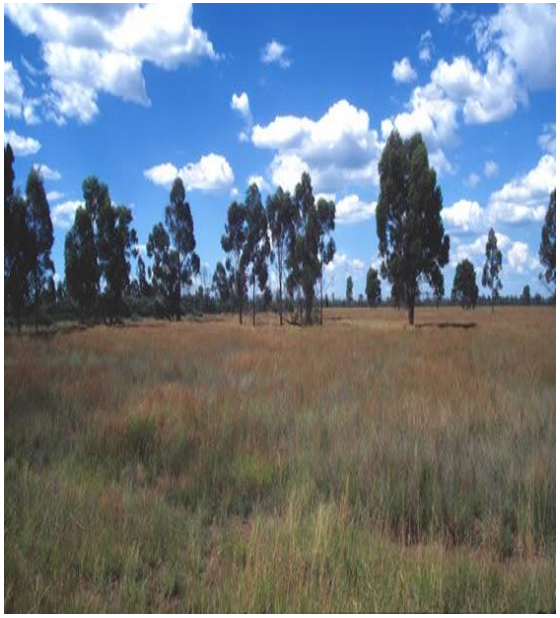
- **Urinary excretion: Minor (10%)**

Therefore, POPs have continuously contaminated our colon

Then, chemical contamination of soil dramatically changes microbiota distribution as a part of self-purification

Clean soil:

Oil degrading Microorganisms <0.1%



Oil contamination

Chemical contaminated soil:

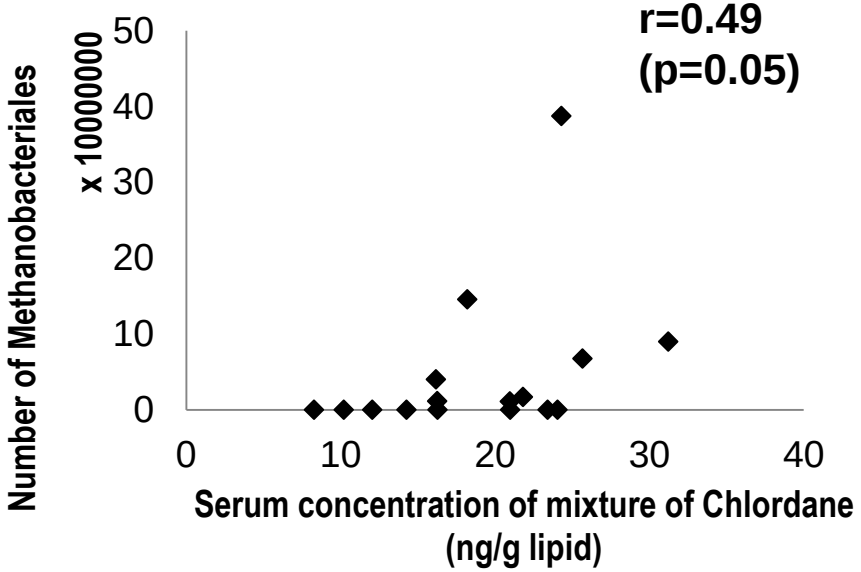
Oil degrading microorganisms ↑↑

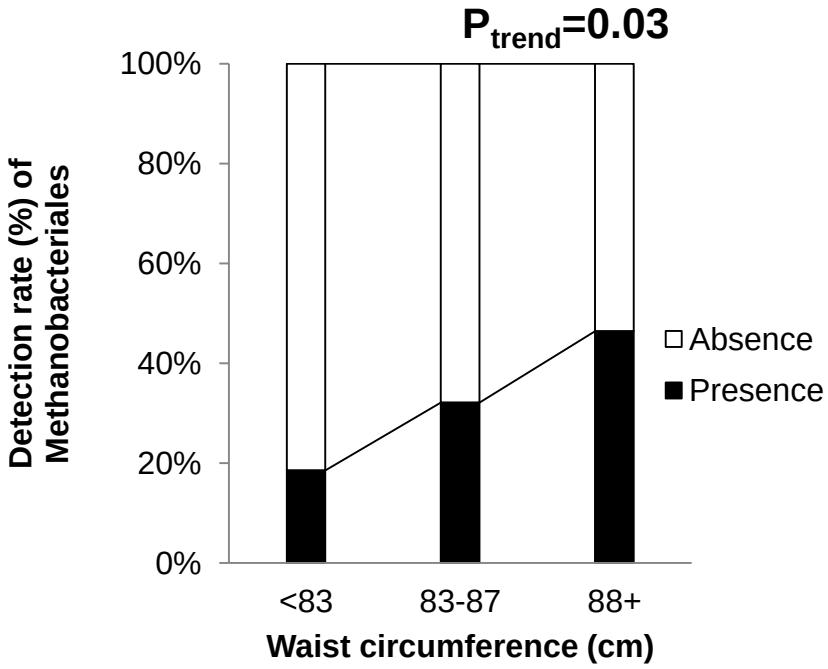
If so, it is sensible to hypothesize that similar situations would happen in our gut as well, isn't it?

POPs can affect gut microbiota

Subjects: 16 Korean women

Results: POPs were correlated with numbers of methanogenic archaea in feces

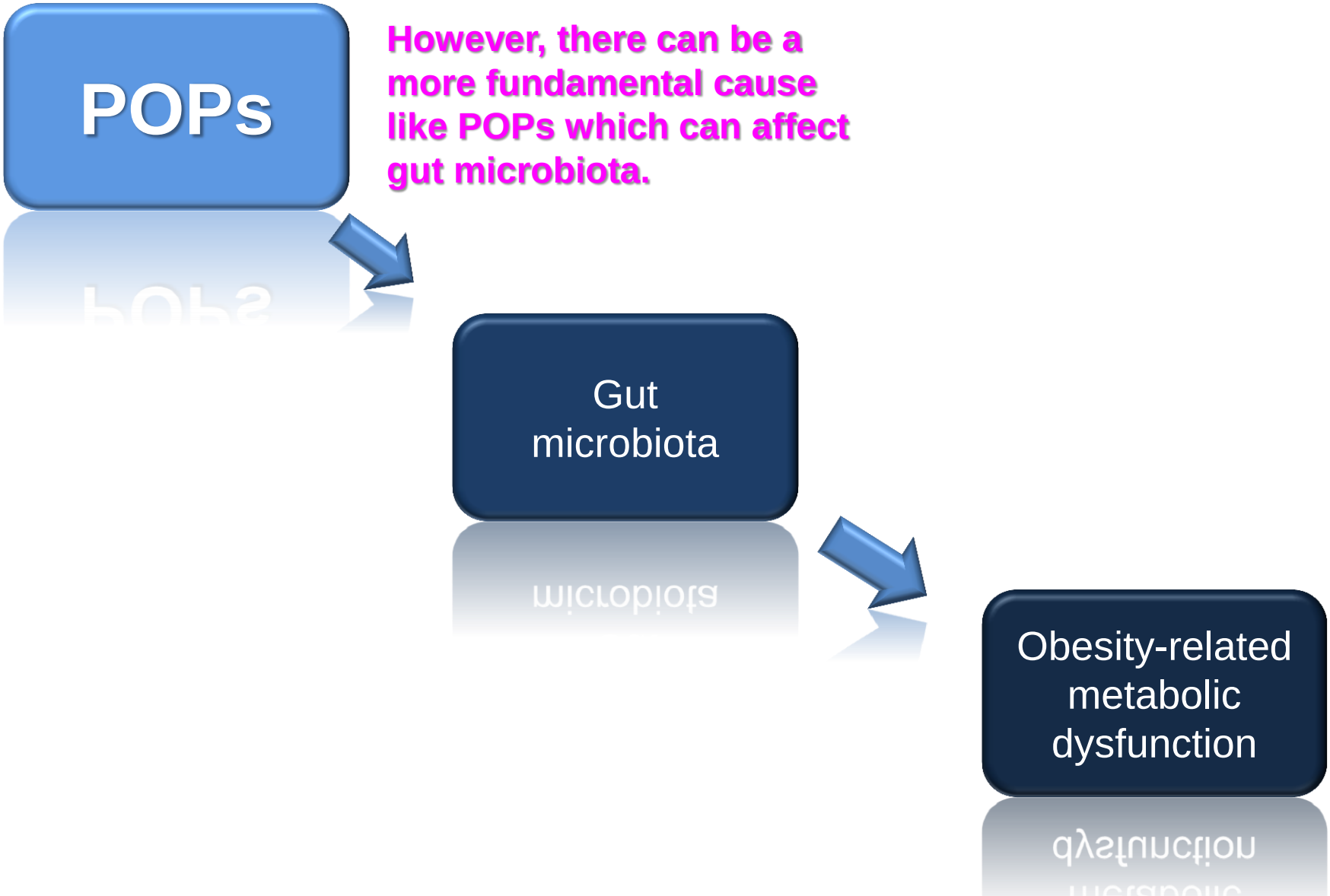




Importantly, methanogenic archaea is POPs-degrading microogranism and also related to obesity as well

Lee HS, et al. PLoS One 2011; e27773

Researchers are just looking at gut microbiota

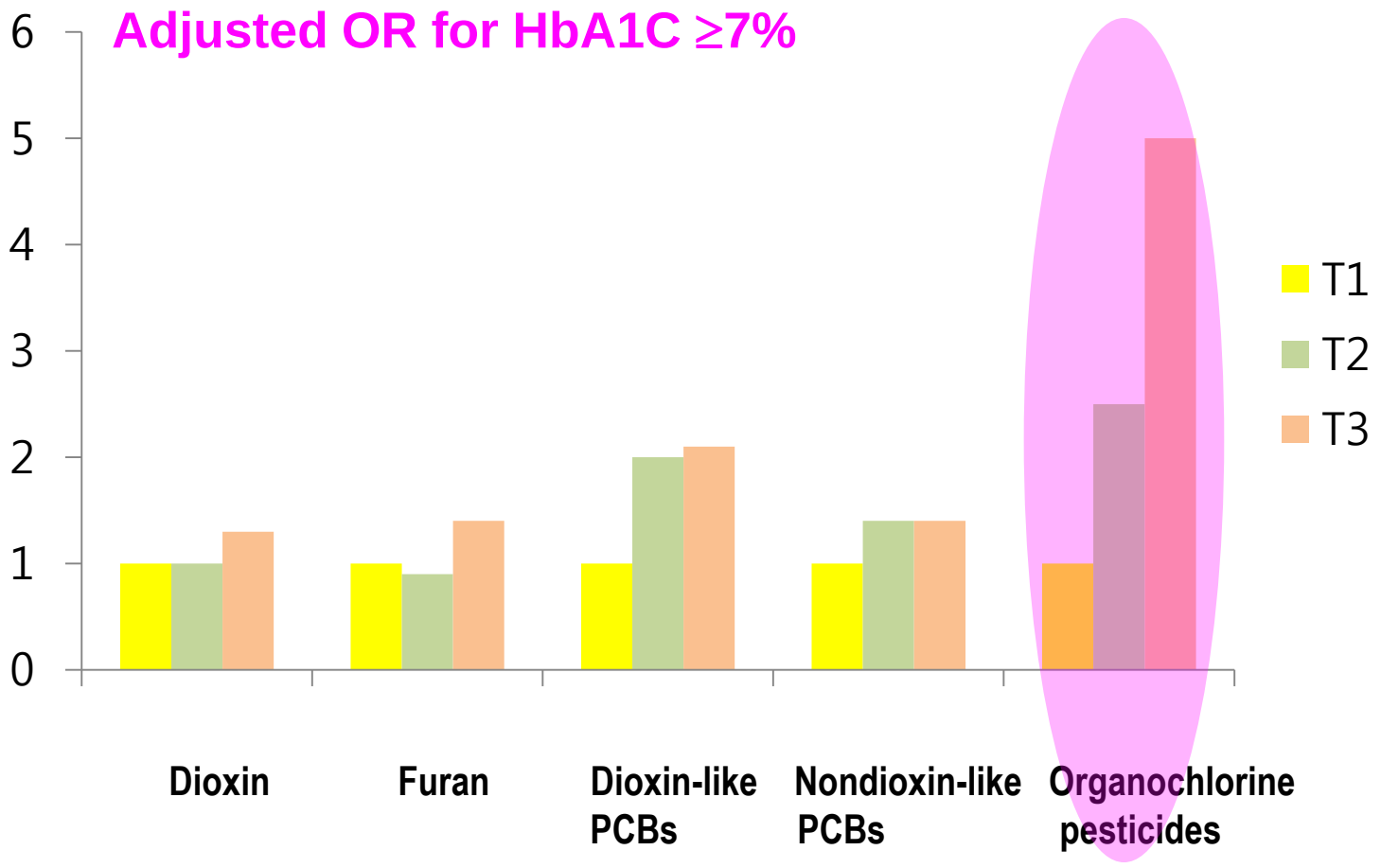


What we exactly don't know

3. Role of POPs in developing complications in patients with T2D

POPs and poor glycemic control in diabetic patients

- Cross-sectional study
- 246 diabetic patients aged ≥ 40 (NHANES dataset)



Lee DH, et al. Diabetes 2008;57:3108-11

POPs and CVD in general population

Cross-sectional study

Association between Serum Concentrations of Persistent Organic Pollutants and Self-Reported Cardiovascular Disease Prevalence: Results from the National Health and Nutrition Examination Survey, 1999–2002

Myung-Hwa Ha,[1,2] Duk-Hee Lee,[1] and David R. Jacobs Jr.[3,4]

[1]Department of Preventive Medicine and Health Promotion Research Center, School of Medicine, Kyungpook National University, Daegu, Korea; [2]National Cancer Center, Ilsan, Korea; [3]Division of Epidemiology, School of Public Health, University of Minnesota, Minnesota, USA; [4]Department of Nutrition, University of Oslo, Oslo, Norway

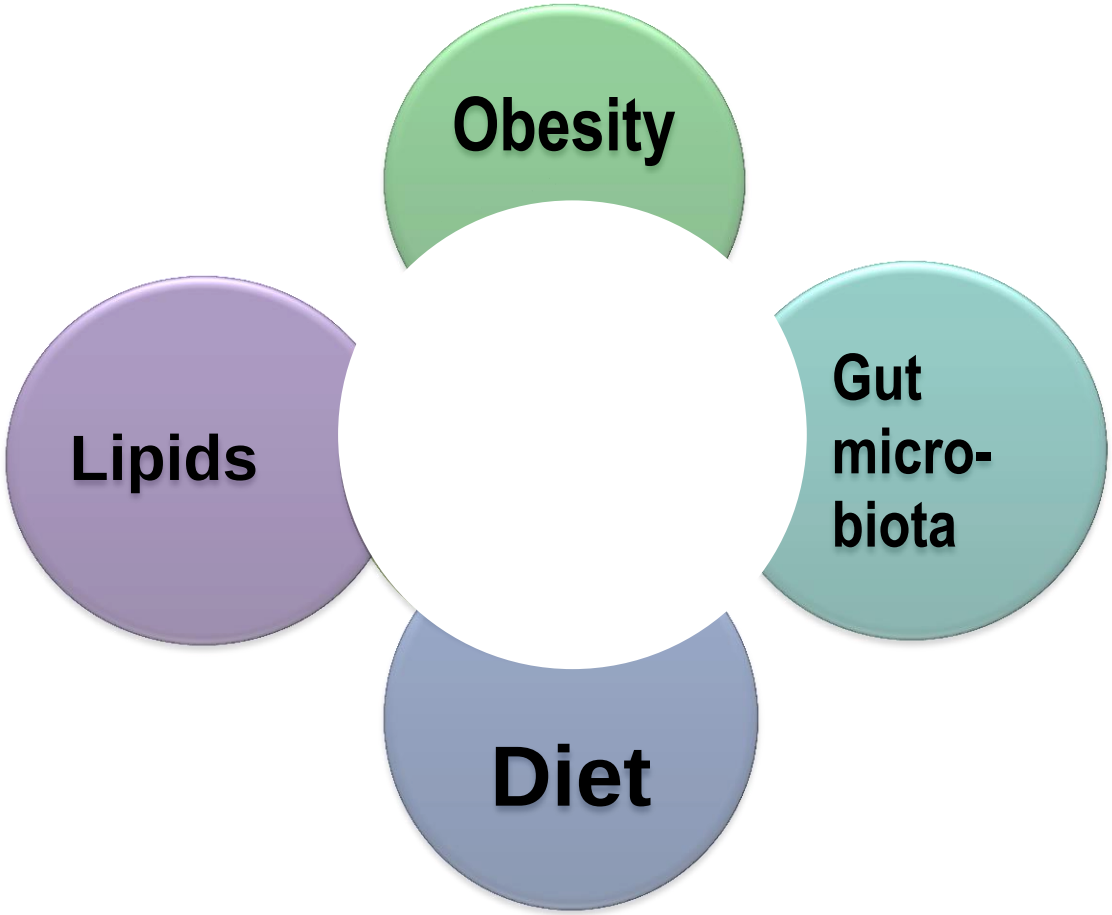
Prospective cohort study

Background exposure to persistent organic pollutants predicts stroke in the elderly

Duk-Hee Lee [a], P. Monica Lind [b], David R. Jacobs Jr. [c,d], Samira Salihovic [e], Bert van Bavel [e], Lars Lind [f,*]

[a] *Department of Preventative Medicine, School of Medicine, Kyungpook National University, Daegu, Republic of Korea*
[b] *Occupational and Environmental Medicine, Uppsala University, Uppsala, Sweden*
[c] *Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN, United States*
[d] *Department of Nutrition, University of Oslo, Oslo, Norway*
[e] *MTM Research Center, School of Science and Technology, Örebro University, Örebro, Sweden*
[f] *Department of Medicine, Uppsala University, Uppsala, Sweden*

Therefore, it is highly plausible that diabetic patients with high POPs levels develop more complications in the future



How valid research findings without consideration of POPs??

A half-truth is often a great lie

-Benjamin Franklin

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Monica Lind
Uppsala University

CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People.™

Thank CDC for free releasing of the precious datasets to foreigners like me!!!

Thank you for your attention !!