The metabolic syndrome (MetS) - dysmitochondrial syndrome and mitochondrial disruptors

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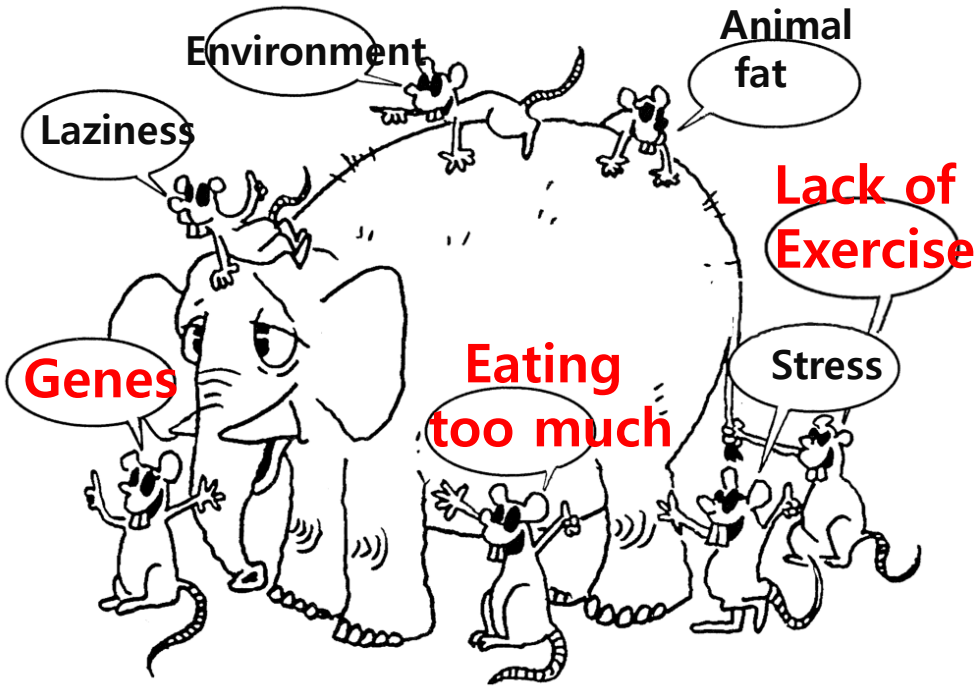
2017 MiP School, July 23-27, Obergurgl, Tyrol, Austria

Insulin Resistance

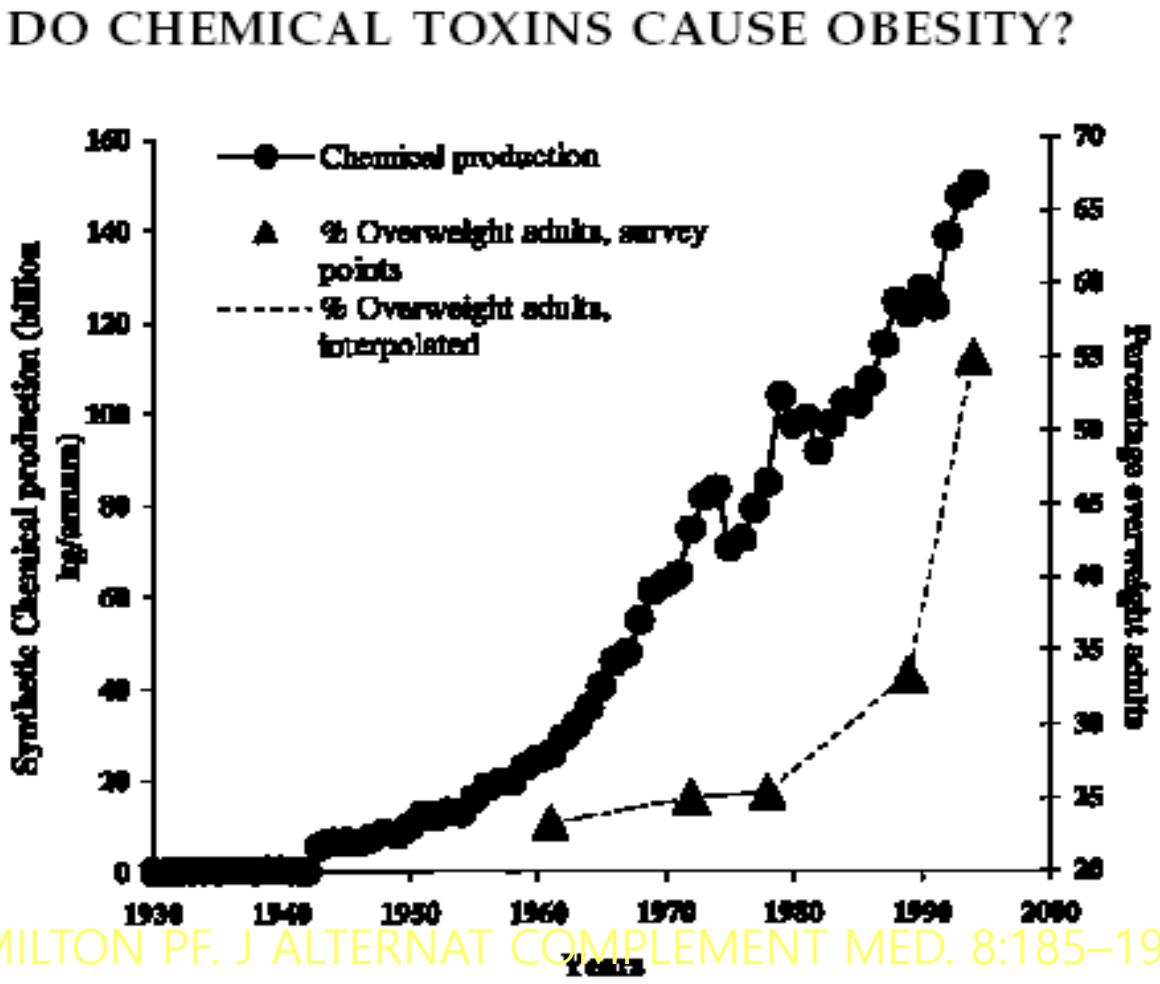
- **IR = high blood insulin level with normal or high blood glucose level**
- **IR is a characteristic of type 2 diabetes, as well as hypertension, abdominal obesity, hypertriglyceridemia and many others, such as increased serum inflammatory markers.**
- **These conditions and many other clinical states frequently occur in one person.**
- **This cluster state (of IR) is called metabolic syndrome.**

Causes of Metabolic Syndrome?

1. Unknown, but lack of exercise and overeating are usually blamed.
2. Genes are considered as major underlying cause.



Baillie-Hamilton PF was the first to propose chemical toxins might be culprits. J Altern Compl Med. 2002



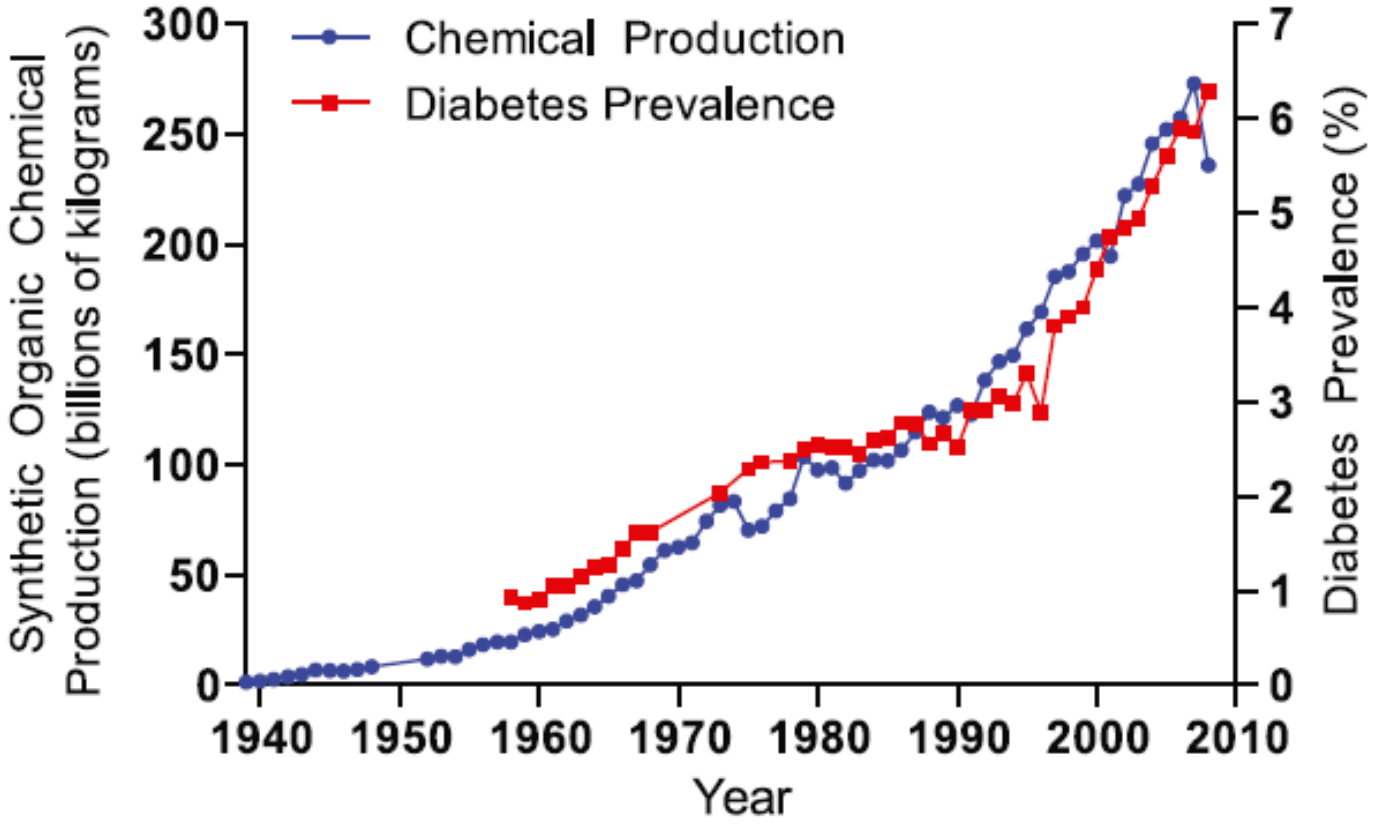
BAILLIE-HAMILTON PF. J ALTERNAT COMPLEMENT MED. 8:185–192, 2002

PERSPECTIVES IN DIABETES

The Paradox of Progress: Environmental Disruption of Metabolism and the Diabetes Epidemic

Brian A. Neel[1] and Robert M. Sargis[2]

Diabetes 60: 1838-1848, July 2011

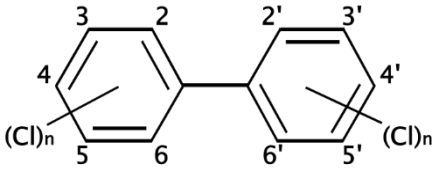


POPs (Persistent Organic Pollutants) were the key suspects

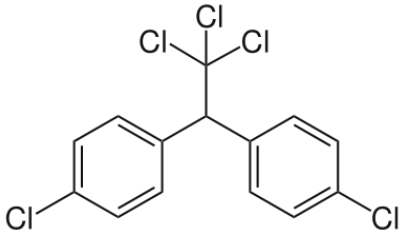
Organic compounds that are resistant to environmental degradation.
(Lipophilic, persistent, bio-accumulated through food chain)
Banned by a UN treaty – Stockholm Convention

Compounds:

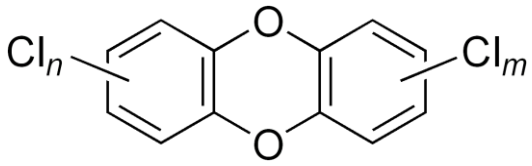
- **organochlorine pesticides (OCP)**
- **polychlorinated biphenyls (PCBs) – dioxin like PCBs and non-dioxin like PCBs**
- **polychlorinated dibenzo-p-dioxins (PCDDs): TCDD**
- **polychlorinated dibenzofurans (PCDFs)**



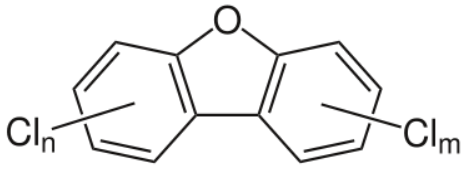
PCBs



DDT

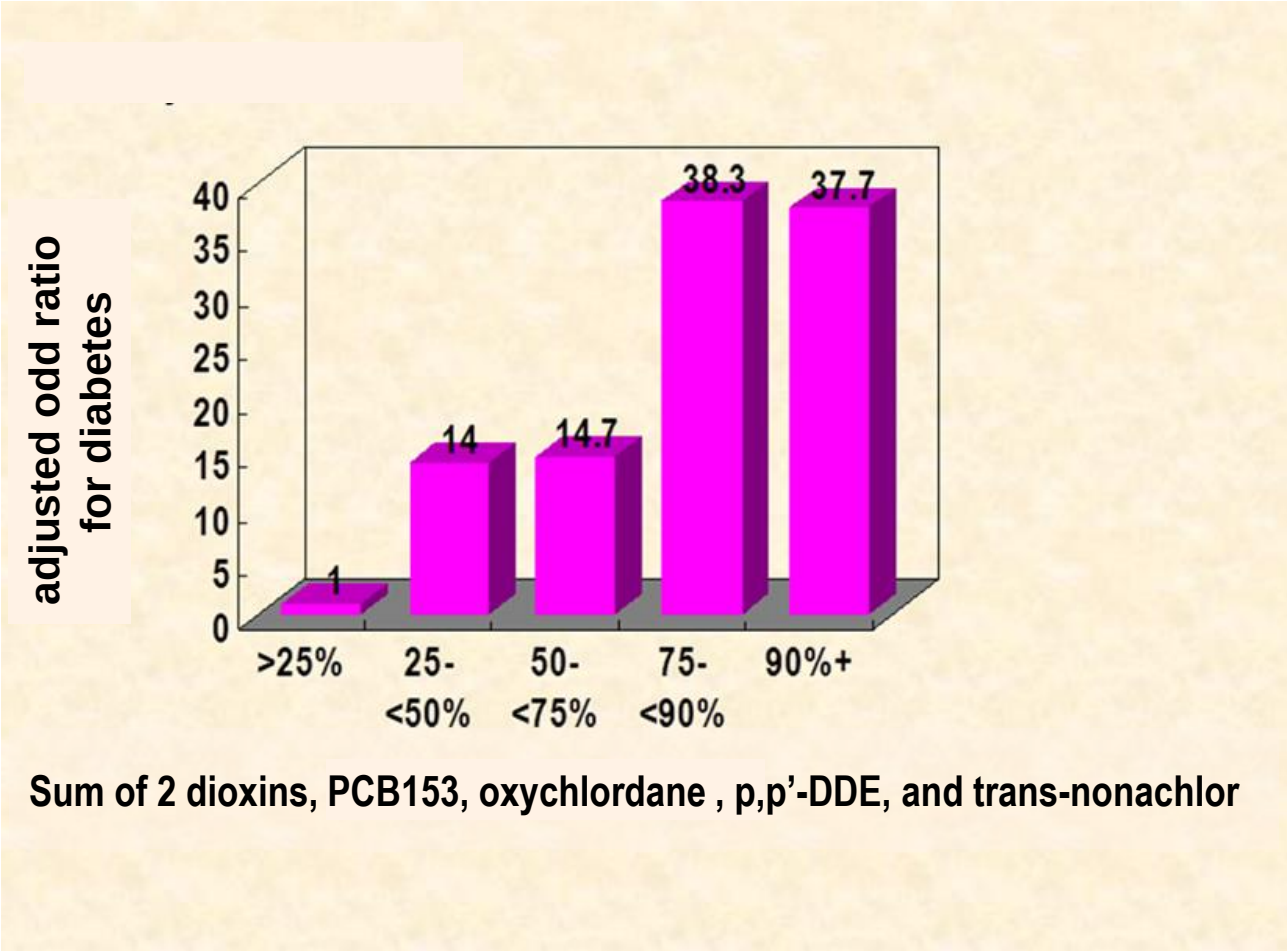


PCDDs



PCDFs

US CDC measured about 50 POPs as a part of NHANES, in a random sample of US population 1999-2002



Lee DH, Lee IK, Jin SH, Steffes M, Jacobs DR Jr. Diabetes Care. 2007

The National Toxicology Program (NTP) at the National Institute of Environmental Health Sciences (NIEHS)

a state-of-the-science workshop, January 2011.

(Taylor KW, et al. Environ Health Perspect. 2013)

- Causal links between exposure and manifestation of disease are substantiated by experimental animal models and are consistent with correlative epidemiological data in humans.
- The evidence was strongest in obesity and diabetes.
- However there were difficulties in drawing broad conclusions, and we must be cautious about inferring causality.

Cause-effect relationship

Chemicals induced insulin resistance in the experimental animals

- Atrazine (with high fat diet) (Lim S et al, PLoS One, 1999)
- POPs mixture (Contaminated Atlantic salmon oil) (Ruzzin et al. EHP, 2010)
- Bisphenol A

- Wei et al. Endocrinology, 2011 - Perinatal exposure to bisphenol A at reference dose predisposes offspring to metabolic syndrome in adult rats on a high-fat diet

-Ding S et al. J Endocrinol. 2014. - High-fat diet aggravates glucose homeostasis caused by chronic exposure to bisphenol A.

IR and mitochondrion

Mitochondrial dysfunction and IR

Lee HK et al.
Diabetes Res Clin Pract 1998

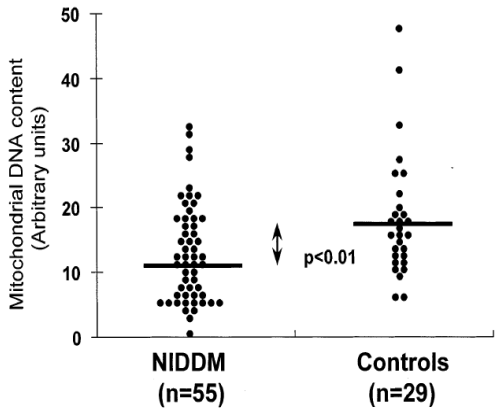


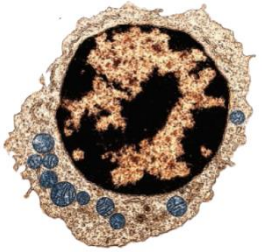
Fig. 1. Distribution of densitometric units of the bands in slot blots, in controls, and in patients with NIDDM.

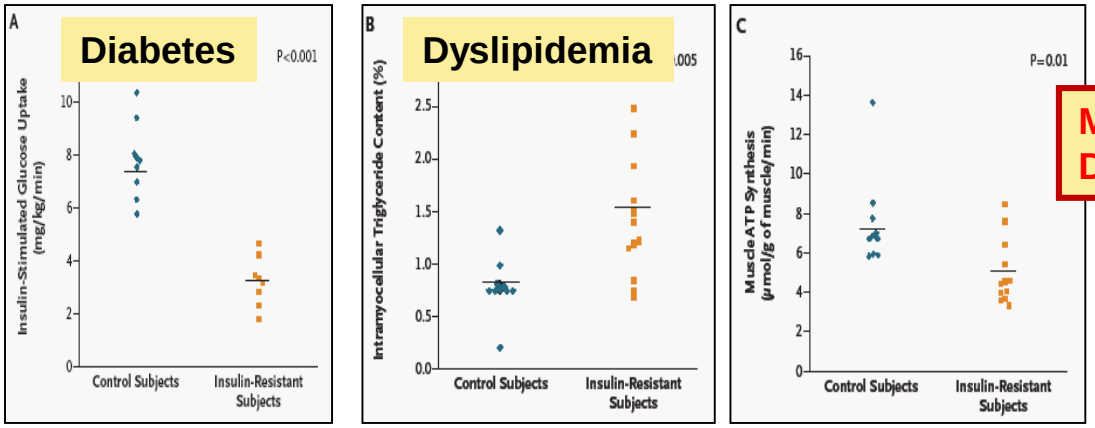
Mitochondrial Dysfunction in the Elderly: Possible Role in Insulin Resistance

Science 300, 1140-1142, 2003

Kitt Falk Petersen,[1] Douglas Befroy,[1,7] Sylvie Dufour,[1,7]
James Dziura,[1] Charlotte Ariyan,[3] Douglas L. Rothman,[4]
Loretta DiPietro,[5,6] Gary W. Cline,[1] Gerald I. Shulman[1,2,7]*

Insulin resistance is a major factor in the pathogenesis of type 2 diabetes in the elderly. To investigate how insulin resistance arises, we studied healthy, lean, elderly and young participants matched for lean body mass and fat mass. Elderly study participants were markedly insulin-resistant as compared with young controls, and this resistance was attributable to reduced insulin-stimulated muscle glucose metabolism. These changes were associated with increased fat accumulation in muscle and liver tissue assessed by ^{1}H nuclear magnetic resonance (NMR) spectroscopy, and with a ~40% reduction in mitochondrial oxidative and phosphorylation activity, as assessed by in vivo $^{13}C/^{31}P$ NMR spectroscopy. These data support the hypothesis that an age-associated decline in mitochondrial function contributes to insulin resistance in the elderly.





Mitochondrial Dysfunction !!!!

Petersen et al (2004)
NEJM 350; 664-671

Insulin stimulates respiration in pigeon muscle

Krebs HA, Eggleston LV. Biochem J, 1938

Table VIII. *Effect of varying quantities of insulin on the O_2 uptake of pigeon breast muscle*

3 g. minced muscle suspended in 15 ml. 0·1 *M* phosphate buffer, *p*H 6·8 + 21 ml. boiled sheep's heart extract (see Table IX) + 2 ml. 0·2 *M* Na_3-citrate.

	Quantity of insulin added (units per ml.)			
	0	0·01	0·1	1·0
µl. O_2 absorbed by 3 ml. suspension after:				
20 min.	333	340	347	370
50 min.	1500	1605	1625	1730
135 min.	3265	3495	3650	3975
270 min.	3845	4155	4540	5785
µl. O_2 absorbed due to insulin		+310	+695	+1940

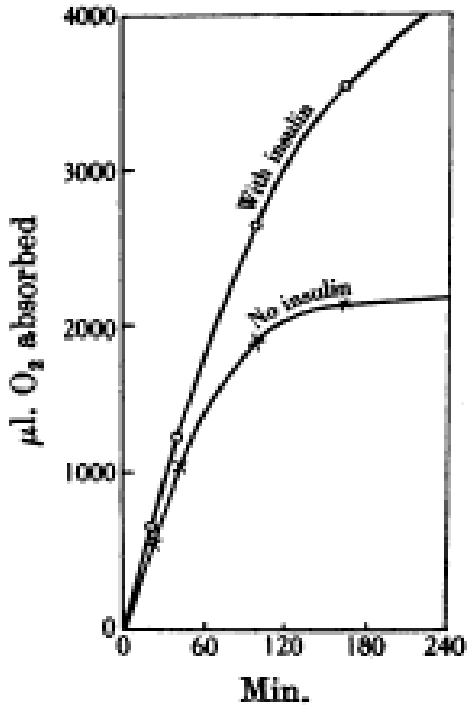


Fig. 2. Effect of insulin on respiration in pigeon muscle (in the presence of boiled muscle extract and of citrate). For data see Table VII, columns 7 and 8.

Do EDCs cause mitochondrial dysfunction?

Difficulties in proving cause-effect relationship (chemicals as cause of MetS)

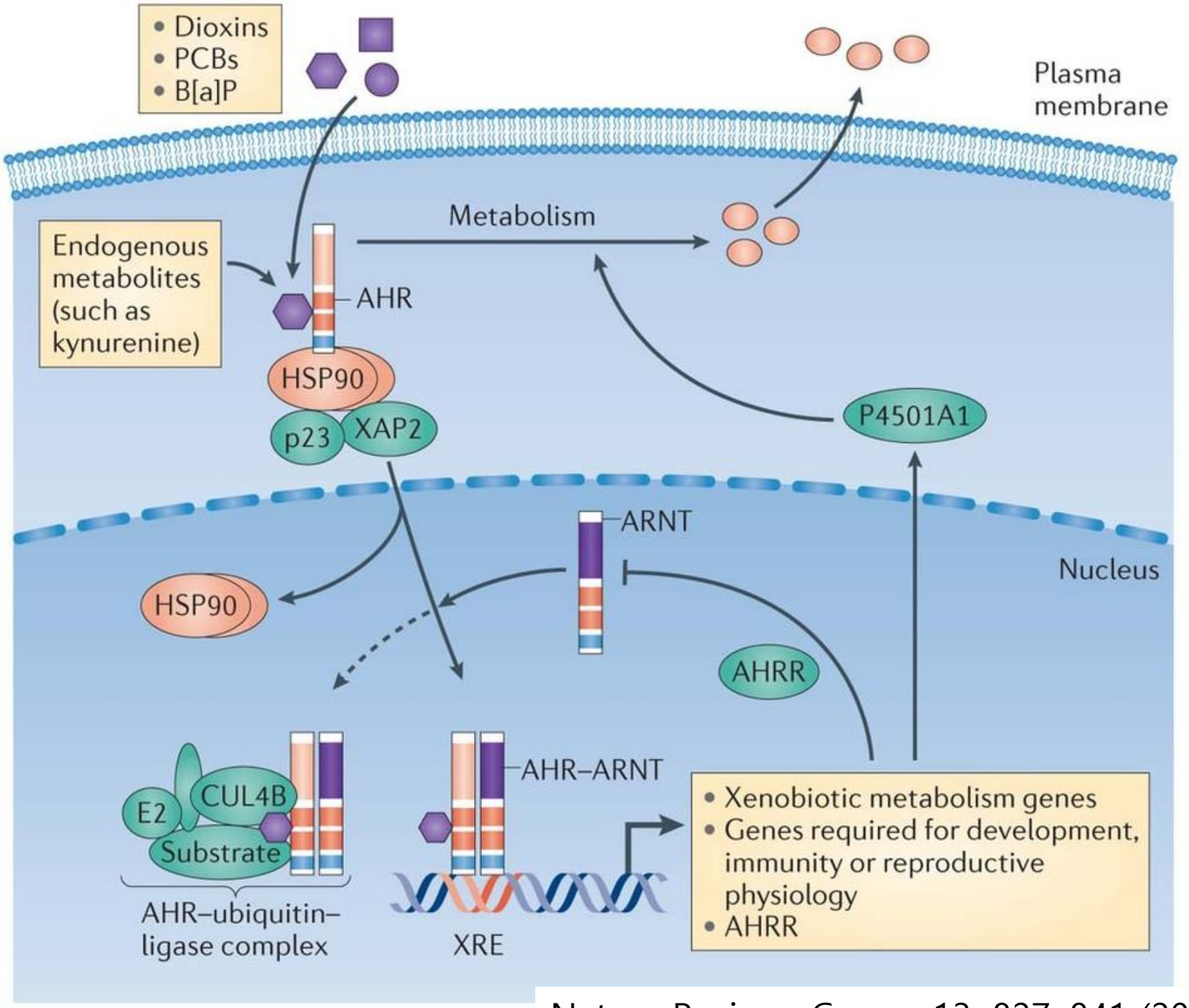
- Chemicals are infinitely diverse and present in mixture
- Some chemicals are known toxic (like POPs), but some are protective, and many are unknown
- Concentrations and compositions of chemicals vary over time
- Metabolic syndrome is a constellation of several disease states

Limitations of current method measuring POPs/EDCs

- High resolution gas chromatography coupled with high resolution mass spectroscopy (hrGC/hrMS).
- Too expensive, need experts and special facilities and reagents, and large amount of blood sample.
- Not all the toxins could be measured.

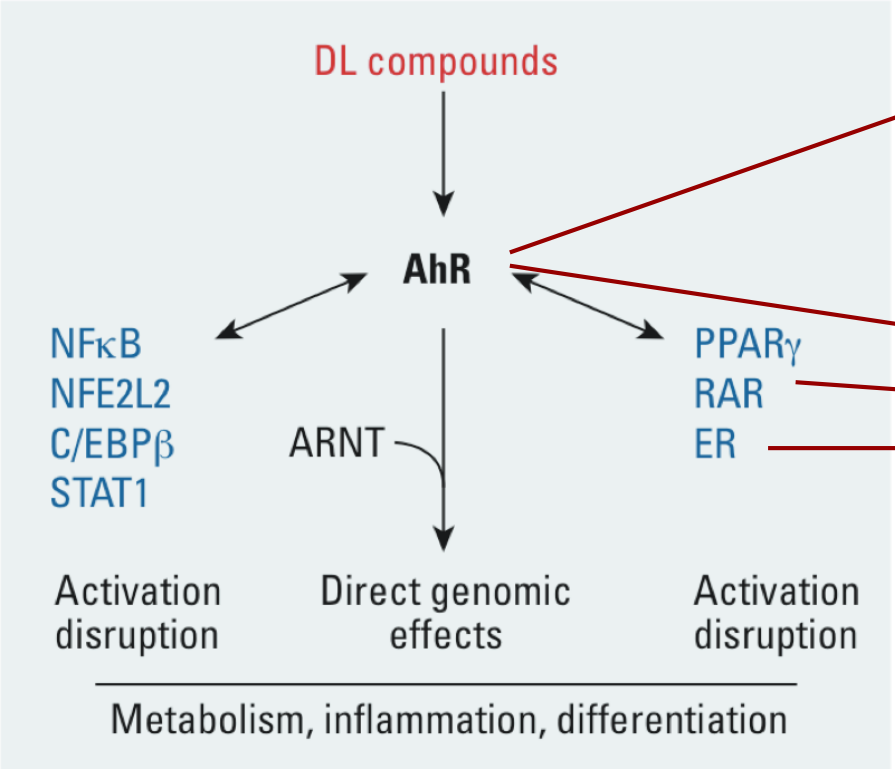
- **Bioassay!**

Chemical Activated LUciferase gene eXpression (CALUX) assay. Murk AJ et al. Fundam Appl Toxicol 33:149. 1996



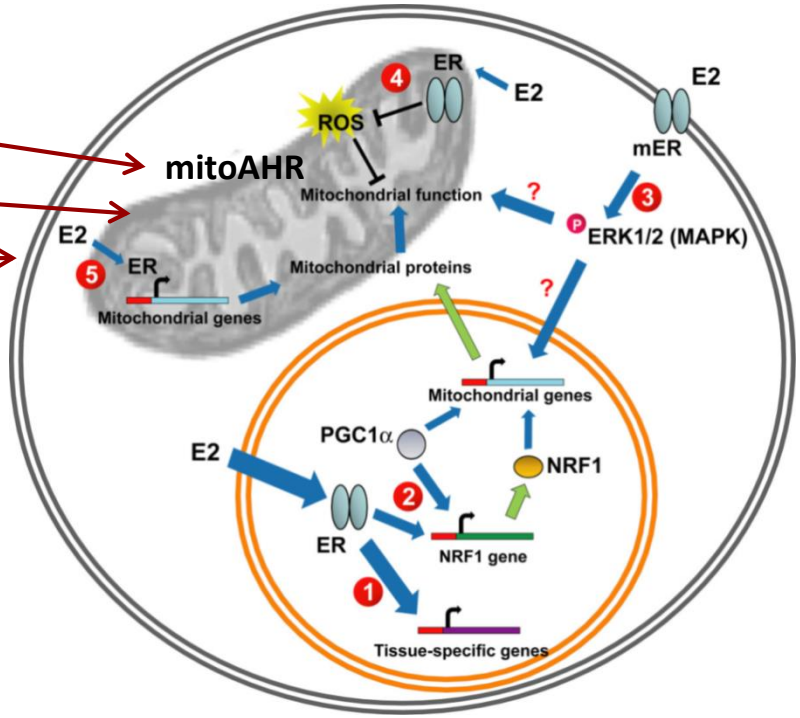
Nature Reviews Cancer 13, 827–841 (2013)

Dioxin like substances affect mitochondrial function acting through AHR (dioxin receptor)



ATP synthase 5α1

(Teppenden DM. ToxicolApplPharmacol 2011)

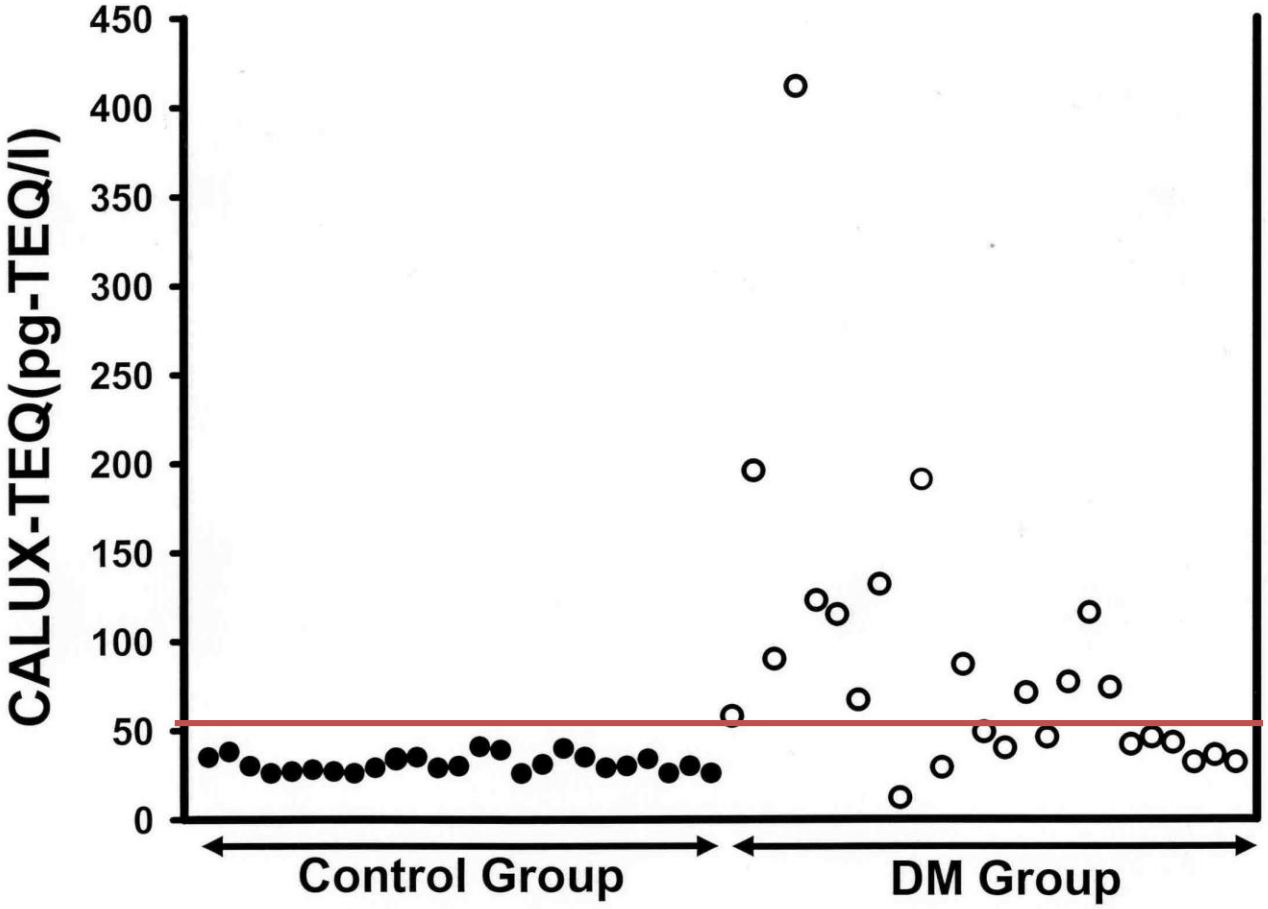


Park WH et al. Biofactors, 2013

Merrill ML et al. EHP, 2013

Velarde MC. Longev Healthspan, 2014

Hwang HJ et al. 2016



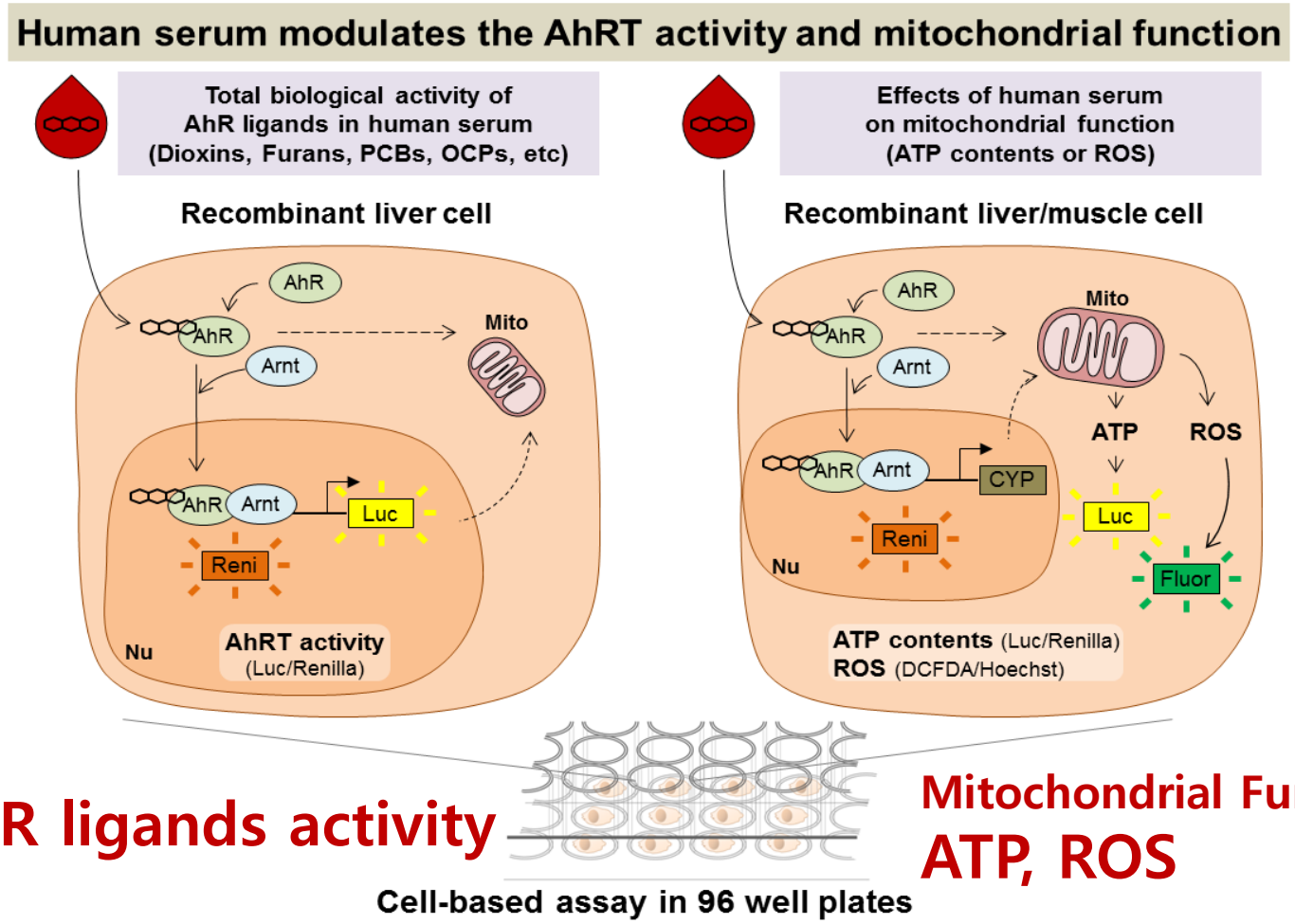
Dioxin like activity in plasma of patients with diabetes with CALUX assay. A collaboration with Prof. YY Shin, Ewha Women's University (unpublished ata)

Problems with the previous CALUX assay

- **Had solvent extraction process, thus needed very expensive reagents**
- **Required rather large amount of serum (>3 ml)**
- **Had isotope addition process to correct loss**
- **Measure lipid concentration and express in per gram of lipids**

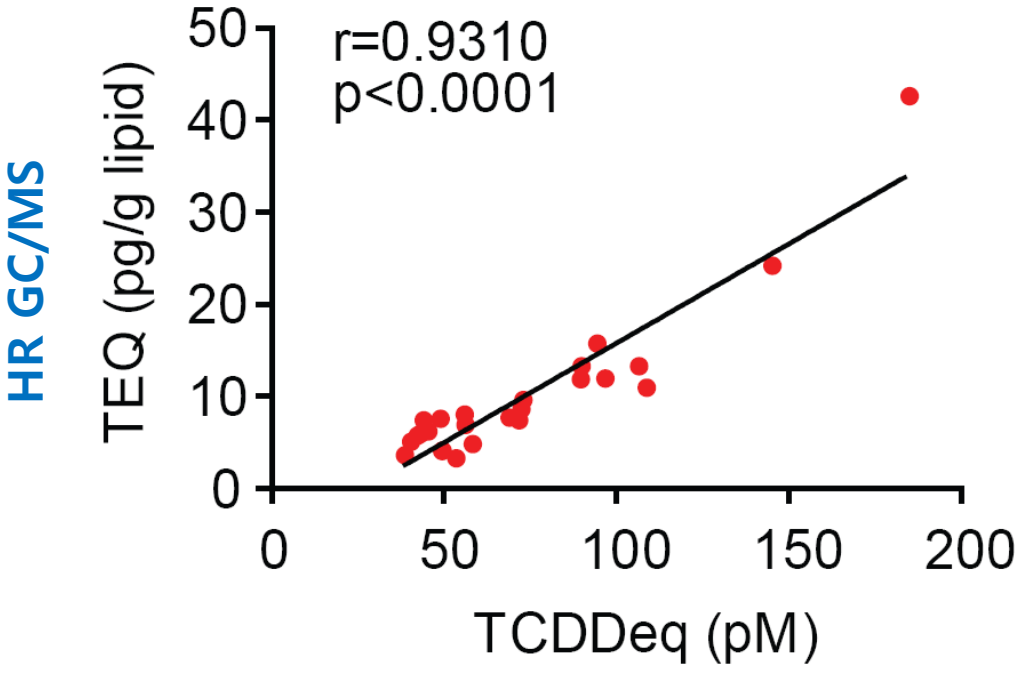
A novel cell based AhR ligands activated luciferase activation (CALA) assay (Biofactors, 2013)

2nd generation CALA (Cell-based AhR Ligand Activity) assay



Did not perform lipid normalization.

A linear correlation between serum TCDDeq (AhR binding activity) and TEQ by high-resolution GC/MS using same human sera (in collaboration with YS Chang, POSTech)



Cell-based AhR Ligand Activity (CALA) assay

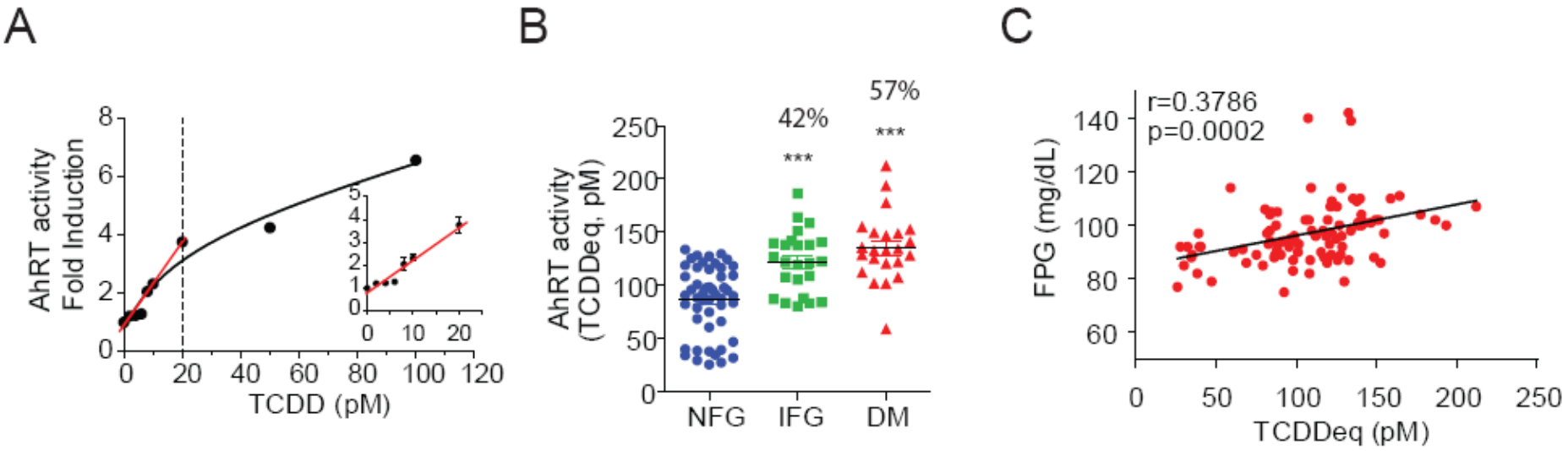
TEF/TEQ concept

- **Toxic equivalency factor** (**TEF**) expresses the toxicity of dioxins, furans, and PCBs in terms of the most toxic form of dioxin, 2,3,7,8 TCDD.
- **4 determinants for TEF**

  1) Structural similarity to polychlorinated dibenzo-p-dioxins or poly-chlorinated dibenzofurans
  2) Capacity to bind to the aryl-hydrocarbon receptor (AhR)
  3) Capacity to elicit AhR-mediated biochemical and toxic responses
  4) Persistence and accumulation in the food chain
- **Toxic equivalency** (TEQ) expresses the toxicity of the mixture of dioxins and dioxin-like compounds in a single number.

  $$TEQ = \Sigma[C_i] \times TEF_i$$
- World Health Organization scheme, represented as **WHO-TEQ$_{DFP}$** is universally accepted.

AhR binding in diabetic sera;
A *cross sectional study*



97 human subjects from Eulji General Hospital who came for health check-up

AhRT = AhR binding = TCDDeq in pM

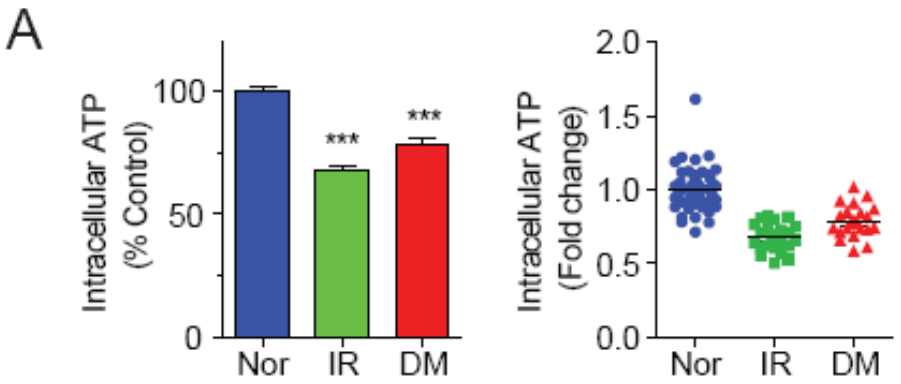
50 Healthy controls

24 IFG subjects

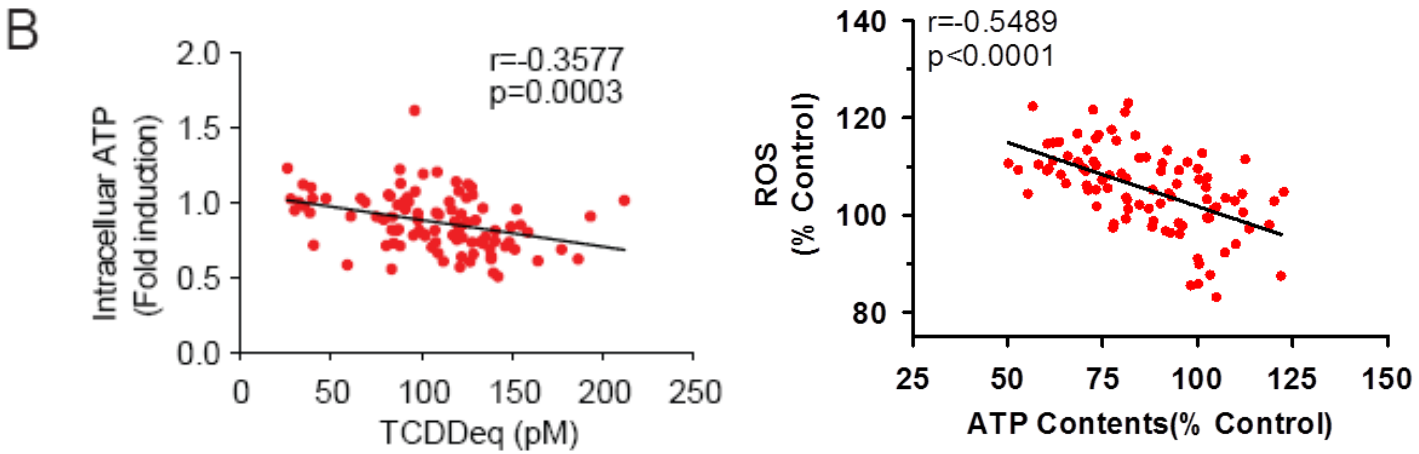
23 Diabetic subjects

Park WH et al. Biofactors 2013

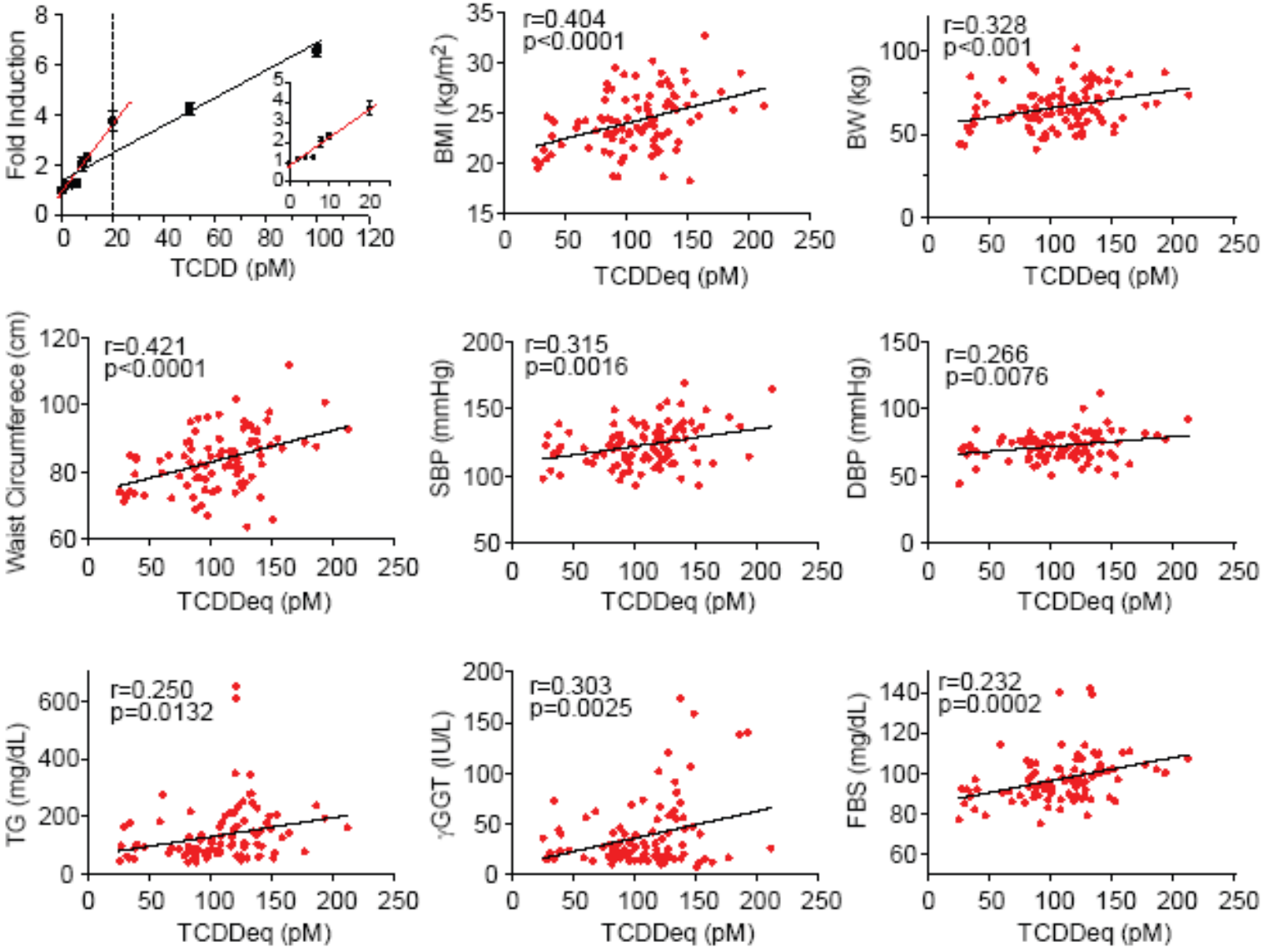
The effect of diabetic sera on the mitochondrial function of cultured C2C12 myoblast



96 subjects
50 Normal
24 IFG (IR)
22 Diabetic

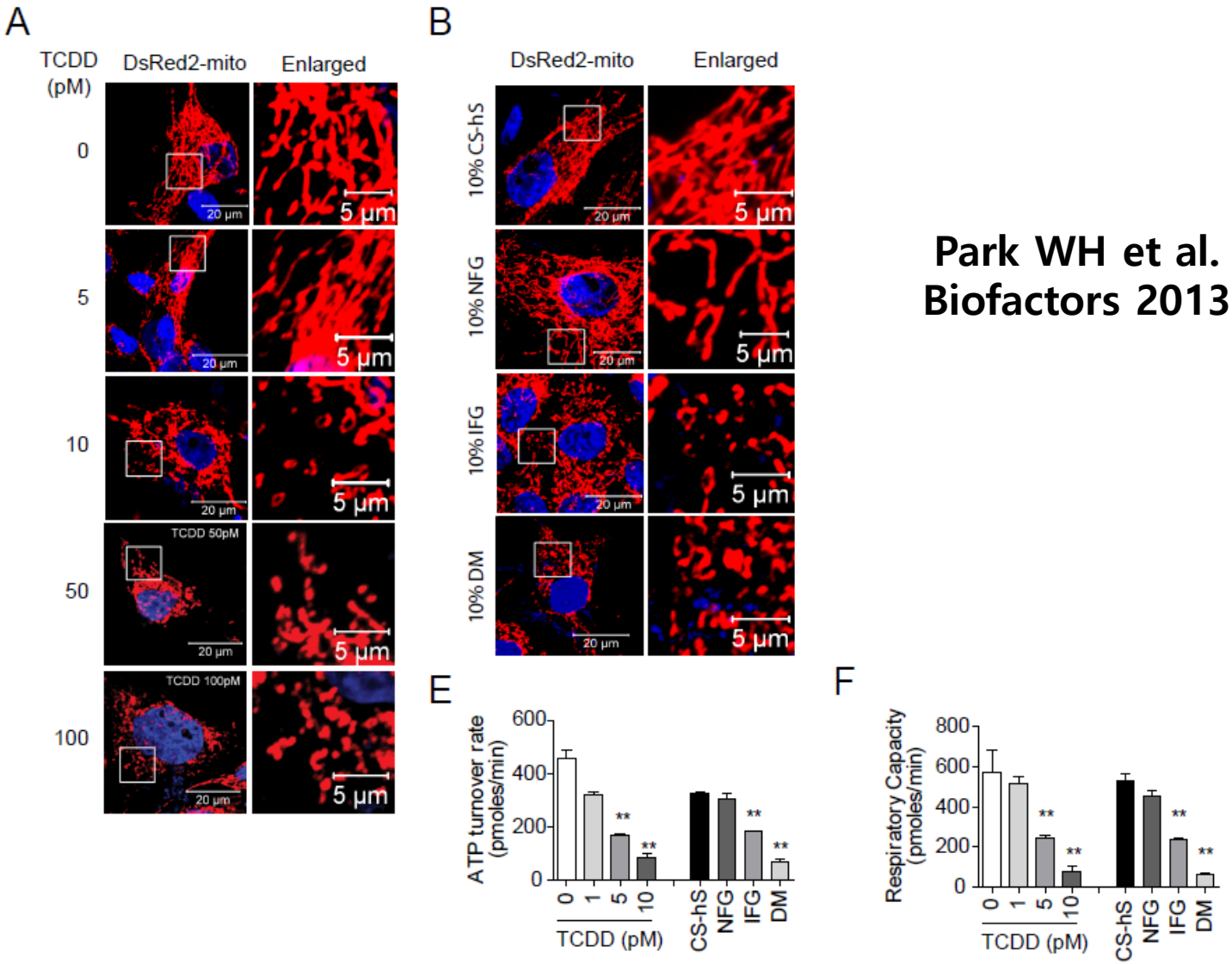


Park WH et al. Biofactors 2013



Park WH et al. Biofactors 2013

Mitochondrial deficits induced by TCDD or diabetic sera



Park WH et al.
Biofactors 2013

- Kim JT et al. **Serum arylhydrocarbon receptor transactivating activity** is elevated in type 2 diabetic patients with **diabetic nephropathy.**

  J Diabetes Investig 4(5):483–491, 2013

- Roh E et al. **Serum aryl hydrocarbon receptor ligand activity** is associated with **insulin resistance** and resulting type 2 diabetes.

  Acta Diabetol 52:489–495, 2015

Could AhR ligands level by CALA assay predict development of diabetes?

Yes, in 3 prospective studies including PIVUS study

(unpublsihed)

Conclusions

- AhR binding and ATP content predicted diabetes development.
- Cell based assays could play critical roles in defining metabolic syndrome and its component phenotypes.

The PIVUS study

Prospective **I**nvestigation of the **V**asculature in **U**ppsala **S**eniors

Investigated 1016 subjects

aged 70

from the general population

50% women

2001-2004

Primary investigators; Lind M & Lind L. University of Uppsala

PIVUS Investigated;

- 3 tests of endothelial function
- 3 tests of arterial compliance
- Echocardiography
- Blood pressure
- Carotid a. ultrasound
- HRV, BRS
- DXA
- Lung function
- ECG
- Medical/drug/social/ smoking/exercise history
- Dietary records -7days
- Blood sampling
- MRI angio+heart+fat (300)
- DNA sampling

Courtesy of Lind L.

And environmental contaminants

Metals

- Al
- Mn
- Cu
- Co
- Cd
- Pb
- Zn
- Mo
- Cr
- Ni
- Hg

POPs

- 16 different PCBs (74-209)
- OCDD (a dioxin)
- DDE (DDT metabolite)
- HCB (Pesticide)
- 3 Chlordanes (Pesticides)
- BDE-47 (flame retardant)
- 15 PFCs

Plastic chemicals

- Bisphenol A
- 10 Phthalate metabolites

Courtesy of Lind L.

Park WH et al. Relationships between serum-induced AhR bioactivity or mitochondrial inhibition and circulating polychlorinated biphenyls (PCBs)

Scientific Reports 2017

Variable	Beta (95% CI)	P-value
TEQ[a]	.0334 (.00751, .05929)	.012
TEQplana[b]	.03226 (.00693, .05759)	.013
TEQortho[c]	.06482 (.02013, .1095)	.0046
PCB74	.03835 (.00561, .07109)	.022
PCB 99	.0307 (.00186, .05954)	.037
PCB138	.0463 (.009, .08361)	.015
PCB153	.05342 (.01195, .09489)	.012
PCB170	.06535 (.01852, .11219)	.0064
PCB180	.05695 (.01086, .10304)	.016
PCB194	.0122 (-.00925, .03364)	.27
PCB206	.04009 (-.00284, .08302)	.068
PCB209	.01768 (-.02056, .05591)	.37
PCB105	.0253 (-.0027, .0533)	.077
PCB118	.03467 (.00324, .06609)	.031
PCB156	.05481 (.01272, .09691)	.011
PCB157	.06506 (.02589, .10424)	.0012
PCB189	.03213 (.00691, .05735)	.013
PCB126	.01982 (.00152, .03812)	.034
PCB169	.0293 (-.00689, .06549)	.11
OCDD	-.00936 (-.03685, .01813)	.50
BDE47	.03189 (.00791, .05587)	.0093

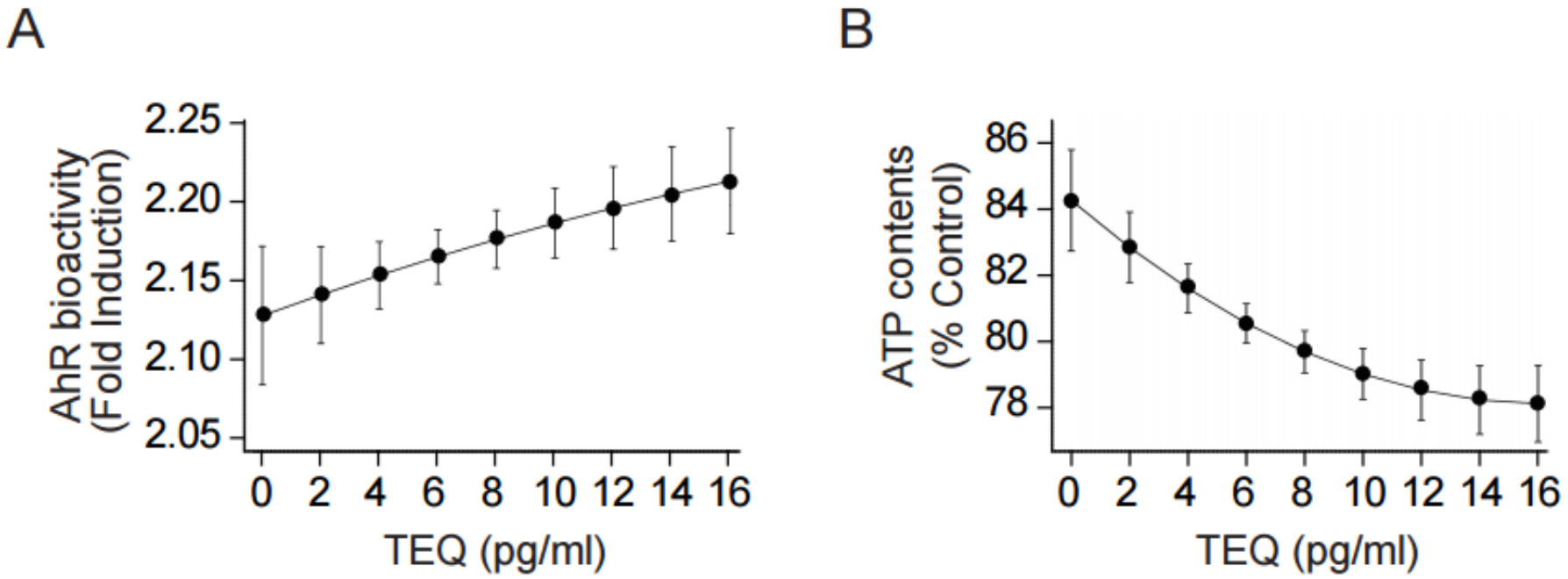
Relationships between AHR binding and calculated TEQ or individual POPs

are given as regression coefficient (Beta), 95%CI and p-value. Linear regression models were adjusted for sex, serum cholesterol and triglycerides. [a]Total TEQ; [b]TEQ for dioxin-like coplanar, non-ortho-substituted PCBs; [c]TEQ for dioxin-like mono-ortho-substituted PCBs; PCB, polychlorinated biphenyl; OCDD, octachlorinated dibenzo-p-dioxin; BDE, brominated diphenyl ether. (Unpublished data)

Variable	β coefficient (95% CI)	P value
TEQ_{total}	-1.84 (-2.71, -0.97)	0.000040*
TEQ_{planar}	-1.80 (-2.65, -0.94)	0.000042*
TEQ_{ortho}	-1.15 (-2.67, 0.37)	0.14
PCB74	-0.53 (-1.64, 0.59)	0.36
PCB 99	-0.055 (-1.035, .093)	0.91
PCB138	-.0167 (-1.44, 1.10)	0.80
PCB153	-0.73 (-2.14, 0.68)	0.31
PCB170	-1.15 (-2.74, 0.45)	0.16
PCB180	-0.98 (-2.55, 0.59)	0.22
PCB194	-0.27 (-0.99, 0.46)	0.47
PCB206	-1.51 (-2.97, -0.055)	0.042*
PCB209	-1.99 (-3.28, -0.70)	0.0025*
PCB105	-.023 (-1.18, 0.72)	0.63
PCB118	-0.33 (-1.40, 0.74)	0.55
PCB156	-0.98 (-2.41, 0.45)	0.18
PCB157	-0.71 (-2.05, 0.63)	0.30
PCB189	-0.66 (-1.51, 0.20)	0.13
PCB126	-1.22 (-1.84, -0.61)	0.00011*
PCB169	-1.857 (-3.08, -0.63)	0.0030*
OCDD	-0.48 (-1.41, 0.45)	0.31
BDE47	0.099 (-0.72, 0.92)	0.81

Relationships between ATP contents and calculated TEQ or individual POPs

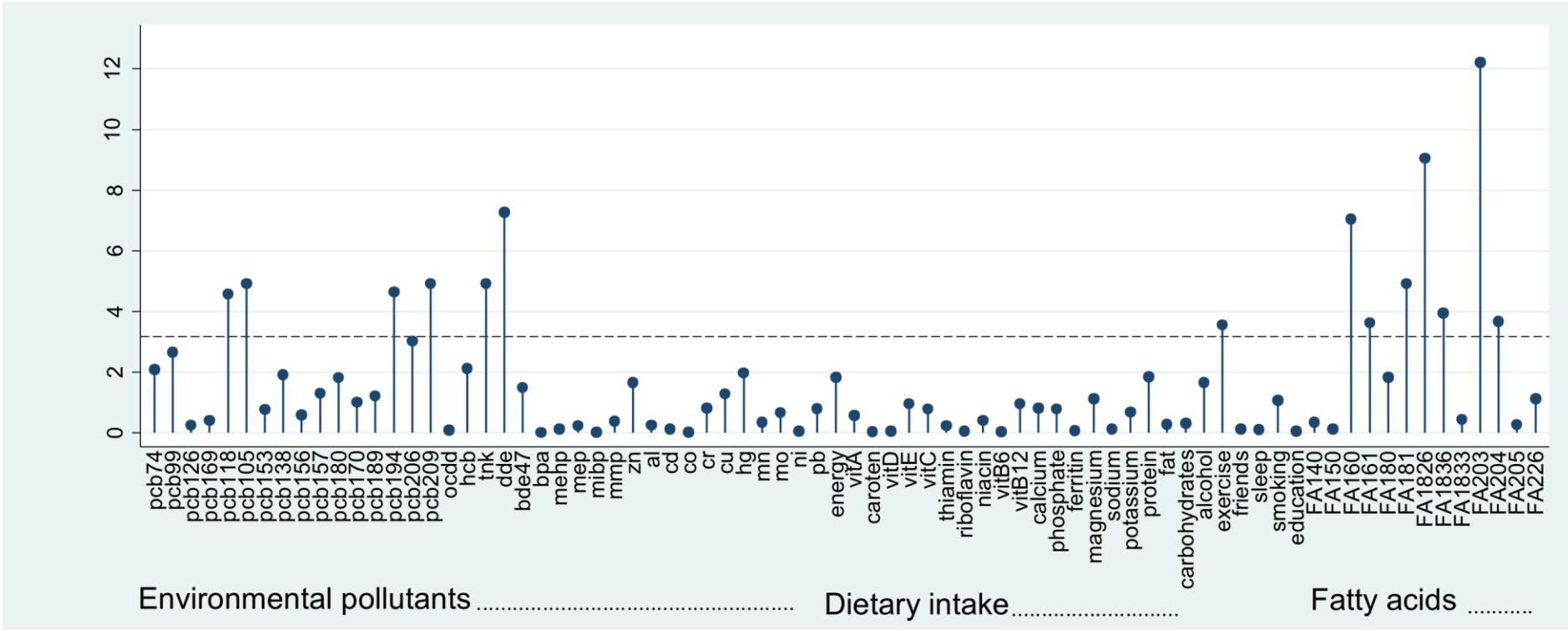
* indicates significant *P* value. CI, confidence interval; TEQ, toxic equivalence; PCB, polychlorinated biphenyl; TEQ_{planar}: TEQ for dioxin-like coplanar, non-ortho-substituted PCBs; TEQortho: TEQ for dioxin-like mono-ortho-substituted PCBs; OCDD, octachlorinated dibenzo-p-dioxin; BDE, brominated diphenyl ether



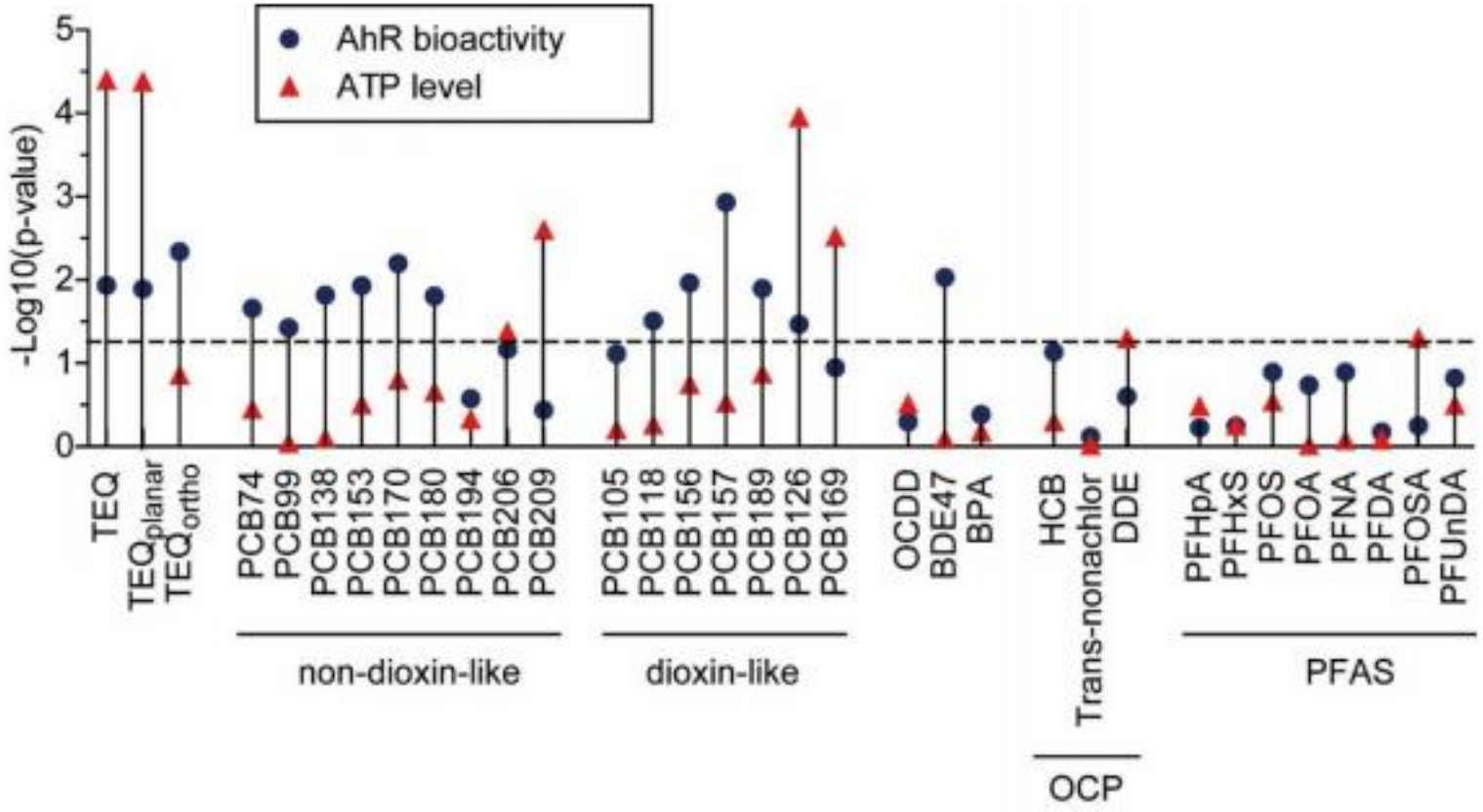
Relationship between TEQ, AHR binding and ATP contents

given as predicted margins from a regression model also including a quadratic term for TEQ, thus allowing for non-linear relationships. The relationship is adjusted for sex, serum cholesterol and triglycerides. PIVUS cohort.
(Revision submitted to Scientific Reports)

-Log10 p-value for 76 environmental factors vs Metabolic Syndrome



Lind et al Environ Int 2013


AhR bioactivity
ATP level
-Log10(p-value)
5
4
3
2
1
0
TEQ
TEQplanar
TEQortho
PCB74
PCB99
PCB138
PCB153
PCB170
PCB180
PCB194
PCB206
PCB209
PCB105
PCB118
PCB156
PCB157
PCB189
PCB126
PCB169
OCDD
BDE47
BPA
HCB
Trans-nonachlor
DDE
PFHpA
PFHxS
PFOS
PFOA
PFNA
PFDA
PFOSA
PFUnDA
non-dioxin-like
dioxin-like
OCP
PFAS

Summary

- Cell based assays of serum AhR binding showed highly significant statistical correlations with serum levels of several chemically measured individual PCB levels and TEQ levels derived.
- AhR binding was higher in metabolic syndrome and predicted diabetes (unpublished data)
- There are many problems to fix in cell based assays.

Conclusions

1. Insulin resistance, metabolic syndrome and type 2 diabetes mellitus are caused by environmental pollutants.
2. Serum of patients with diabetes and MetS are harmful to mitochondrion.
3. Cell based analysis of biological samples, especially on their effects on the mitochondrial function should be explored further as a diagnostic tests of metabolic diseases.
4. Metabolic syndrome could be defined as dysmitochondrial syndrome.

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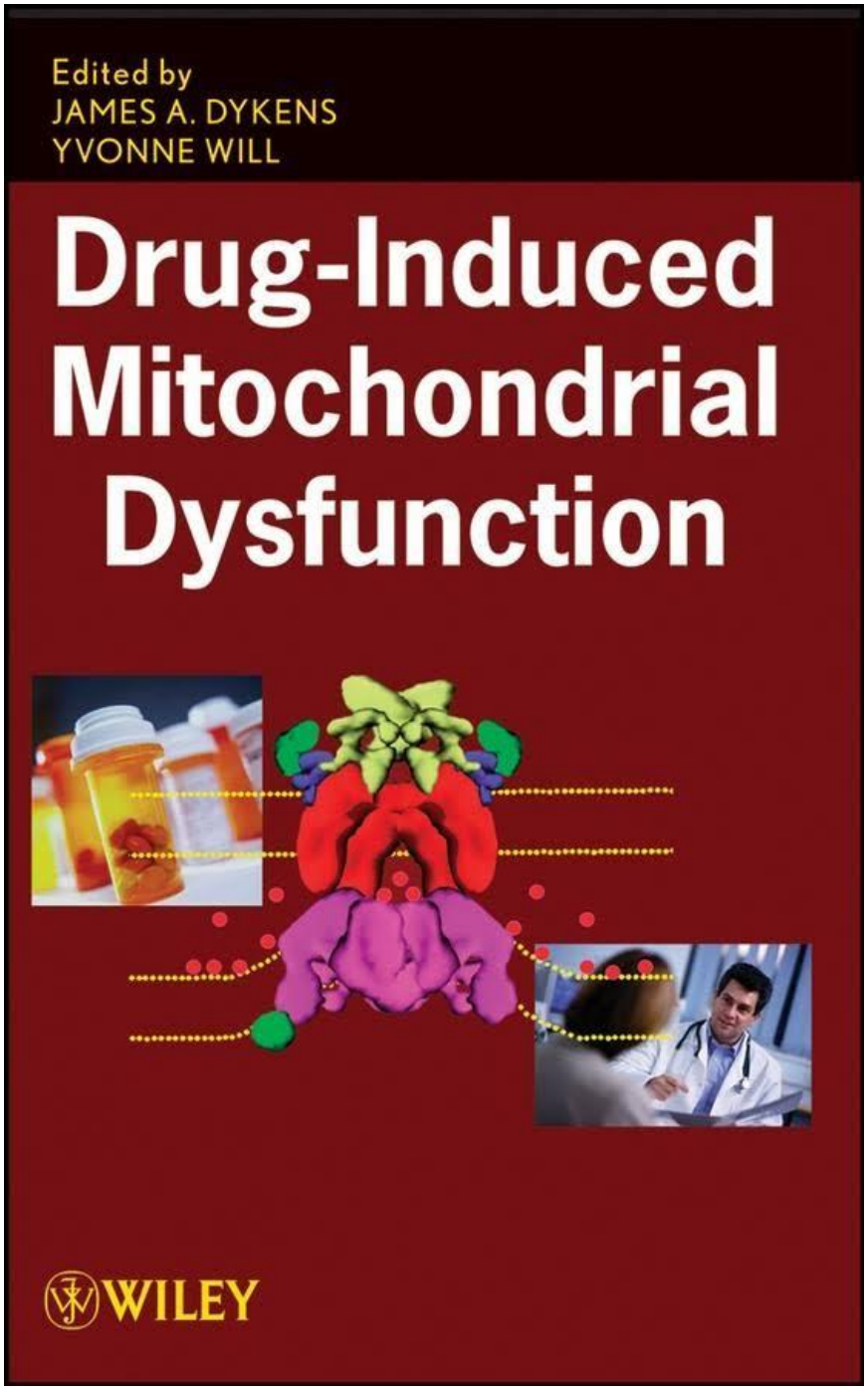
WHO/UNEP-report 2012:

Close to 800 chemicals are known or suspected to be capable of interfering with hormone receptors, hormone synthesis or hormone conversion.

http://www.who.int/ceh/publications/endocrine/en/



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