Epigenetic pollution and prevention of cardio-metabolic diseases

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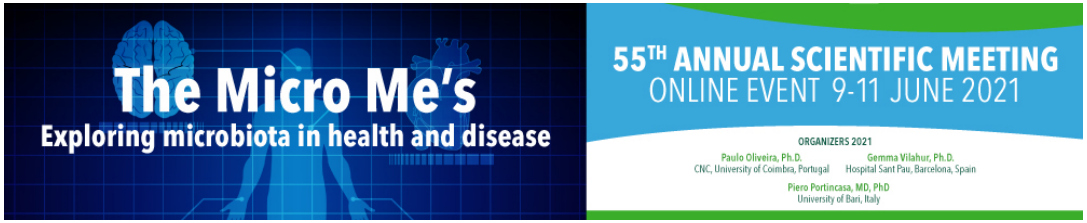

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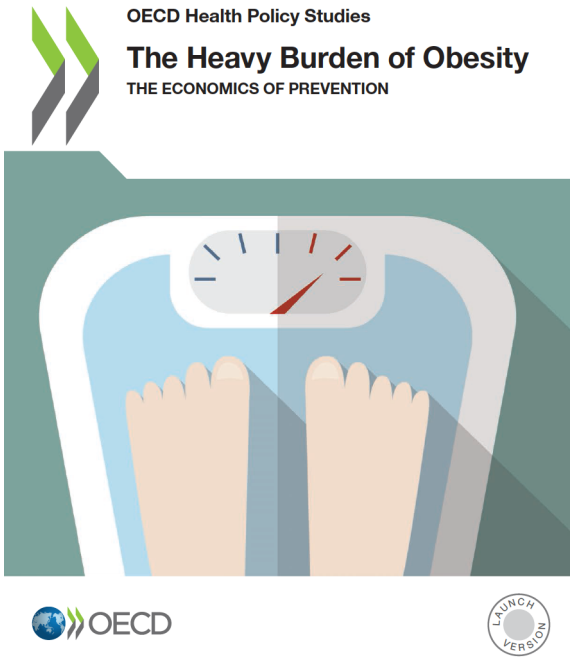
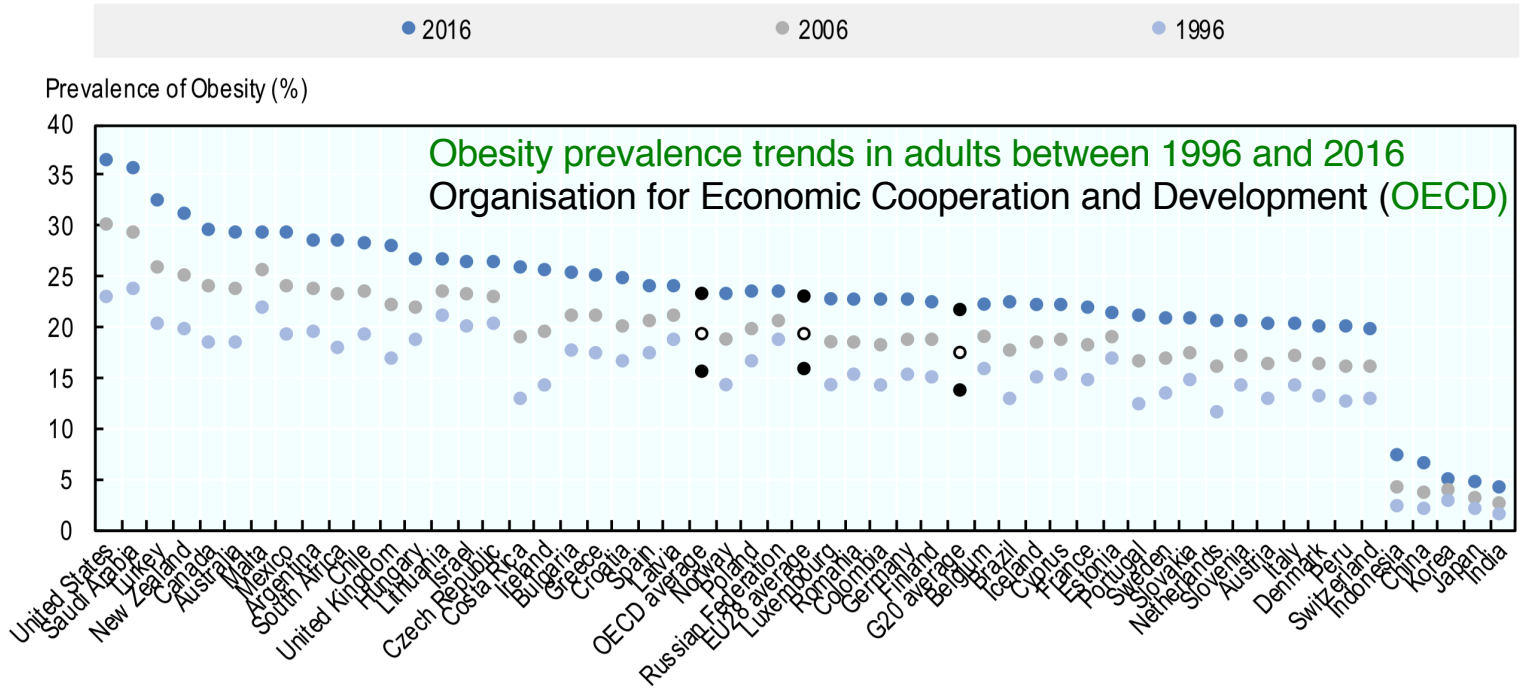


Organisation for Economic Cooperation and Development ***(OECD) - 2019***

- *"OECD countries have made **substantial progress on policies to tackle unhealthy diets and lack of physical activity in the last decade**".*
- *Virtually **all OECD countries have a national action plan on obesity** …, and a **vast majority of countries have a specific action plan to tackle obesity in children** as well as national guidelines to promote healthy diets and active lifestyles"*

"**Despite this**, the growing overweight rates show that, so far, **the response has not fully met the challenge**"

Health policies oriented to improve diet and lifestyle are widely employed, but the prevalence of cardio-metabolic diseases is increasing worldwide

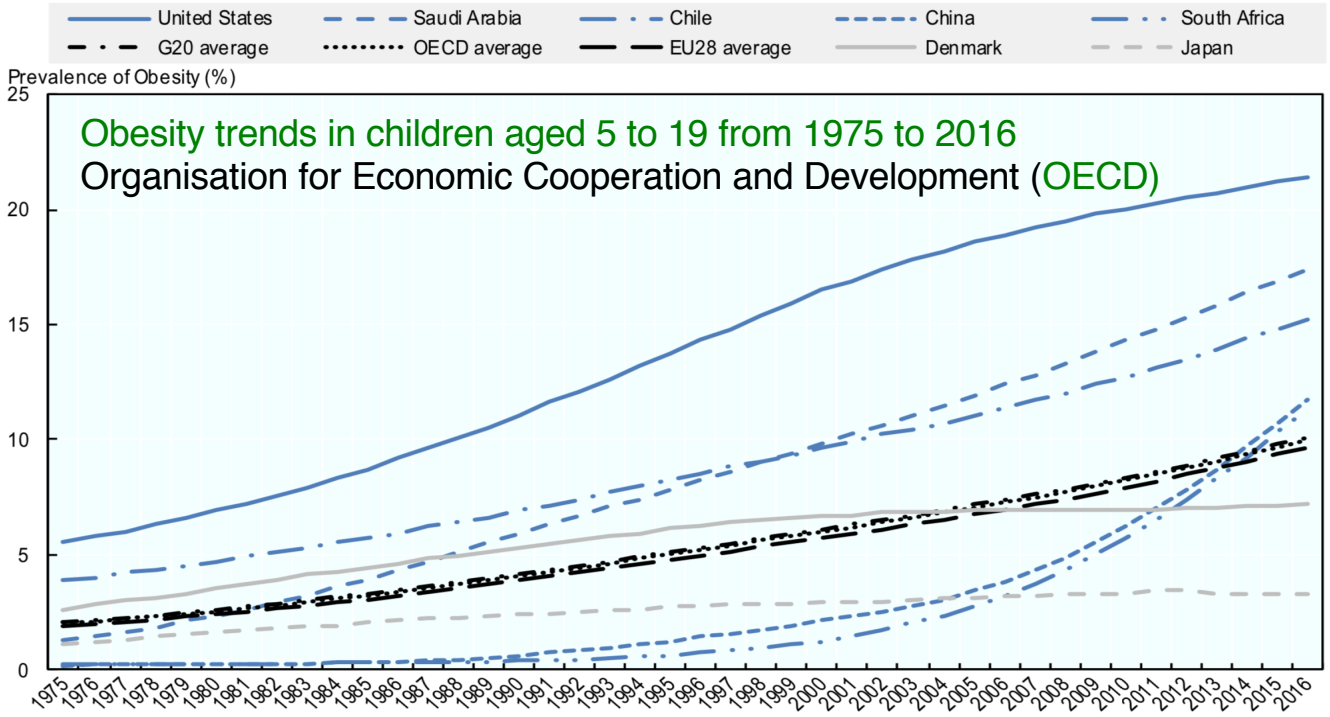


World Health Organization:

- Worldwide obesity has nearly tripled since 1975
- 39% of adults aged 18 years and over were overweight in 2016, and 13% were obese

(OECD – 2019)

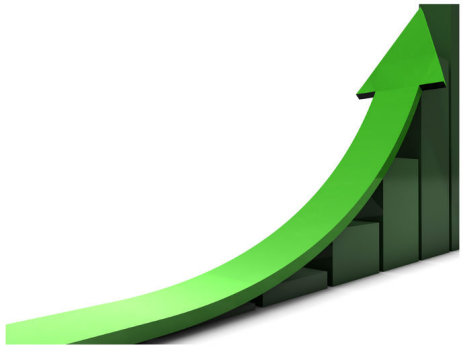
Health policies oriented to improve diet and lifestyle are widely employed, but the prevalence of cardio-metabolic diseases is increasing worldwide



World Health Organization:

- 38 million children under the age of 5 were overweight or obese in 2019
- Over 340 million children and adolescents aged 5-19 were overweight or obese in 2016

(OECD – 2019)



Similar increasing trends:

NAFLD

- One of the most popular chronic disorders in western countries (10-46% of prevalence in USA)
- The most frequent chronic liver disease
- Median prevalence is about 20% worldwide, with a progressive increasing trend

Type 2 Diabetes

- The number of people with diabetes rose from 108 million in 1980 to 422 million in 2014
- Between 2000 and 2016, there was a 5% increase in premature mortality from diabetes
- In 2019, an estimated 1.5 million deaths were directly caused by diabetes

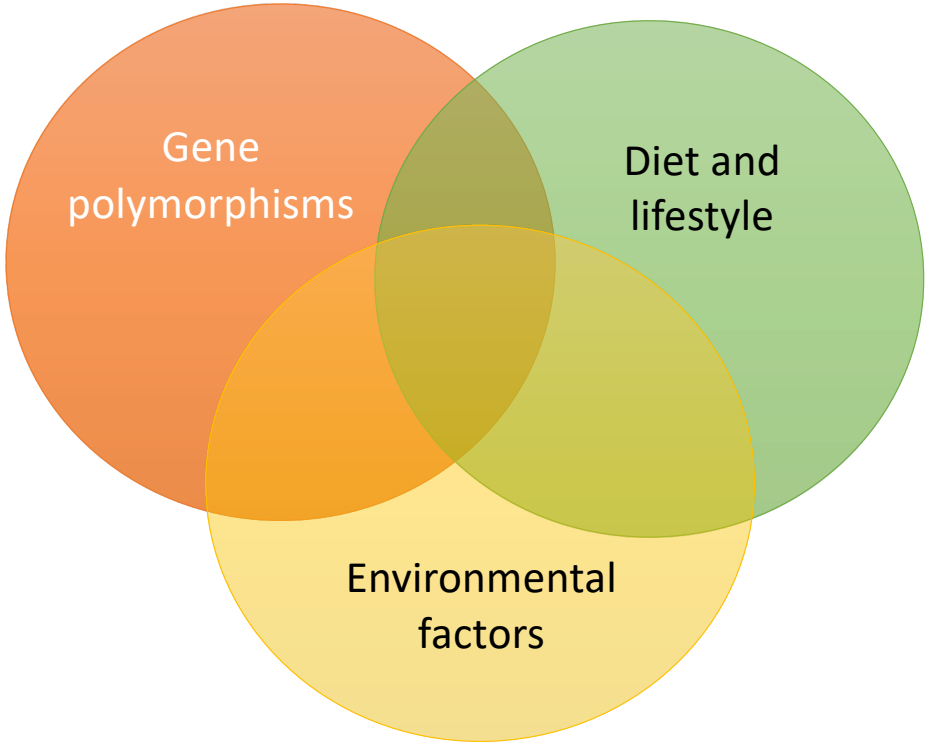
(WHO)

The majority of metabolic diseases are preventable

Epidemiologic data show that, in the last decades, we have not been able to prevent the majority of these diseases…

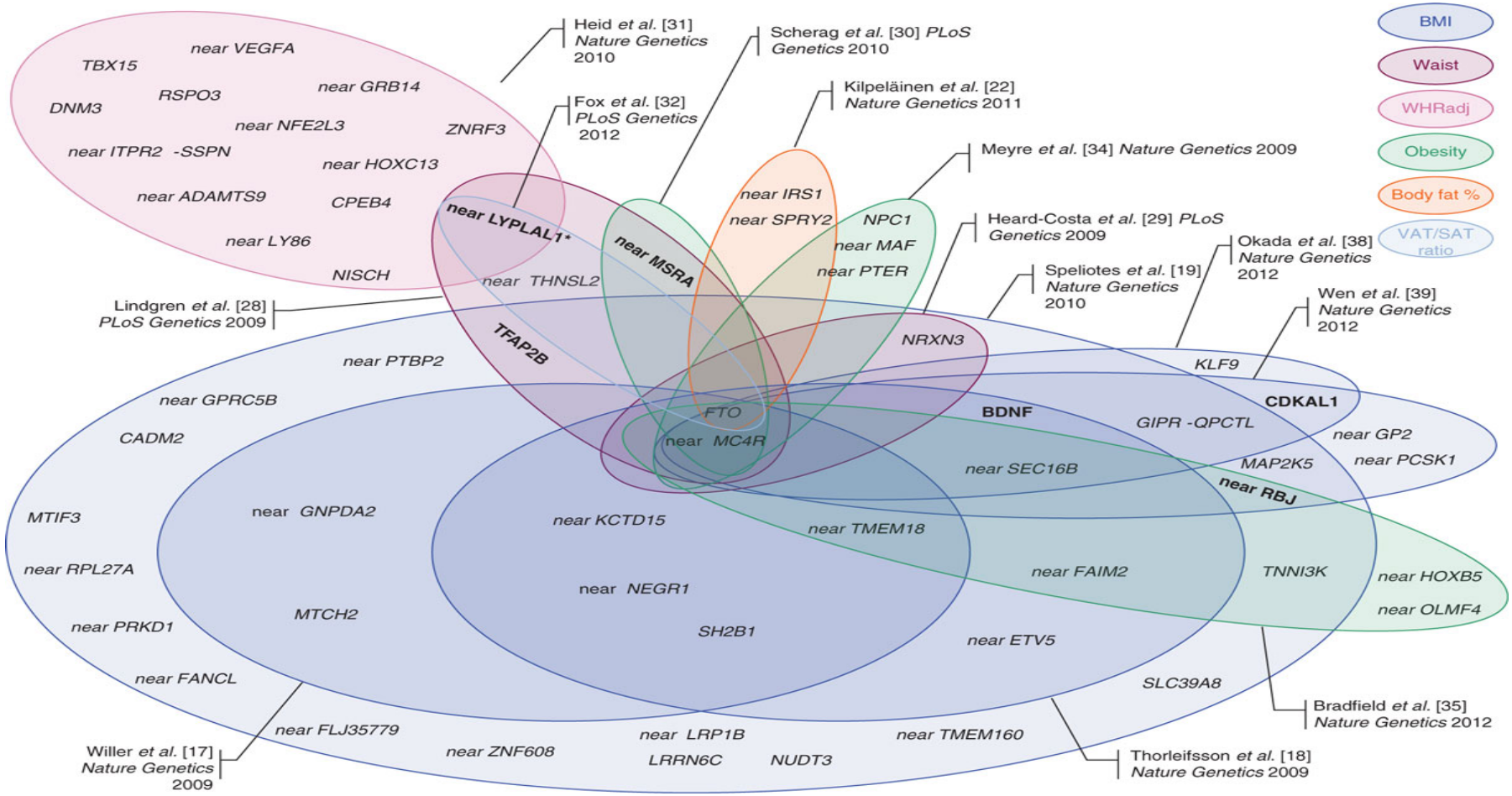
The question is: Do we really, comprehensively know how to prevent these chronic diseases?

Pathogenesis of metabolic diseases



Who takes the responsibility ?

(Lu and Loos Genome Medicine 2013; Locke et al, Nature 2015)



Genome-Wide Association Studies (GWAS) have identified 97 genetic loci associated with BMI and over 500 associated with obesity-susceptibility

Fels Longitudinal Study (USA) (Demerath et al, Hum Ered 2013)

Description of the sample data: 907 Fels Longitudinal Study adults aged 25–64 years.

		Birth Year					
	All	**<=1939**	**1940–1949**	**1950–1959**	**1960–1969**	**1970+**	**P (X^2 statistic)**
			N or Percent				
N	907	177	171	196	183	180	
Sex (% Female)	47.4%	49.7%	49.0%	44.8%	48.9%	46.3%	0.56
Adulthood Obesity (N, %)	188 (20.7%)	28 (15.8%)	34 (19.9%)	42 (21.4%)	40 (21.9%)	44 (24.4%)	<0.001
Adulthood Underweight (N, %)	16 (1.8%)	5 (2.8%)	2 (1.2%)	2 (1.0%)	4 (2.2%)	3 (1.7%)	0.44
			Mean (SD) [range]				P (F statistic)
Age	43.3 (11.4) [25–65]	51.2 (11.0) [26–65]	51.5 (9.4) [25–65]	45.9 (8.0) [25–61]	36.8 (7.1) [26–51]	31.5 (4.1) [25–41]	<0.001
Birth Year	1955 (15.0) [1901–1986]	1931 (7.3) [1901–1939]	1944.5 (3.0) [1940–1949]	1954.5 (2.8) [1950–1959]	1964.4 (3.0) [1960–1964]	1975.6 (4.2) [1970–1986]	<0.001
Genetic Risk Score, no. risk alleles	28.46 (3.45) [18–38]	28.9 (3.5) [19–38]	28.1 (3.6) [18–38]	28.5 (3.7) [18–37]	28.5 (3.5) [29–37]	28.3 (3.4) [21–39]	0.31

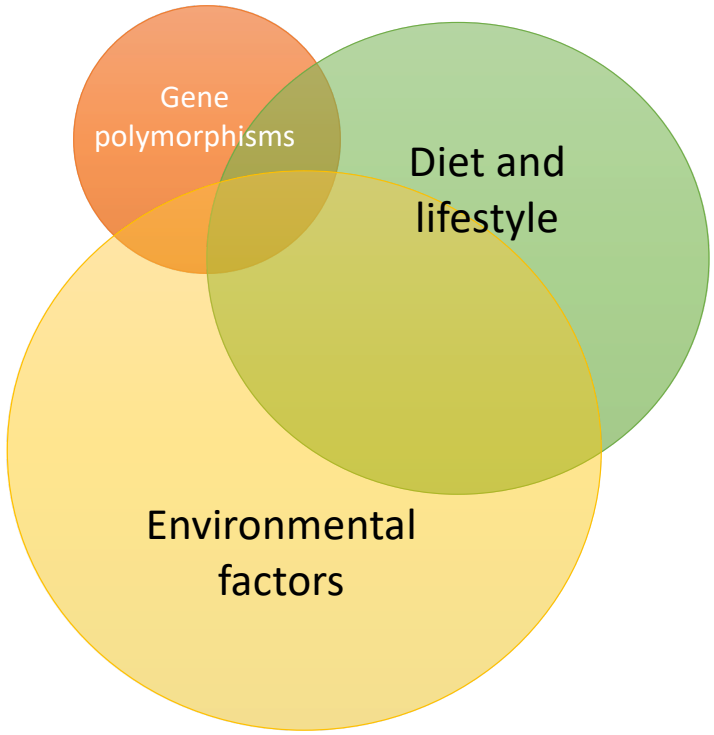
The obesity rate increased according to birth year

The genetic risk score remained stable

“Genes may co-determine who becomes obese, but our environment determines how many become obese.”

(J Lennert Veerman, PLoS Med 2011)

Evidence points to a limited responsibility for genetic factors and to external factors as major drivers of this epidemic increase



Modifiable factors

Possible primary prevention of metabolic diseases

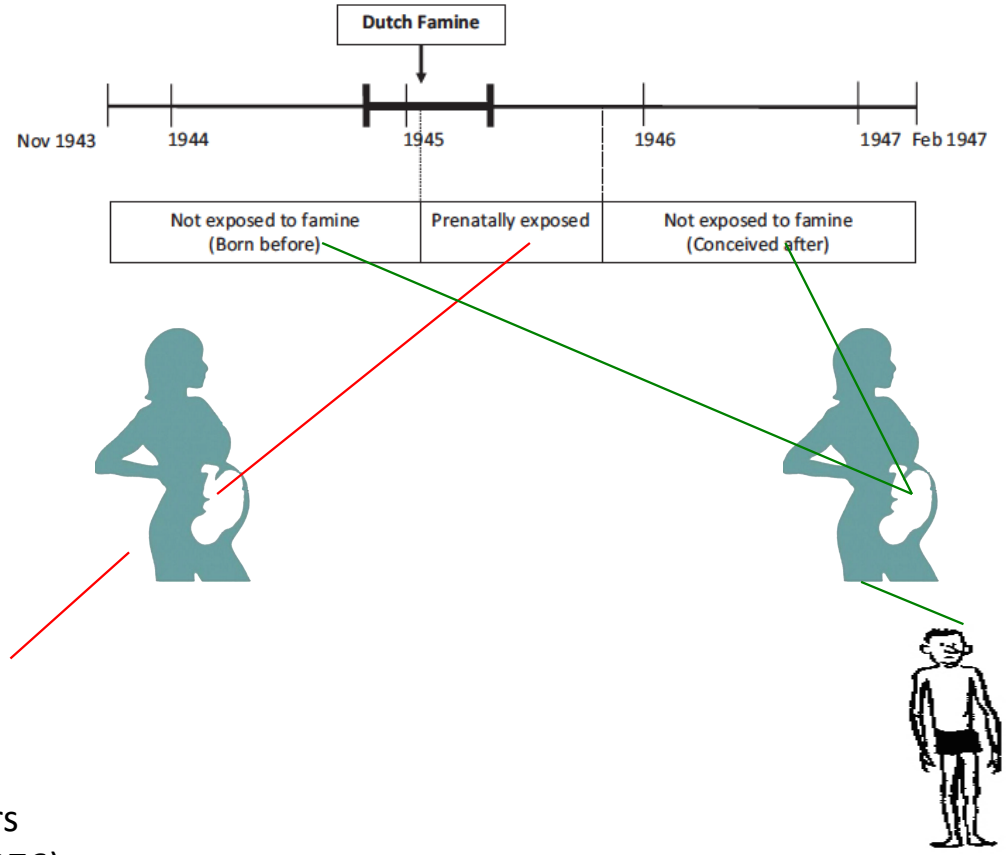
Dutch famine during the German occupation in 1944

Unique nutritional challenge:

- started and ended abruptly
- lasted only 5 mo
- was preceded and followed by adequate nutrition

Opened the windows on the key role played by epigenetic factors

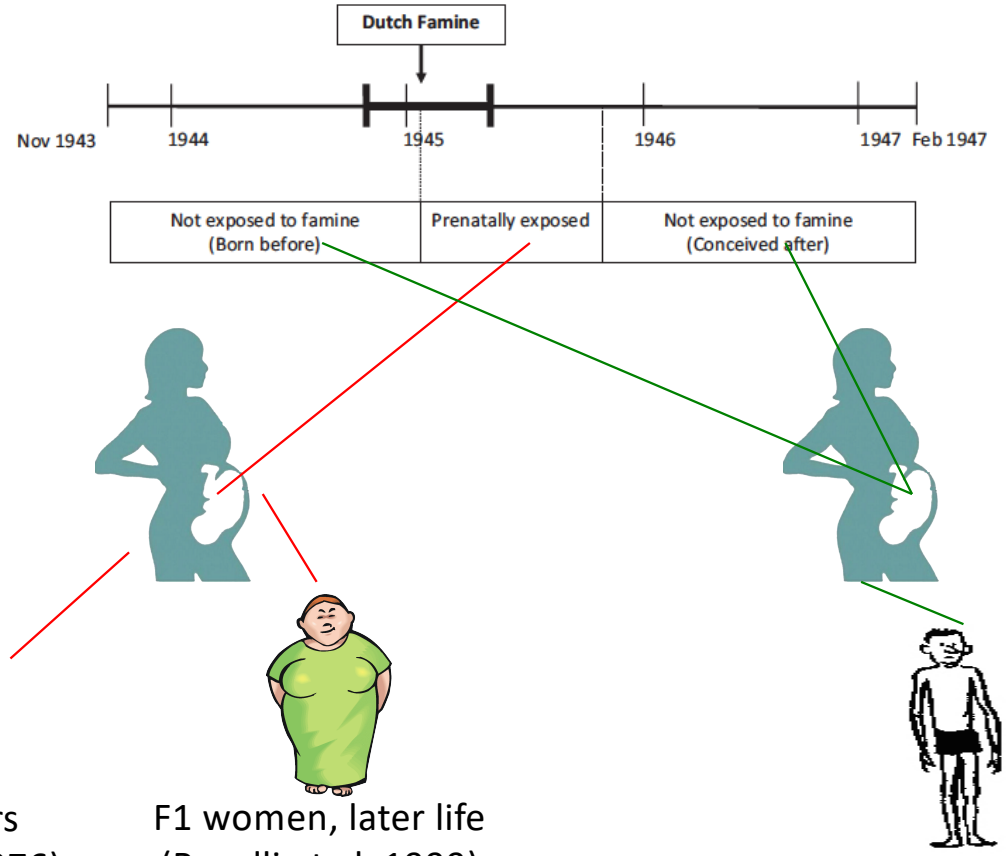
Results from the Dutch famine studies



F1 men, 19yrs
(Ravelli et al, 1976)

Men who were conceived during the Dutch famine had higher rates of obesity than those conceived before or after it

Results from the Dutch famine studies

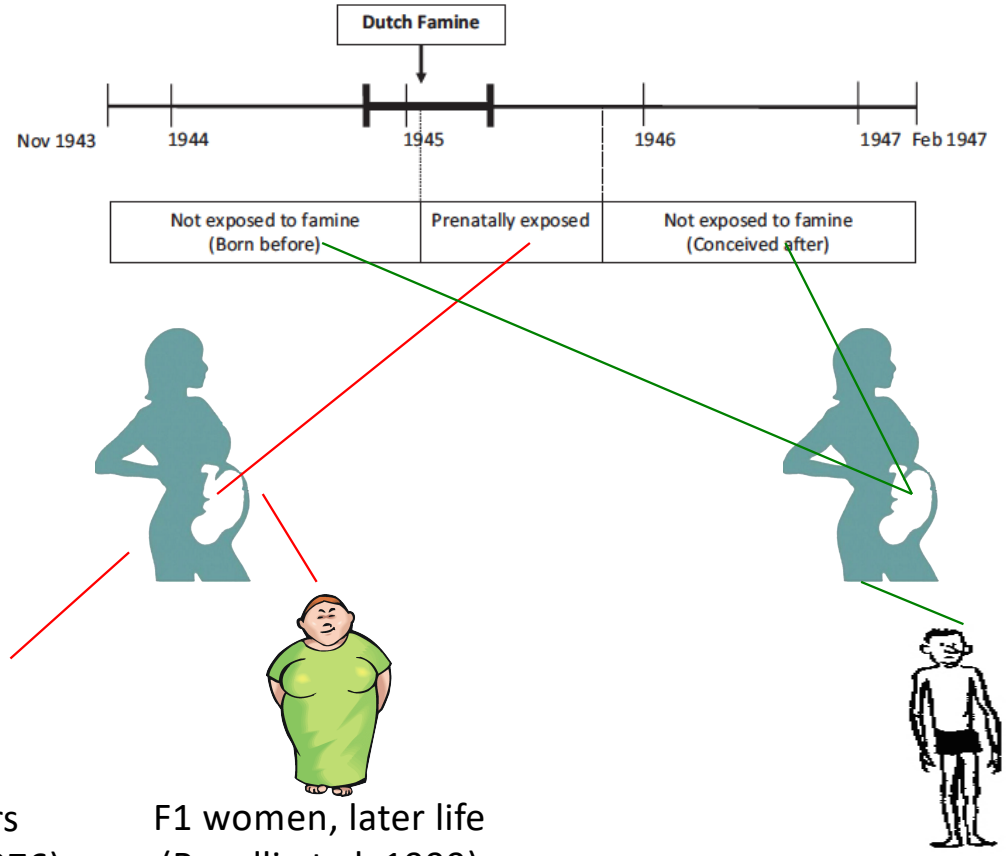


F1 men, 19yrs
(Ravelli et al, 1976)

F1 women, later life
(Ravelli et al, 1999)

Maternal malnutrition in early gestation was also associated with higher BMI and waist circumference in 50-y-old women

Results from the Dutch famine studies



F1 men, 19yrs
(Ravelli et al, 1976)

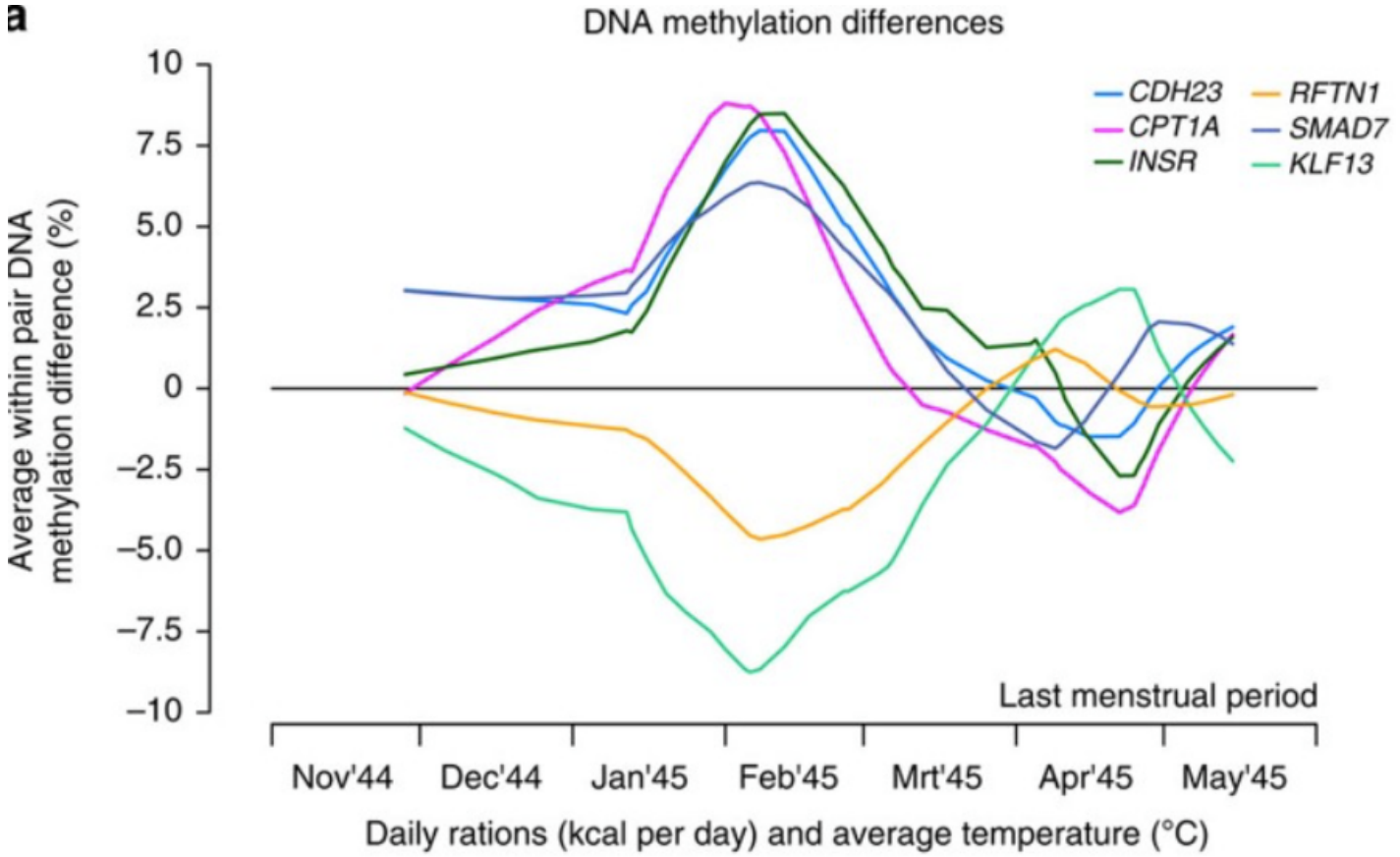
F1 women, later life
(Ravelli et al, 1999)

Higher BMI in F2 offsprings
(Veenendaal et al, 2012)

Grandchildren had increased BMI in adult age

Results from the Dutch famine studies

Exposed subjects had different DNA methylation patterns as compared with their unexposed, same-sex siblings



(Tobi et al, Nat Commun 2014)

- decreased expression of genes involved in oxidative phosphorylation; carbohydrate, amino acid, and lipid metabolism;

- increased expression of genes involved in inflammation and glycan degradation

In monozygotic twin pairs, candidate genes for obesity and T2D can be differentially expressed in discordant twins

(Nilsson et al. Diabetes 2014;63:2962-2976)

Diet, lifestyle and epigenome

- High-fat/high calorie diets
- Excessive fructose consumption
- Dietary fatty acids
- Fiber consumption

- Low physical activity
- Smoking
- Excessive alcohol intake
- Chronic stress

Associated with epigenetic variations
(mainly DNA methylation)

Personal choices as dietary habits and lifestyle can affect the epigenome, and epigenetic changes are emerging as early markers of metabolic dysfunction

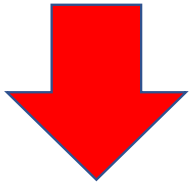
The CENTRAL randomized controlled trial

- Intrahepatic fat (IHF%, MR imaging)
- CpG specific DNA-methylation levels of 41 selected candidate genes

120 participants with abdominal obesity or dyslipidemia

18-month regimen

- low-fat or Mediterranean-low carbohydrate diets
- with or without physical activity (PA)



Baseline- **IHF% was inversely correlated with DNA-methylation** at individual CpGs within within *AC074286.1, CRACR2A, A2MP1, FARP1*

- significant **reduction in IHF%** (no difference between diets)
- **Differential methylation pattern after diet**
- PA+ reduced more IHF% compared with PA-, with **differential methylation pattern**

(Meir AY et al, Liver Int 2021)

- However, the responsibility for the epidemic rise of cardio-metabolic diseases cannot be entirely attributed to individuals

- A major role is also played by the involuntary exposure to widely diffused environmental toxics as endocrine disrupting chemicals and air pollutants

The Lancet: Global Burden of Diseases Study

2015 rank

1 High systolic blood pressure
2 Smoking
3 High fasting plasma glucose
4 High total cholesterol
5 Ambient particulate matter pollution
6 Diet high in sodium
7 High body-mass index
8 Diet low in whole grains
9 Diet low in fruits
10 Household air pollution from solid fuels
11 Impaired kidney function
12 Alcohol use
13 Diet low in nuts and seeds
14 Diet low in vegetables
15 Low physical activity
16 Diet low in seafood omega3 fatty acids
17 Unsafe sex
18 Childhood undernutrition
19 Unsafe water source
20 No handwashing with soap
21 Second-hand smoke
22 Unsafe sanitation
23 Diet high in processed meat
24 Suboptimal breastfeeding

- Air pollution is ranked at the 5th place as determinant of global burden of disease
- 9 million premature deaths in 2015

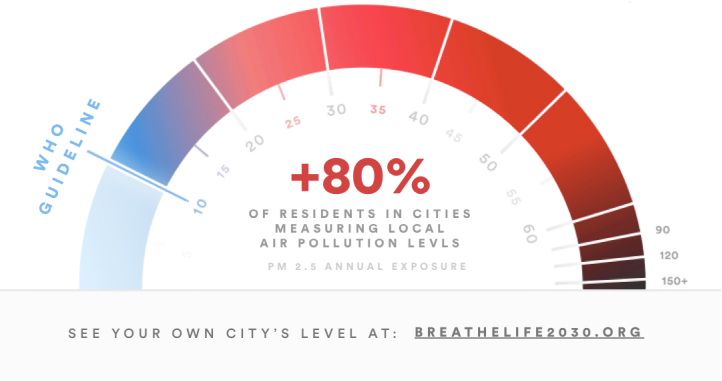
- "three times more deaths than from AIDS, TBC and malaria combined"

- "15 times more than from all wars and other forms of violence"

World Health Organization

AIR POLLUTION ISSUE GLOBALLY

Over 80% of urban residents are exposed to air quality levels that exceed WHO limits



SEE YOUR OWN CITY'S LEVEL AT: BREATHELIFE2030.ORG

Estimating Burden and Disease Costs of Exposure to Endocrine-Disrupting Chemicals in the European Union

Leonardo Trasande, R. Thomas Zoeller, Ulla Hass, Andreas Kortenkamp, Philippe Grandjean, John Peterson Myers, Joseph DiGangi, Martine Bellanger, Russ Hauser, Juliette Legler, Niels E. Skakkebaek, and Jerrold J. Heindel

probability of causation *(epidemiological/toxicological evidences),* ***median costs calculation*** *(Monte Carlo simulations)*

- There is a 40–69% probability of **phthalate** exposure causing **53,900 cases of obesity** and 20,500 new onset cases of diabetes in older women annually
- **Prenatal BPA** exposure was identified to have a 20–69% probability of causing **42,400 new cases of childhood obesity** annually, with **associated lifetime costs of € 1.54 billion**
- **Phthalate-attributable adult obesity** was the second **largest driver of costs, at € 15.6 billion per year**

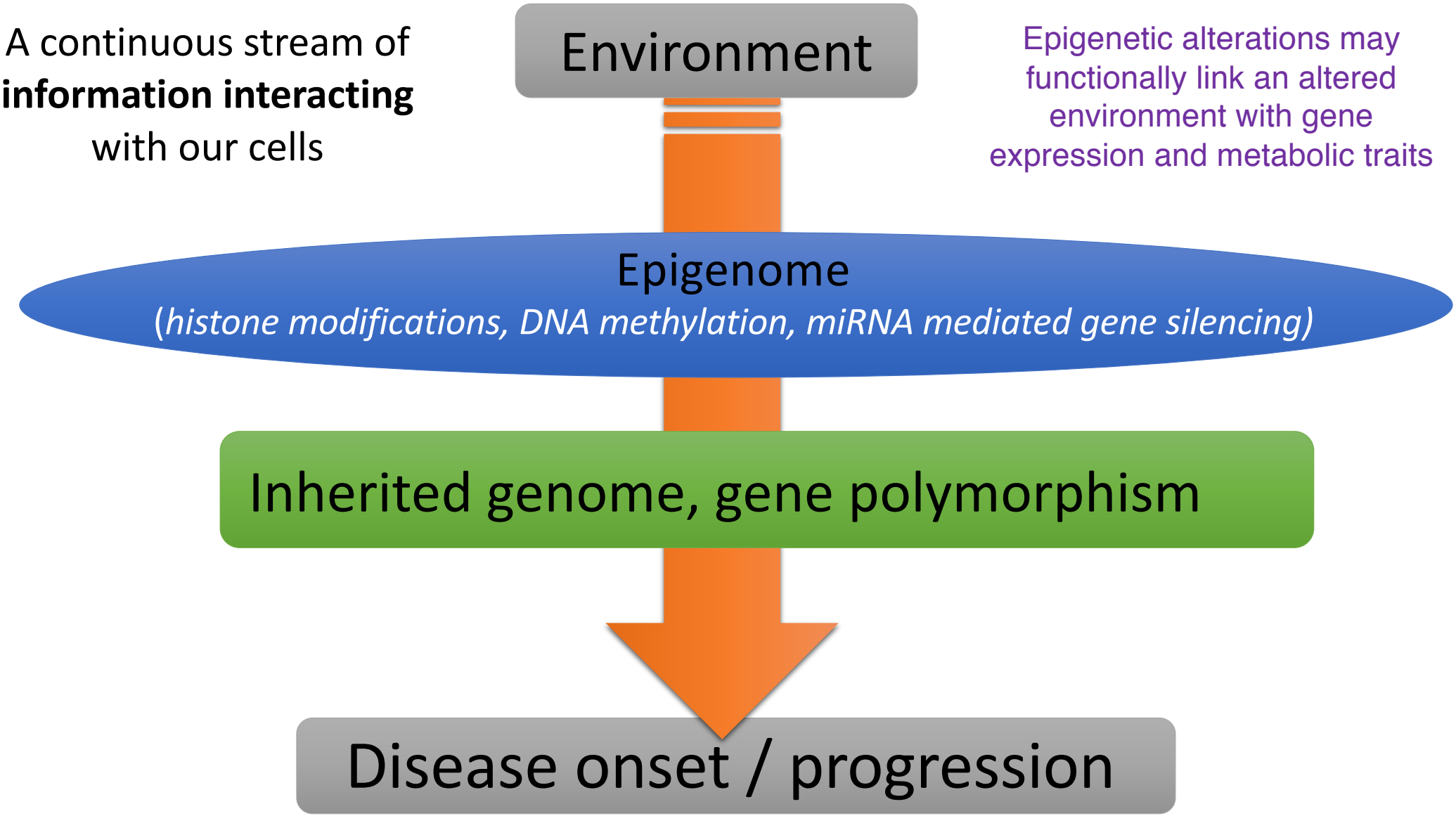
(J Clin Endocrinol Metab 2015)

The obesogenic environment

Fetal life

Elderly

A continuous stream of **information interacting** with our cells



Epigenetic alterations may functionally link an altered environment with gene expression and metabolic traits

Environmental Pollution

Chromatin remodeling by histone tail modifications
(*acetylation, methylation, phosphorylation and ubiquitination)*

- Air pollution
- Endocrine disruptors

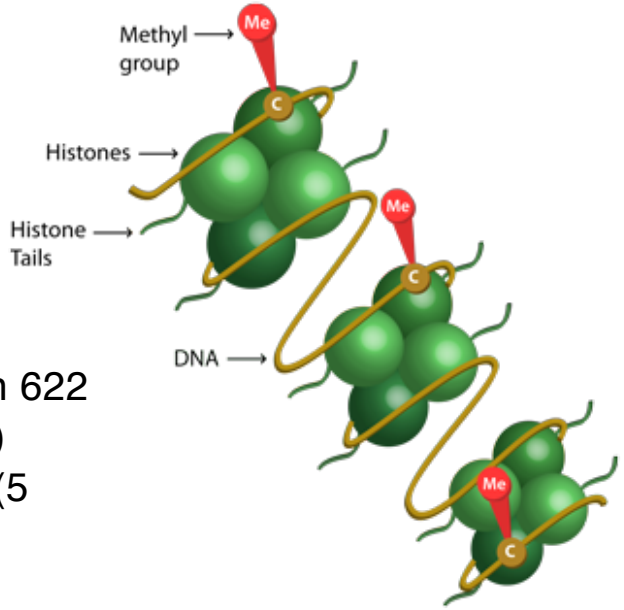
DNA methylation in CpG islands

miRNA mediated gene silencing

- Alter gene expression preceding metabolic diseases (glycolipid homeostasis, hormone dynamics, insulin resistance, chronic low-grade inflammation)
- Alter parental gametes and promote transgenerational transmission of the risk
- Promote fetal programming and late onset of diseases

An epigenome-wide association study identifies multiple DNA methylation markers of exposure to endocrine disruptors

Xueling Lu[a,b,1], Eliza Fraszczyk[a,c,1], Thomas P. van der Meer[d], Martijn van Faassen[e], Vincent W. Bloks[f], Ido P. Kema[e], André P. van Beek[d], Shuang Li[g], Lude Franke[g], Harm-Jan Westra[g], BIOS Consortium, Xijin Xu[b], Xia Huo[h], Harold Snieder[a,*,1], Bruce H.R. Wolffenbuttel[d], Jana V. van Vliet-Ostaptchouk[a,g,i,*,1]



- A blood-based epigenome-wide association study was performed in 622 adult participants from the Lifelines DEEP cohort (The Netherlands)
- Methylation data were linked with EDC excretions in 24-hour urine (5 parabens, 3 bisphenols, 13 metabolites of 9 phthalates)

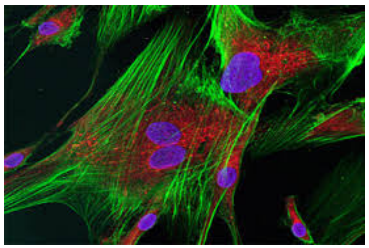
→ 20 differentially methylated cytosine-phosphate-guanines (CpGs) were associated with 10 EDCs

→ 18 out of 20 EDC-associated CpGs were annotated to genes functionally related to metabolic syndrome, hypertension, obesity, type 2 diabetes, insulin resistance

(Lou X et al, Environ Intern 2020)

EDCs

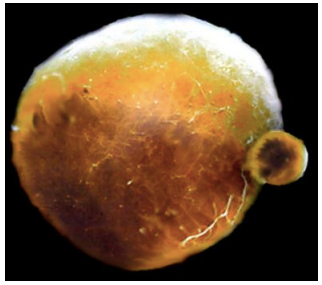
- increased histone deacetylase
- increased/decreased DNA methylation



Multipotent stromal stem cell

- Promotion of the adipogenic lineage
- Increased recruitment of MSCs to the adipose depots
- Transcriptional activation of adipogenic genes
- Regulation of major signaling pathways that commit MSCs or progenitor cells to the adipogenic lineage
- Promotion of the differentiation of pre-adipocytes via direct activation of PPARγ
- Increased lipogenesis, inhibited lipolysis in the adipocyte
- Decreased apoptosis

Adipocyte



Increased Adipocyte formation and increased fat storage

Bisphenol A (BPA)	Orally, during pregnancy and lactation, from GD0 to PND21 (40 μg/kg/day); SD rats	F1: sperm F2: blood, liver	DNA methylation changes in F1 sperm and in F2 liver, although not similar Hypermethylation of Gck promoter and altered gene expression in liver of F2 rats	Li et al. 2014 [40]
	Orally, during pregnancy and lactation, from GD0 to PND21 (40 μg/kg/day); SD rats	F1: sperm F2: blood, pancreatic islets	Pancreatic β-cell dysfunction and glucose intolerance Increased DNA methylation at *Igf2* DMR2 in F1 sperm Decreased Igf2 expression in F1 sperm DNA hypermethylation of *Igf2* in pancreatic islets in the F2 generation	Mao et al. 2015 [41]
	Diet, 2 weeks prior to mating until weaning, 2 doses: 10 μg/kg/day and 10mg/kg/day); C57BL/6J mice	F1: pancreatic islets (16–21 weeks) F2: pancreatic islets (adult)	F1 and F2 males: impaired insulin secretion and increased levels of pro-inflammatory cytokines Dose- and sex-specific effects in gene expression levels related to inflammation and mitochondrial function, in F1 and F2 Altered DNA methylation at *Igf2* DMR1 and increased *Igf2* expression in F1 and F2	Bansal et al. 2017 [42]

Methylation aberrancies in sperm in F1 generation

Maternal BPA exposure during gestation and/or lactation:

- Disrupts glucose homeostasis in F2 offspring rats (*DNA hypermethylation of Glucokinase promoter*)
- Induces beta-cell dysfunction and glucose intolerance in F2 offspring rats (*DNA hypermethylation of Igf2 in pancreatic islets*)
- Impairs insulin secretion, increases levels of proinflammatory cytokines in F1 and F2 offspring rats (*DNA hypermethylation of Igf2 expression*)

(Van Cauwenbergh et al. Clinical Epigenetics 2020)

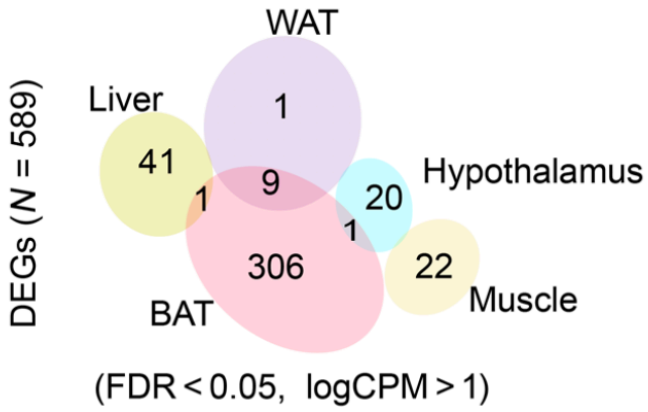
3 wk-old mice observed after a early life exposure to PM2.5 or filtered air, over a 14-week period

- Effects on phenotype, transcriptome, chromatin accessibility
- Comparison with HFD
- Effects of cessation of exposure on phenotype reversibility

PM2.5 exposure:

- Impaired glucose and insulin tolerance
- Reduced energy expenditure
- Reduced glucose uptake in brown adipose tissue
- Increased hepatic cholesterol, lipid deposition and fibrosis
- Induced hepatic steatosis and glycogen depletion
- Induced a marked downregulation of liver adiponectin levels

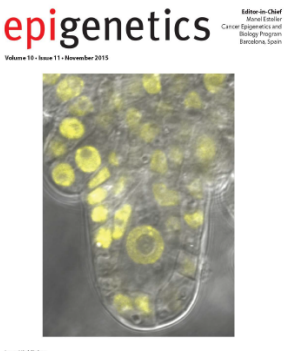
- Several similarities with effects induced by HFD
- Effects linked with multiple differentially expressed genes in White and Brown AT tissues, Liver, Hypothalamus, Muscle



→ Mice exposed to air pollution did not develop adiposity, similar to the human phenotype of lean IR/NAFLD

→ After 8 weeks of cessation of PM2.5 exposure, glucose tolerance and insulin sensitivity improved, with restoration of chromatin accessibility and of exposure-induced changes in the transcriptome

(Rajagopalan et al, J Clin Invest 2020)



- 1,207 adults participating in the Multi-Ethnic Study of Atherosclerosis (MESA)

- Epigenome-wide study of DNA methylation in CD14+ monocytes / long-term ambient air pollution exposure to fine particulate matter (PM2.5) and NO_X (one-year average concentrations of outdoor pollutants estimated at participants' homes)

→ Several differentially methylated regions (DMRs) located on chromosomes 5, 6, 7, and 16 were linked with chronic exposure to PM2.5 and/or NO_X

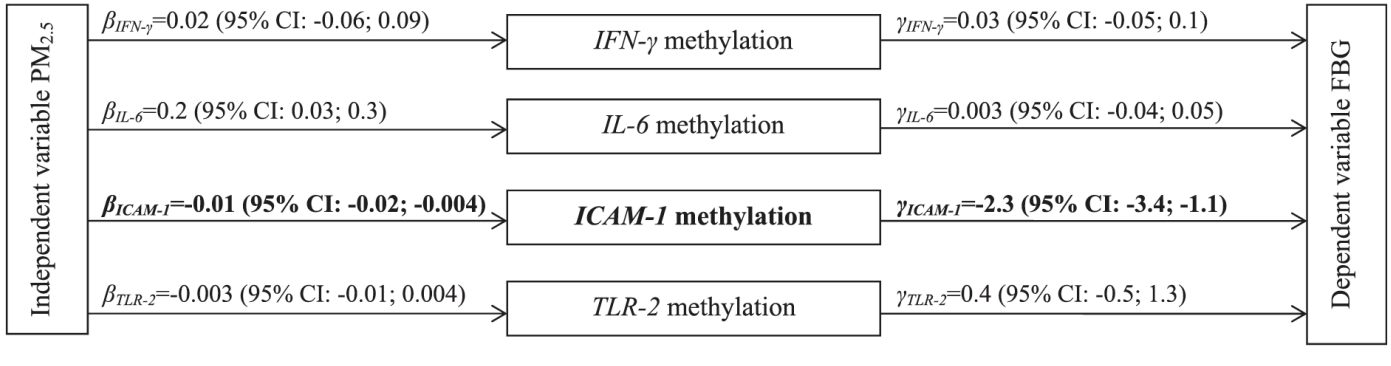
→ DMRs were within or near genes involved in endothelial inflammation, regulation of chronic inflammation, cholesterol homeostasis and mitochondrial dysfunction *(SDHAP3, ZFP57, HOXA5*, *HOXA9*, and *HOXA10, PRM1*, *TOMM20, ZNF347, MRPL36* and *DEXI, ARID4B, IRF2BP2)*

(Chi GC et al, Epigenetics 2021)

Normative Aging Study, 2000–2011

- 551 nondiabetic adults
- Relationships between fasting blood glucose (FBG), DNA methylation of four inflammatory genes (IFN-γ, IL-6, ICAM-1, and TLR-2), short- and medium-term (1-, 7-, and 28-day) exposure to PM2.5

→ short- and medium-term PM2.5 were associated with higher FBG



→ PM2.5 showed a negative association with *ICAM-1* methylation, which, in turn, was negatively associated with FBG

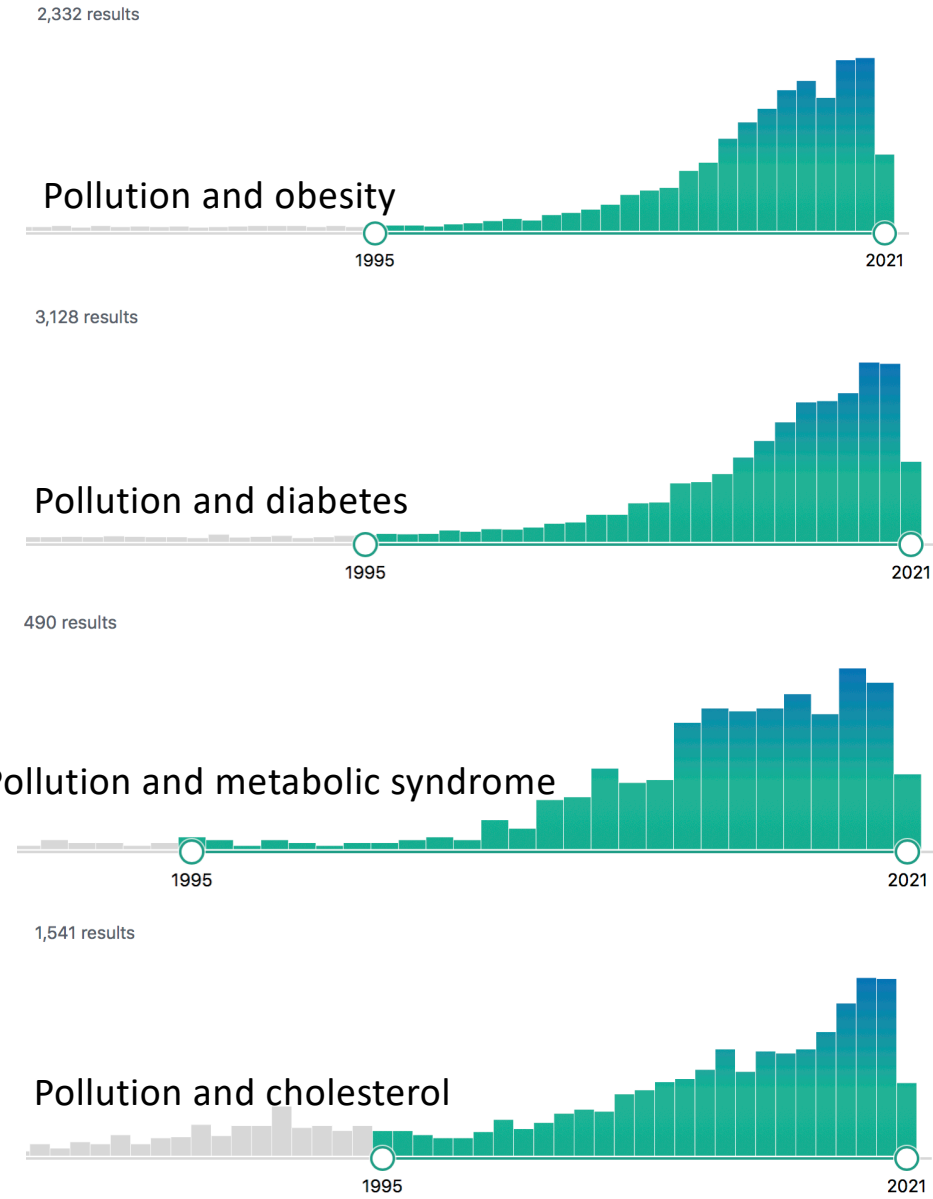
ICAM-1 glycoprotein facilitate migration of leukocytes from the blood to:

- Adipose tissue, resulting in local inflammation and insulin resistance
- Pancreas, possibly affecting beta-cell function and resulting in impaired insulin secretion.

(Peng C et al, EHP 2016)

Experimental, in vitro and animal studies

Epidemiologic and **clinical** studies linking air pollution and EDCs with metabolic diseases as obesity, type 2 diabetes, dyslipidemia, atherosclerosis, metabolic syndrome, NAFLD



Conclusions

- Besides individual food consumption/lifestyle, other widely diffused, involuntary and modifiable environmental factors have a key role in the rapid epidemiological growing of metabolic diseases

- Strong evidences underline the importance of epigenetic mechanisms with trans-generational, short-term and/or later effects both in children and in adults

- Policies extensively acting on the “epigenetic pollution” could significantly improve primary prevention. However, this goal should be reached looking not only to diet and lifestyles but also to a decreased release of toxic chemicals into the environment.


Many thanks for your attention