Part 1. What is Metabolic Syndrome?

Hong Kyu Lee, MD, PhD.

**Department of Internal Medicine,
Eulji University and Eulji Hospital,
Seoul, Korea**

Insulin resistance and diabetes

- Banting F and Best C (1921): found insulin and started to treat patients.

- Himsworth H (1938): two types of diabetes - insulin sensitive and insensitive.

- Yalow RS & Berson SA (1959): developed immunoassays of insulin and found its level is high in **obesity** and obese diabetes.

- Diabetes occurred when insulin resistance (IR) is not compensated by insulin deficiency → IR is primary cause.

- Hypertension, hyperlipidemia and other diseases (other than obesity) were found to have IR.

Diabetes and obesity

- More than two-thirds of adult-onset diabetics in the developed world are obese.

- Elliot P Joslin (1921): Diabetes is largely a penalty of obesity, and the greater the obesity, the more likely is Nature to enforce it.

- Kelly West (1978): Obesity is the most important environmental risk factor for diabetes. Indeed there is some evidence that its importance equals or exceeds the strong influence of diabetes related genetic factors"

Metabolic syndrome

- Clustering of phenotypes with IR in one person has been known for long time (Kylin E,1923, Himsworth H, 1938).

- Reaven G (1988) proposed to study this state as a disease, syndrome X.

- In 1998, WHO expert group (led by Alberti KGMM, Zimmet P) gave a new name, metabolic syndrome and made a diagnostic criteria.

- Other scientific organizations, i.e., US NIH (National Cholesterol Education Program, NCEP), made their own criteria.

Metabolic syndrome:
The NCEP ATP III definition*

In order to make a diagnosis of the metabolic syndrome a patient must present with **three or more** of the following five risk factors:

Risk Factor	Defining Level

Abdominal obesity
  Men
  Women
	Waist circumference
	>102 cm (>40 in)
	>88 cm (>35 in)

Triglycerides	≥150 mg/dL (1.7 mmol/L)

HDL cholesterol
  Men
  Women
	
	<40 mg/dL (1.04 mmol/L)
	<50 mg/dL (1.29 mmol/L)

Blood pressure	≥130/ ≥85 mmHg
Fasting glucose	≥100 mg/dL (5.6 mmol/L)

*2001, updated 2005

High blood glucose

High TG

Low HDL

High BP

NAFLD

Abdominal obesity

High uric acid

Resistance to insulin action
Increased inflammatory markers
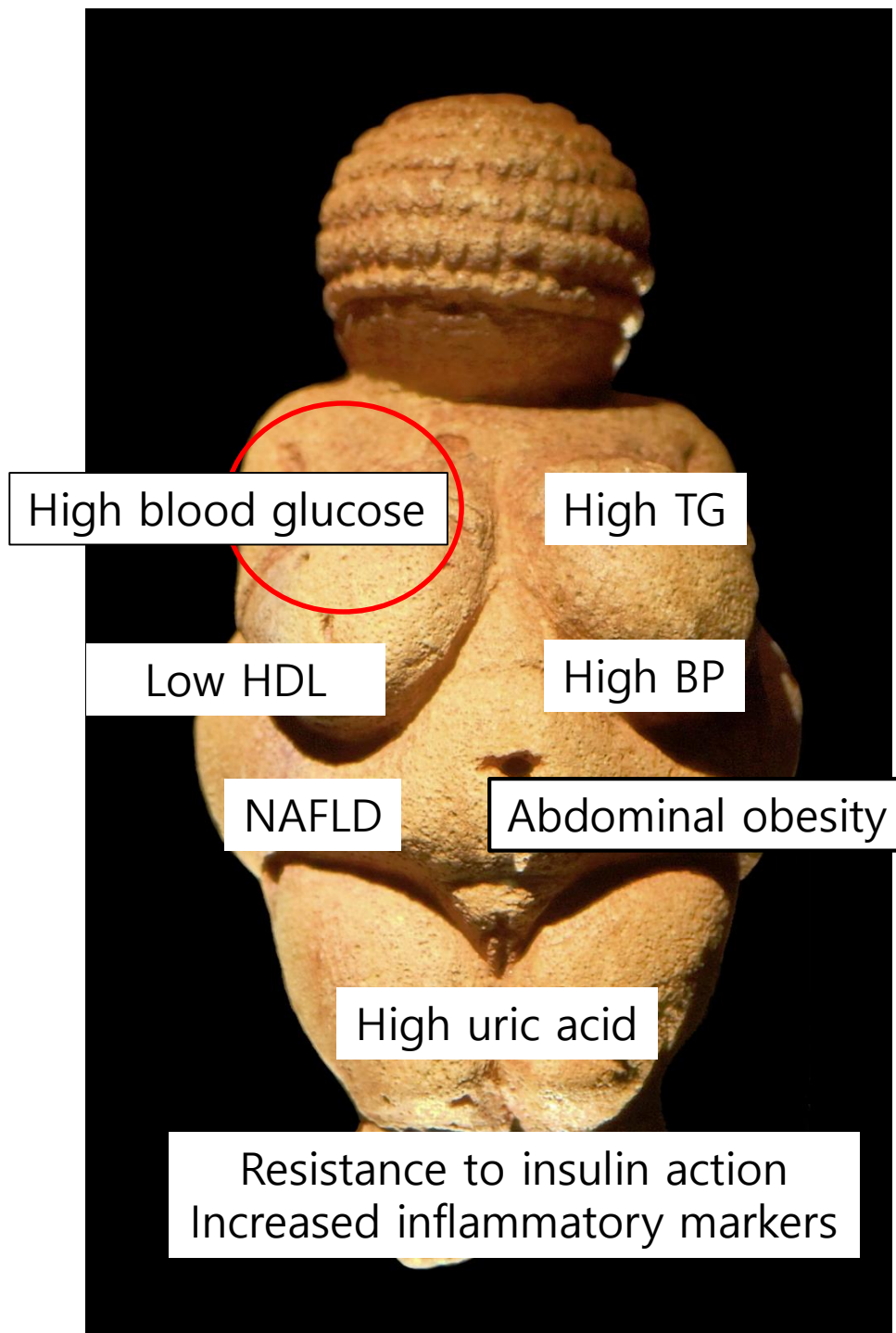
Systemic changes and continuous variables.

Why?

Genetic causes

- Nuclear genes

- Mitochondrial genes

Environmental causes

- Over-eating and less-exercise $\rightarrow$ obesity

- Fetal malnutrition

- Ageing

- Environmental pollutants

Genetic cause(s) of diabetes was suggested by twin study then it was largely denied.

- Pyke DA (1972): diabetes of older age was highly concordant in twin study and concluded the diabetes in older age is genetic disease.

- Later twin studies denied high concordance of diabetes: Swedish twin study (Hong Y et al. 1997) and Danish twin study (Poulsen P, 1999).

- Danish study concluded **non-genetic factors might play a predominant role.**

Genetic causes of diabetes

- Extensive genetic studies revealed that less than 10% of T2D is attributed to genetic cause and the most of them **are related to insulin secretion**, very few genes for insulin sensitivity.

  (Stančáková A, Laakso M. Endocr Dev. 2016)

- GWAS identified >80 common variants for T2D with small effect sizes (risk increased by 5-40%).

- Epidemiological studies revealed a predominantly maternal transmission of T2D (mitochondrial implication).

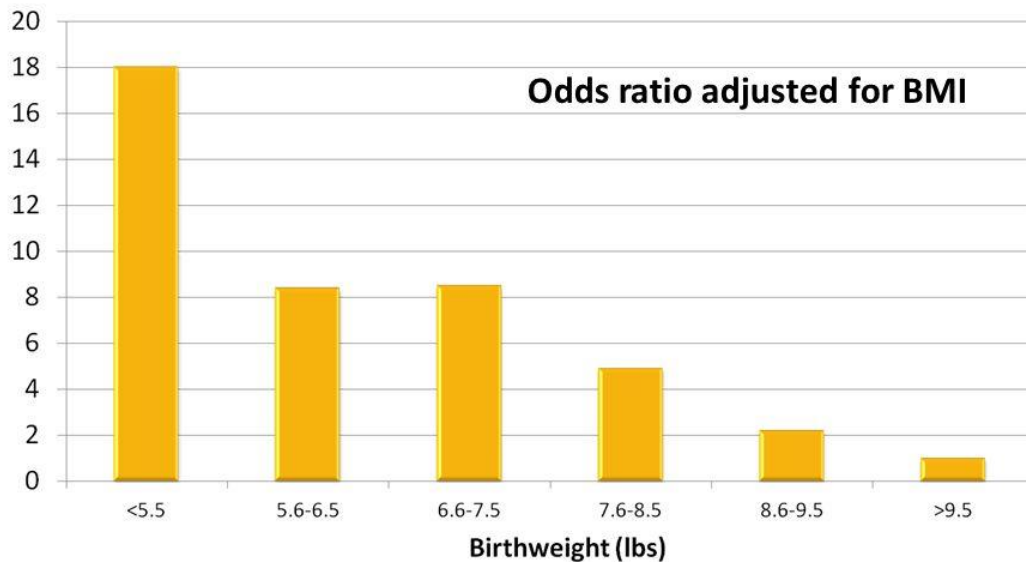# Mitochondrial (mt) DNA association with diabetes and IR

- Diabetes is frequently associated with mitochondrial diseases (mtDNA mutations and deletions).

- Poulton J et al (1992): common polymorphism in D-loop is associated with IR.

- Wilson FH et al. (2002): variations in mtDNA is associated with hypertension and hyperlipidemia.

- mtDNA haplotypes are associated with T2D and IR (Fuku N, Park KS et al. (2007)
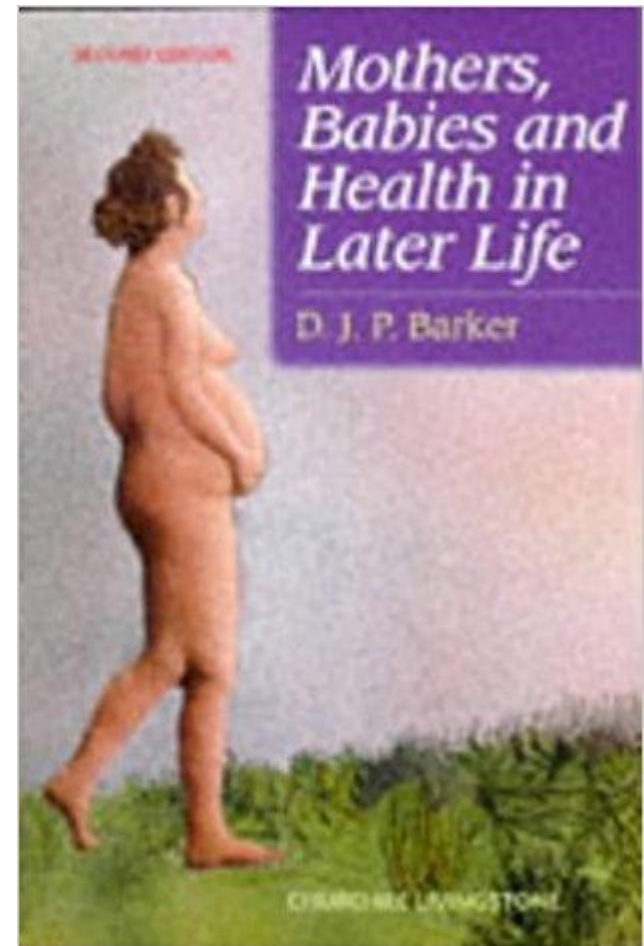
Fetal or early life malnutrition

Ravelli G-P et al. (N Eng J Med, 1967)

- In a historical cohort of 300,000 19-year-old men exposed to the Dutch famine of 1944 - 45, exposure during the first half of pregnancy resulted in significantly higher obesity rates (P<0.0005).

- However exposure during the last trimester of pregnancy and the first months of life, produced significantly lower obesity rates (P<0.005).

- Barker DJP systemically studied UK birth cohorts born in 1920s → Thrifty phenotype or Barker hypothesis.

- Poor mitochondrial biogenesis could explain this phenomenon (Lee HK, 1999).

Barker Hypothesis: Birth Weight and Insulin Resistance Syndrome

Odds ratio adjusted for BMI

Chart: Odds ratio (y-axis 0 to 20) vs Birthweight (lbs) (x-axis)
- <5.5: 18
- 5.6-6.5: ~8.4
- 6.6-7.5: ~8.5
- 7.6-8.5: ~5
- 8.6-9.5: ~2.2
- >9.5: ~1

Birthweight (lbs)

Barker DJP, Hales CN, Fall CHD, Osmond C, Phipps K, Clark PMS. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (Syndrome X): Relation to reduced fetal growth. Diabetologia 1993;36:62-67.

CONTRA COSTA
HEALTH SERVICES

Mothers, Babies and Health in Later Life

D. J. P. Barker

CHURCHILL LIVINGSTONE

Cause(s) of insulin resistance – insulin receptor and post-receptor defects

- Insulin act by binding it's receptor on the cell.

- Kasuga M & Kahn CR (1982): found insulin stimulates insulin receptor phosphorylation.

- Many workers found various (genetic) 'post receptor' abnormalities, but no 'garden variety' genes.

- Insulin stimulates mitochondrial respiration (Krebs H, Eggleston LV. 1938).

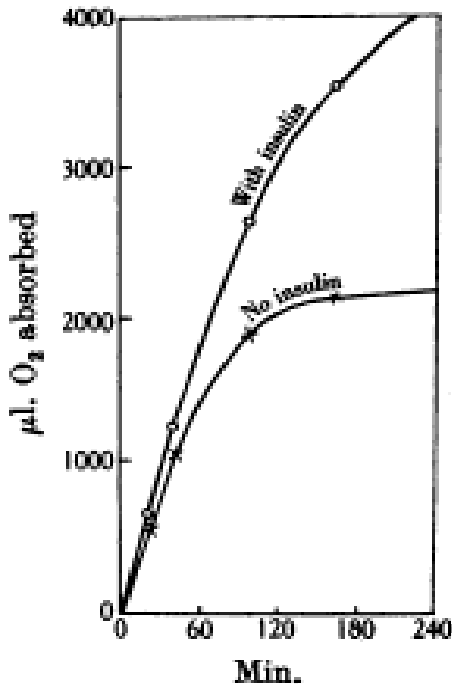- Mitochondrial dysfunction as 'cause' of insulin resistance.

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.

Insulin stimulates mitochondrial respiration.

What if mitochondria preparation is not good ?

Krebs HA, Eggleston LV. Biochem J, 1938

mtDNA content

- Shin CS (1994) found mtDNA content in the peripheral blood of diabetic subjects is decreased.

- Antonetti DA et al. (1995) reported mtDNA density decreased in diabetic muscles, of both NIDDM and IDDM, thus concluded it is secondary to insulin deficiency.

- Lee HK et al (1998) confirmed mtDNA content decrease is correlated with parameters of MetS before the onset of diabetes and precede the onset of diabetes.

- mtDNA depletion made insulin resistance in mtDNA depleted rho-0 cell (Park KS et al. 2001).

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 ^1H nuclear magnetic resonance (NMR) spectroscopy, and
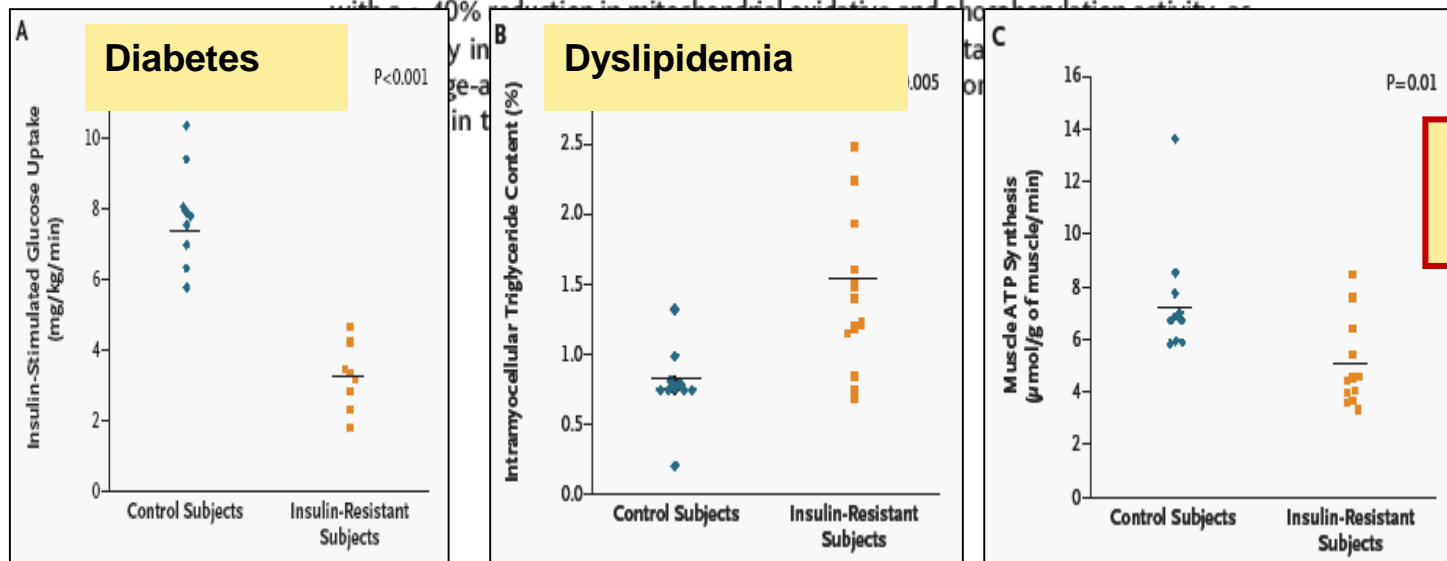
Petersen et al (2004)
NEJM 350; 664-671

Mitochondrial function and insulin sensitivity

Eleven generations of selective breeding of high running capacity rats resulted in two divergent strains.
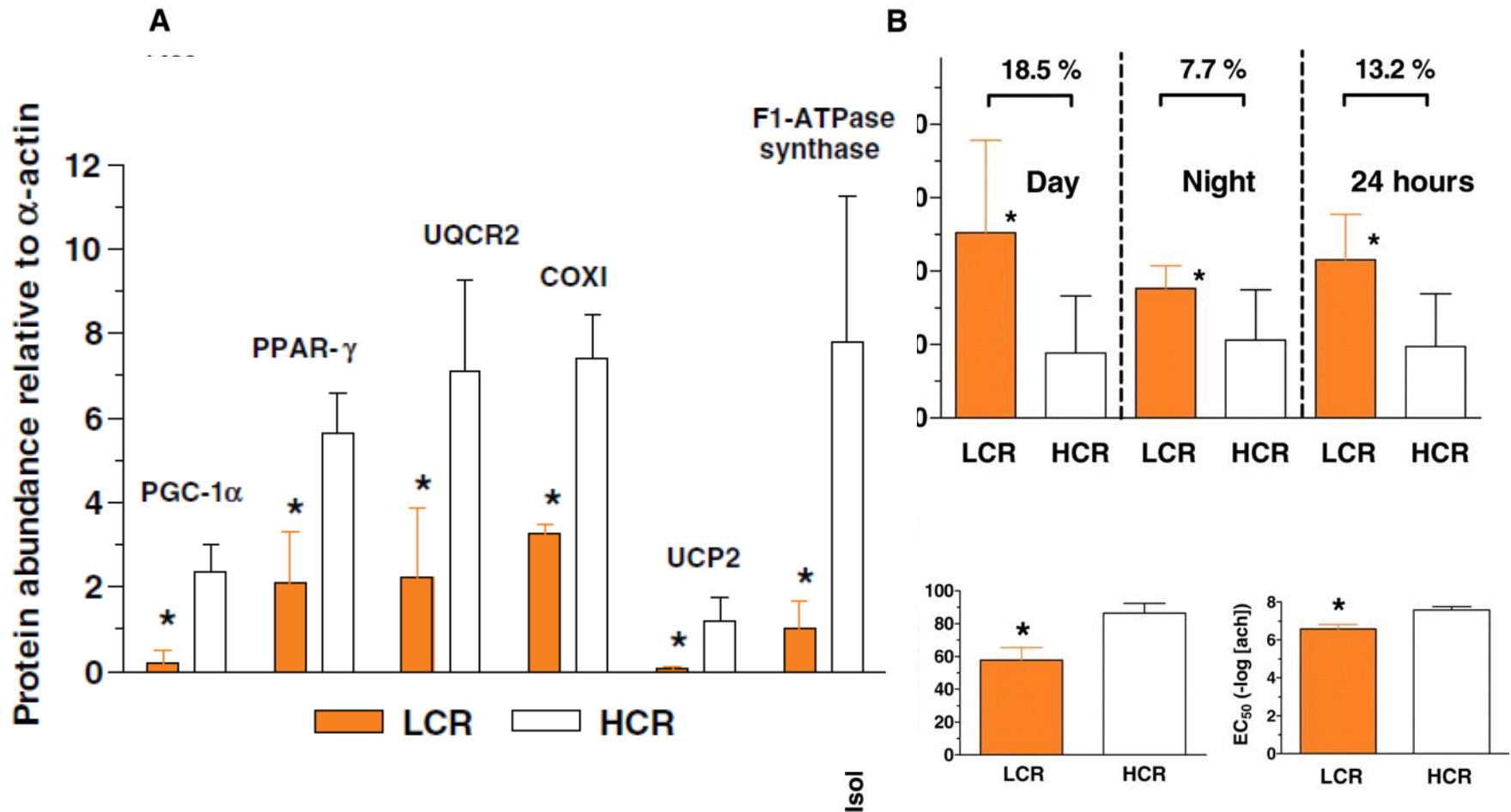
Table 1. LCR and HCR rats differed significantly for carbohydrate and lipid metabolic measures. Measurements were taken from male LCR ($n = 8$) and HCR ($n = 8$) rats. Blood was drawn at 0900 hours with food and water ad libitum to measure random blood sugar. Other metabolic measures were made on blood drawn after 12 hours of food and water deprivation.

	LCR	HCR	% Difference LCR vs. HCR	P value
Random glucose (mg/dl)	86 ± 6	75 ± 12	15%	0.036
Fasting glucose (mg/dl)	110 ± 9	92 ± 5	20%	0.0007
Insulin (pM)	684 ± 195	296 ± 172	131%	0.002
C-peptide (pM)	1590 ± 338	1077 ± 565	48%	0.061
C-peptide/insulin	2.4 ± 0.4	3.8 ± 1.2	−58%	0.013
Visceral adiposity/body weight (%)	1.55 ± 0.39	0.95 ± 0.32	63%	0.005
Triglycerides (mg/dl)	67 ± 24	25 ± 4	168%	0.013
Free fatty acids (meq/l)	0.64 ± 0.22	0.33 ± 0.04	94%	0.031

Ulrik Wisløff et al. Science 2005;307:418-420

Pollution, diabetes and obesity
Baillie-Hamilton PF, 2002

DO CHEMICAL TOXINS CAUSE OBESITY?
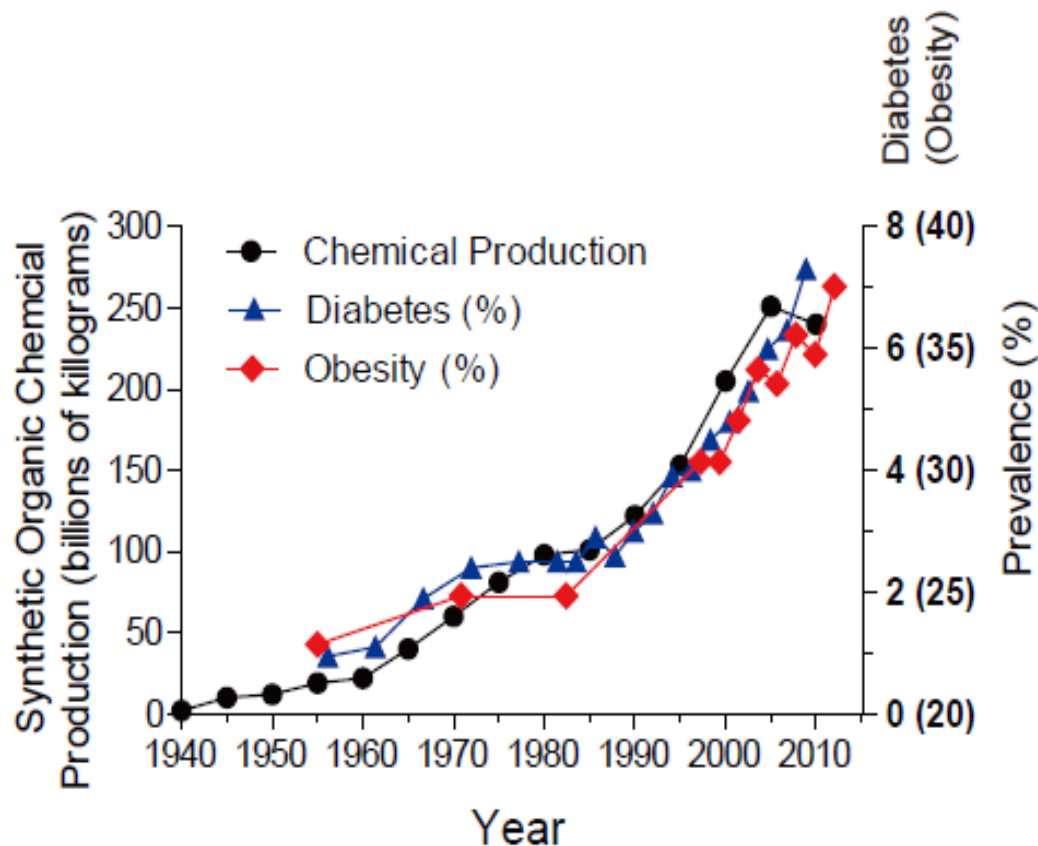
Neels BA, Sargis RM, 2011, Y-K Pak, HK Lee, 2017

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 is strongest in obesity and diabetes.

Summary of my MiP School 2017 presentation

1. Insulin resistance, metabolic syndrome and type 2 diabetes mellitus are caused by environmental pollutants.

2. Cell based assays (serum AhR binding activity and mitochondrial function inhibitor activity assays) should be explored further for a diagnostic tests of metabolic diseases.

Part II. Functional Differences of Mitochondrial Genome and Its Association with Phenotypes of Metabolic Syndrome.

Gene expression pattern in transmitochondrial cytoplasmic hybrid cells harboring type 2 diabetes-associated mitochondrial DNA haplogroups.
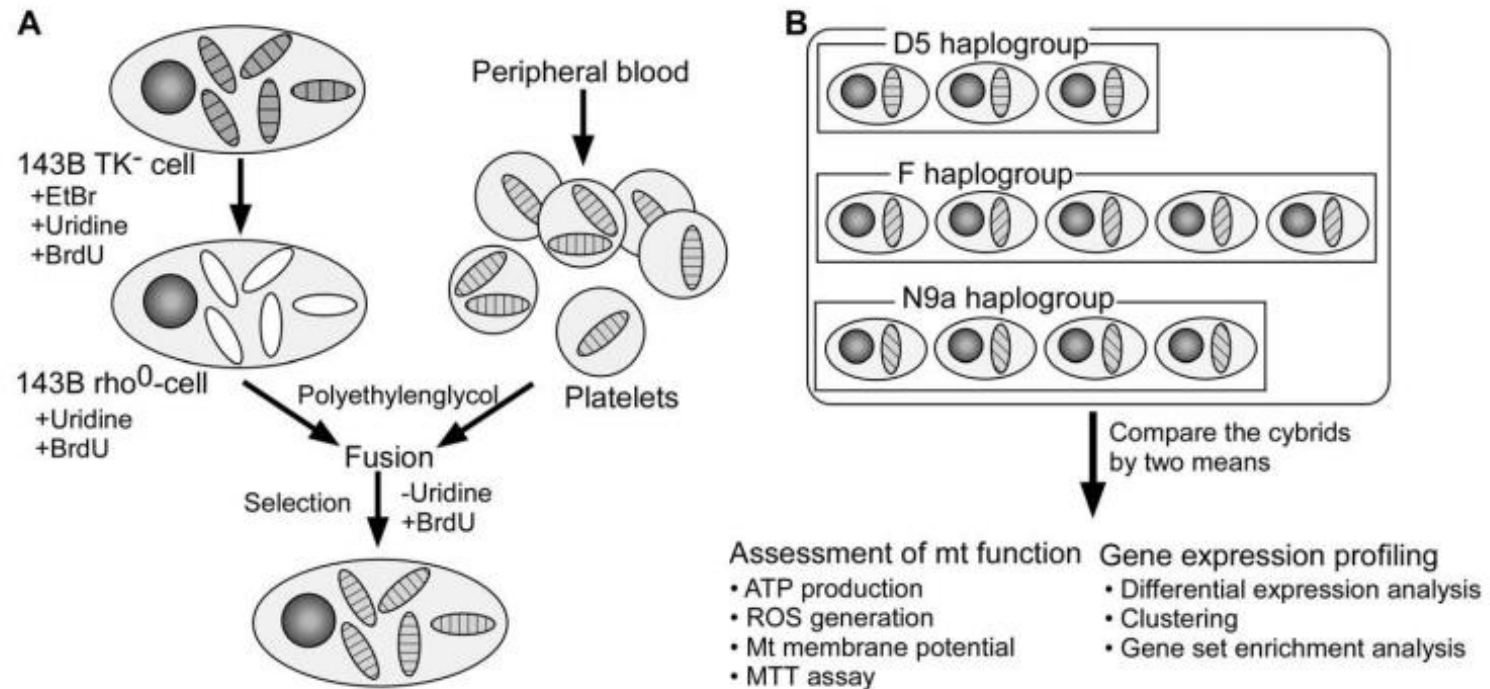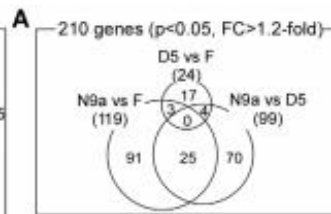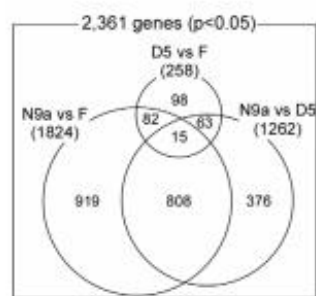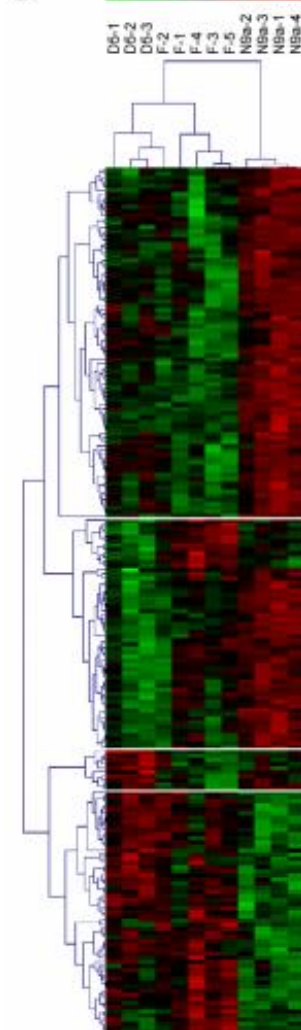## Hwang S et al. PLoS One, 2011

Figure 1. Schematic of cybrid generation and data analysis. (A) Generation of cybrids. Round circle: nucleus, filled ovals: intact mitochondria, unfilled ovals: mtDNA-depleted mitochondria. (B) Comparison of cybrids originating from three mtDNA haplogroups.
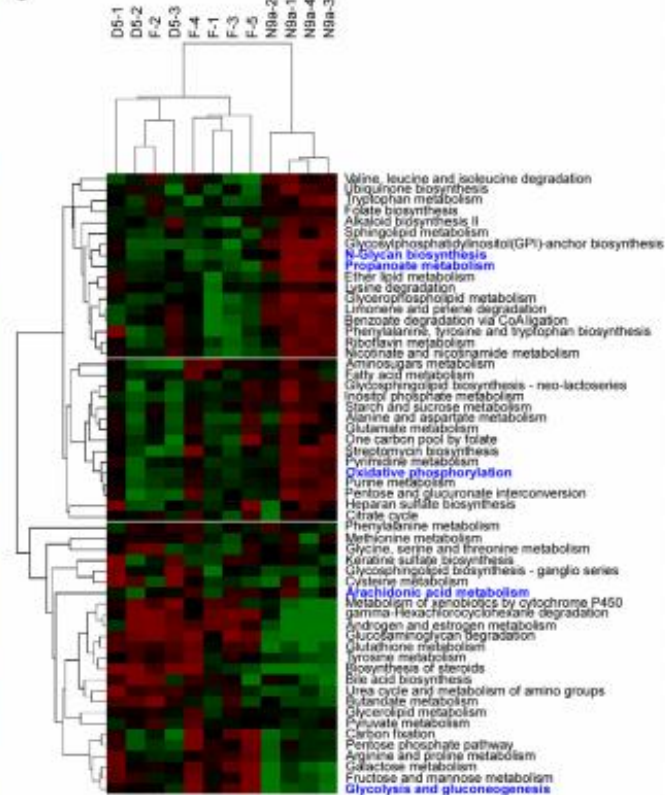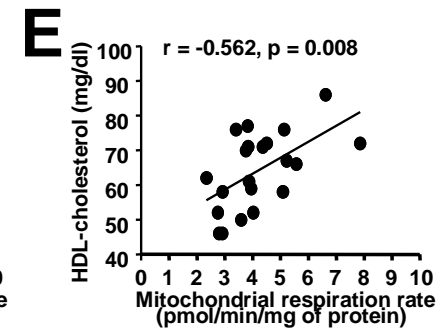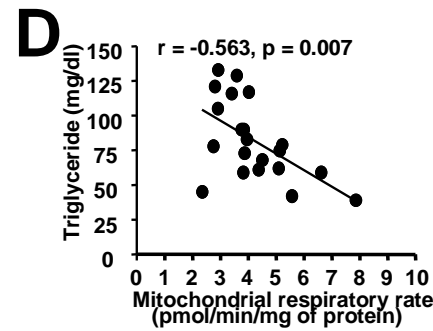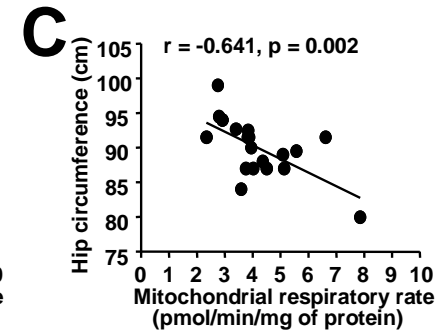doi:10.1371/journal.pone.0022116.g001

A

2,361 genes (p<0.05)

D5 vs F
(258)

N9a vs F
(1824)

N9a vs D5
(1262)

98

82   83

15

919   808   376

210 genes (p<0.05, FC>1.2-fold)

D5 vs F
(24)

N9a vs F
(119)

N9a vs D5
(99)

17

3   4

0

91   25   70

B

-3.0   3.0

D5-1
D5-2
D5-3
F-2
F-4
F-3
F-5
N9a-2
N9a-3
N9a-1
N9a-4

C

D5-1
D5-2
F-2
D5-3
F-4
F-1
F-3
F-5
N9a-2
N9a-1
N9a-4
N9a-3

Valine, leucine and isoleucine degradation
Ubiquinone biosynthesis
Tryptophan metabolism
Folate biosynthesis
Alkaloid biosynthesis II
Sphingolipid metabolism
Glycosylphosphatidylinositol(GPI)-anchor biosynthesis
N-Glycan biosynthesis
Propanoate metabolism
Ether lipid metabolism
Lysine degradation
Glycerophospholipid metabolism
Limonene and pinene degradation
Benzoate degradation via CoA ligation
Phenylalanine, tyrosine and tryptophan biosynthesis
Riboflavin metabolism
Nicotinate and nicotinamide metabolism
Aminosugars metabolism
Fatty acid metabolism
Glycosphingolipid biosynthesis - neo-lactoseries
Inositol phosphate metabolism
Starch and sucrose metabolism
Alanine and aspartate metabolism
Glutamate metabolism
One carbon pool by folate
Streptomycin biosynthesis
Pyrimidine metabolism
Oxidative phosphorylation
Purine metabolism
Pentose and glucuronate interconversion
Heparan sulfate biosynthesis
Citrate cycle
Phenylalanine metabolism
Methionine metabolism
Glycine, serine and threonine metabolism
Keratan sulfate biosynthesis
Glycosphingolipid biosynthesis - ganglio series
Cysteine metabolism
Arachidonic acid metabolism
Metabolism of xenobiotics by cytochrome P450
gamma-Hexachlorocyclohexane degradation
Androgen and estrogen metabolism
Glucosaminoglycan degradation
Glutathione metabolism
Tyrosine metabolism
Biosynthesis of steroids
Bile acid biosynthesis
Urea cycle and metabolism of amino groups
Butanoate metabolism
Glycerolipid metabolism
Pyruvate metabolism
Carbon fixation
Pentose phosphate pathway
Arginine and proline metabolism
Galactose metabolism
Fructose and mannose metabolism
Glycolysis and gluconeogenesis

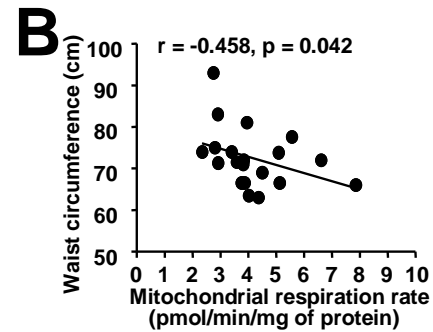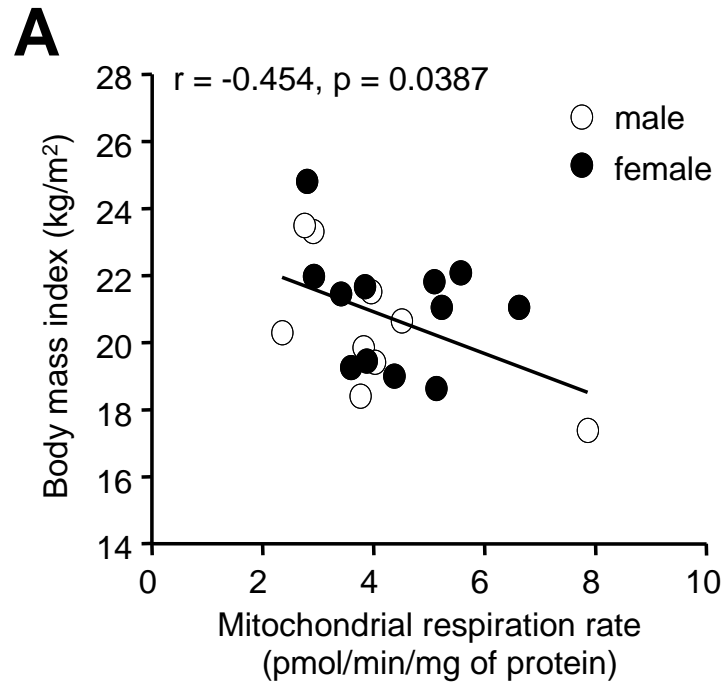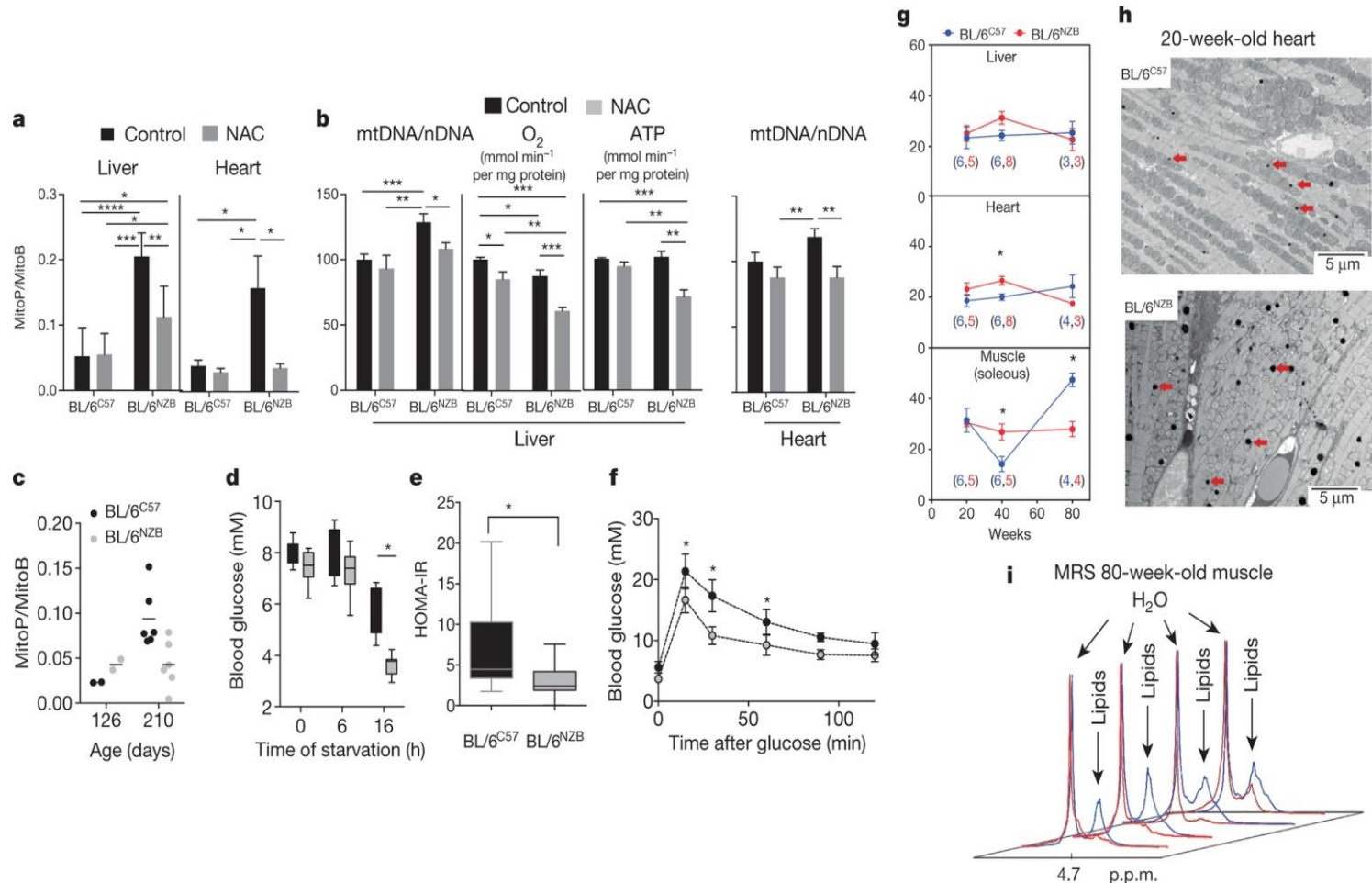**Cho YM et al. bioXriv, 2017.**

Summary

- T2D-susceptible haplogroup F cybrids showed down-regulation of oxidative phosphorylation and up-regulation of glycolysis than haplogroup N9a cybrids.

- These results suggest that variations in mtDNA can affect the expression of nuclear genes regulating mitochondrial functions or cellular energetics.

- Cybrids showed varying degree of tumorigenicity both *in vitro* and *in vivo*. Especially, the cybrids harboring mtDNA haplogroup D had a significantly slower growth rate.

- The mitochondrial oxygen consumption rates of cybrids were associated with multiple components of metabolic syndrome.

Functional differences of mtDNA was proved by a study with conplastic strains of mice

Latorre-Pellicer *A et al. Nature*, **2016**

- Conplastic animals are individuals with the same nuclear genome but different mtDNAs.

- The mtDNA of C57BL/6 and NZB/OlaHsd mice differ by 12 missense mutations, 4 transfer RNA (tRNA) mutations, 8 ribosomal RNA (rRNA) mutations, and 10 non-coding-region mutations.

- Authors compared the conplastic mouse strain with the C57BL/6 nuclear genome with the NZB/OlaHsd mtDNA (BL/6NZB) with the original C57BL/6 strain with the C57BL/6 nuclear genome and C57BL/6 mitochondrial genome (BL/6C57) in details.

Functional differences between the conplastic strains of mice

Authors conclude that mtDNA haplotype profoundly influences mitochondrial proteostasis and reactive oxygen species generation, insulin signalling, obesity, and ageing parameters including telomere shortening and mitochondrial dysfunction, resulting in profound differences in health longevity.
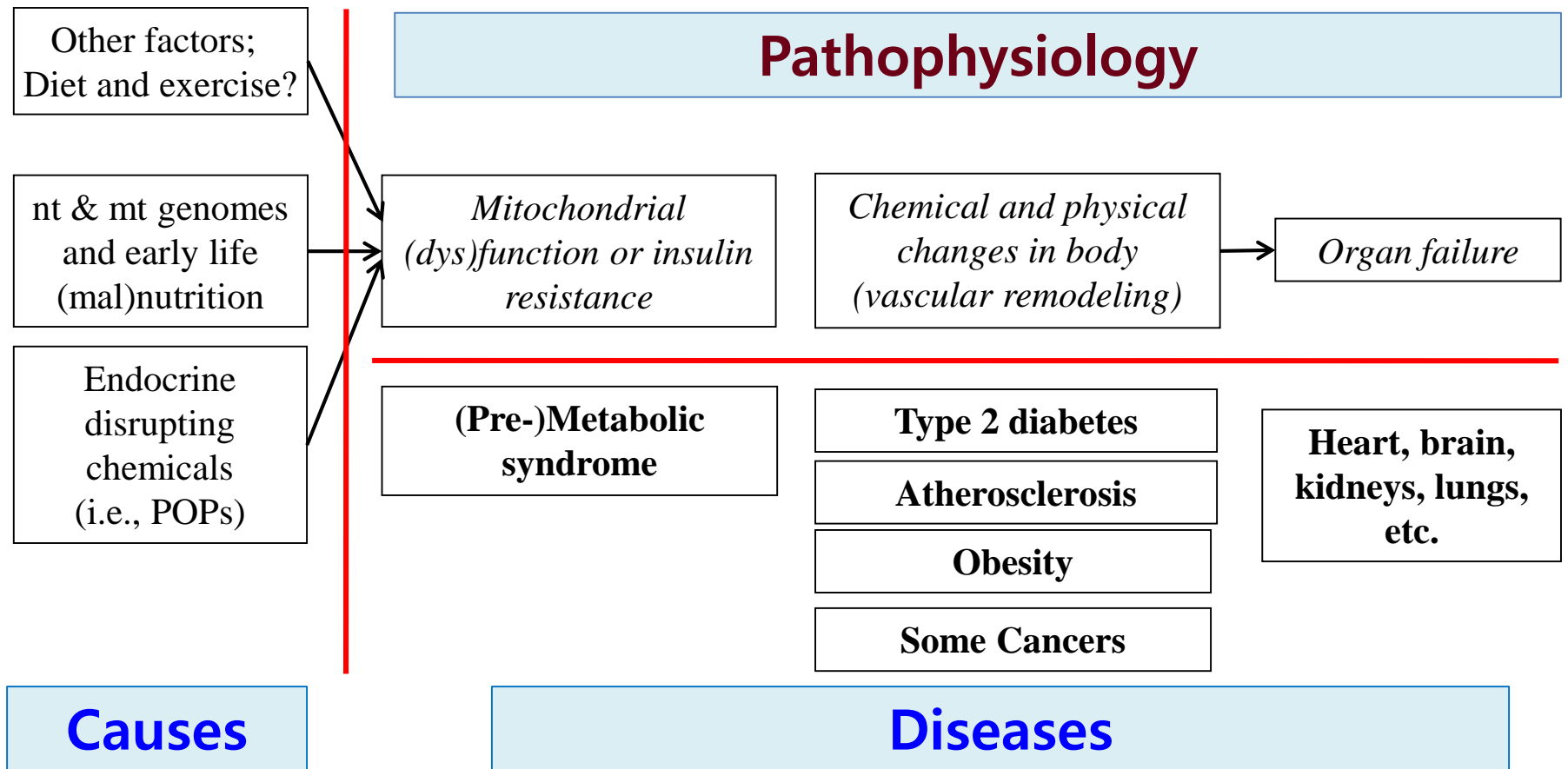
Conceptual framework

Lee HK, Shim EB, Extension of Mitochondria Dysfunction Hypothesis of Metabolic Syndrome to Atherosclerosis with Emphasis on the Endocrine Disrupting Chemicals and Biophysical Laws. *J Diab Invest,* 2013, 4: 19-33.

Acknowledgments