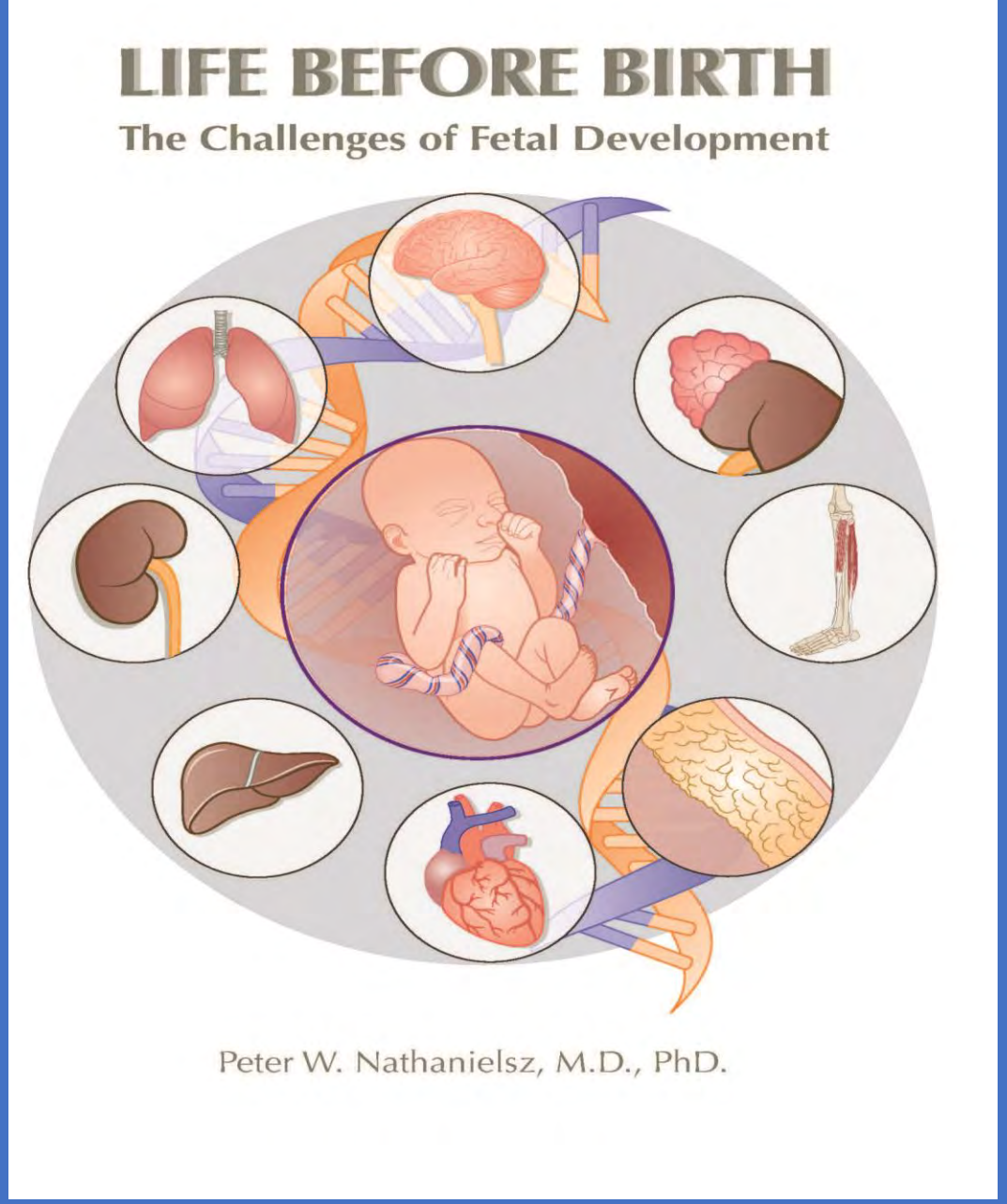
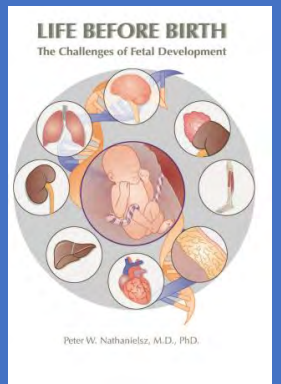




All illustrations showing the cover of *Life Before Birth: The challenges of fetal development* are free for use by anyone as long as the origin from the book is acknowledged.

Why did I write
Life Before Birth: The Challenges of Fetal Development?

This second edition of *Life Before Birth: The Challenges of Fetal Development* updates the wondrous story of the developmental biology that each one of us has passed through and that occurs in our children and grandchildren. Since the first edition, there have been enormous advances in molecular biology, genomics, epigenomics, proteomics and many other omics that have been applied to unlock the secrets of fetal development. For example, Develop[mental Programming was only just appearing over the horizon in the 1990's

However, many other processes still remain fundamental and unchanging. The placenta will always be a fetal lung, a fetal gut, a fetal kidney and a set of endocrine glands producing many hormones.

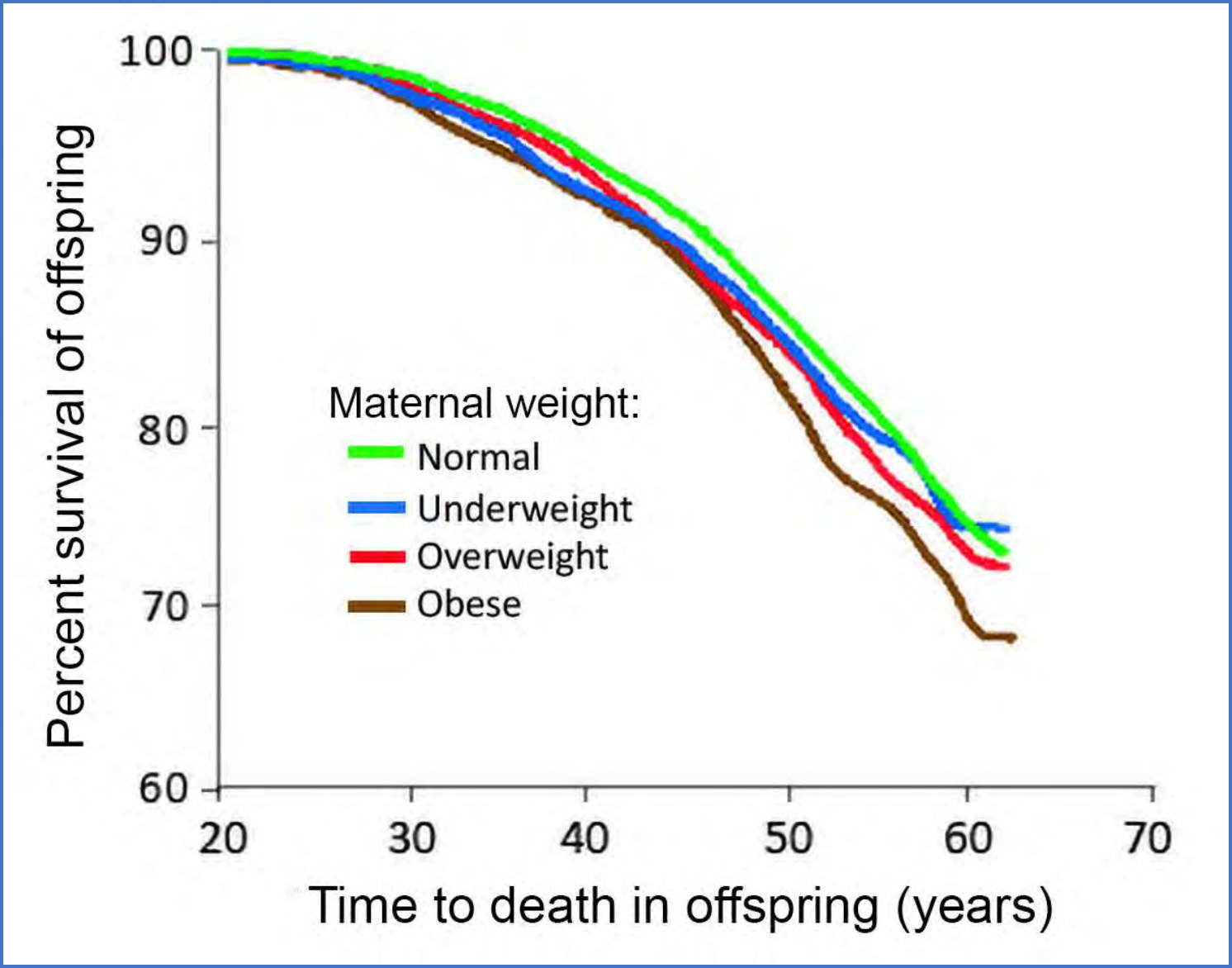
We pass more biological milestones before birth then we pass in the rest of our lives. Is it surprising that how we pass those milestones will determine the health we will enjoy for a lifetime? From a single cell at fertilization, the fetus produces a liver, heart, brain, kidneys and a myriad of other organs. As an added challenge the fetus must function in one environment, the uterus, while at the same time preparing to live in a completely different one, the outside world.

Developmental Programming.

Developmental programming is the fetal response to a specific challenge during a critical developmental time window that alters organ development with resulting effects on offspring' health that can persist throughout life.

Challenges that result in fetal programming include – maternal undernutrition, overnutrition, stress and now we know environmental pollution. Recent studies have also shown effects of paternal under or over nutrition, as we shall see.

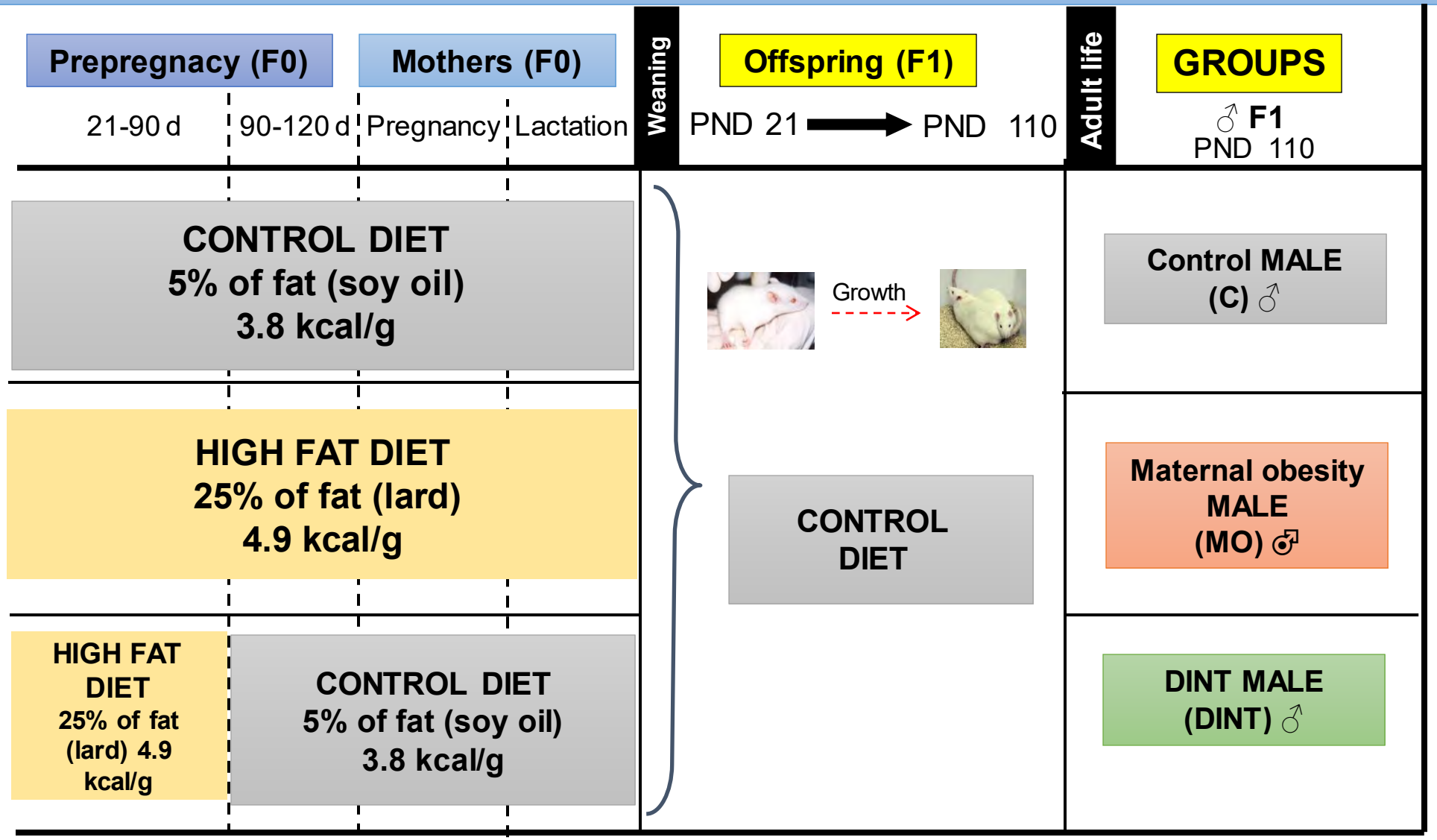
Programming by Maternal Obesity and Overnutrition.



Death rates in humans according to different levels of obesity in their mothers.

Adapted from Reynolds, BMJ. 2013 13;347

Programming of rat offspring by maternal obesity: effect of maternal dietary intervention (DINT).

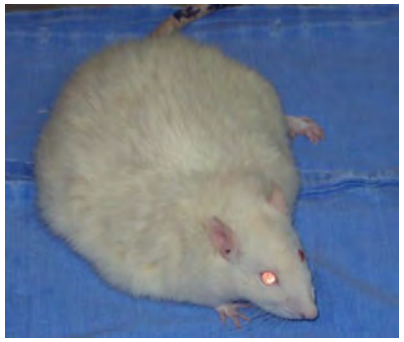


CONTROL n=6; MO n=5; DINT n=5.

In the setting of maternal obesity the offspring liver may be vulnerable to steatosis because immature fetal adipose depots cannot buffer the excess of transplacental lipid delivery from the obese mother

F0

Maternal obesity



Excess weight gain
Excess lipids/glucose

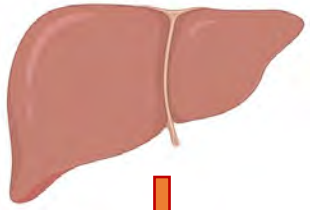
↓

Insulin resistance
Increases fuel to the fetus

↓

F1

Fetal liver



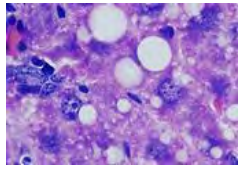
Hepatic fat accumulation

↓

Ongoing fat accumulation

F1

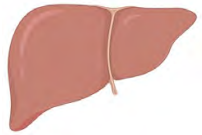
Childhood and Adult life

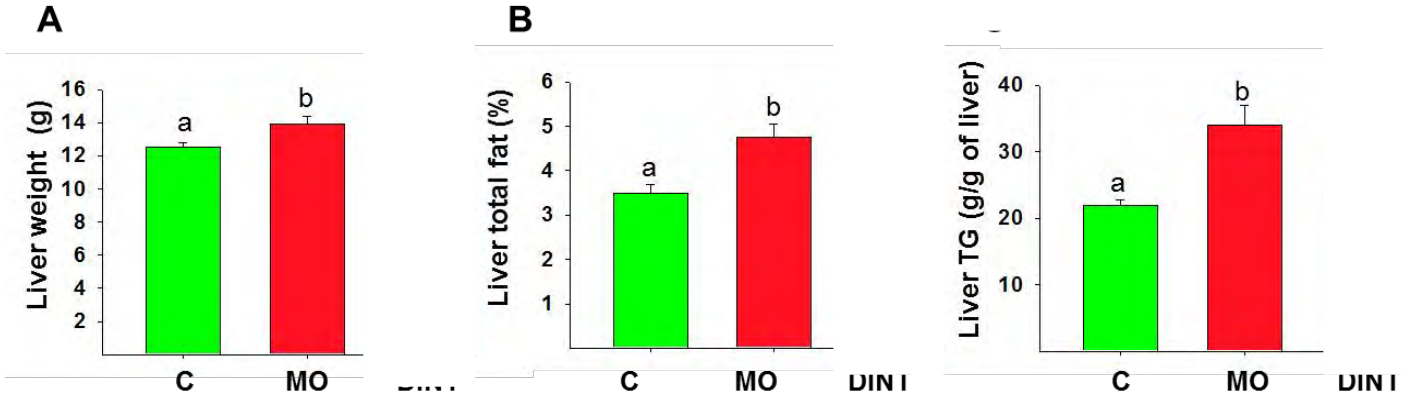


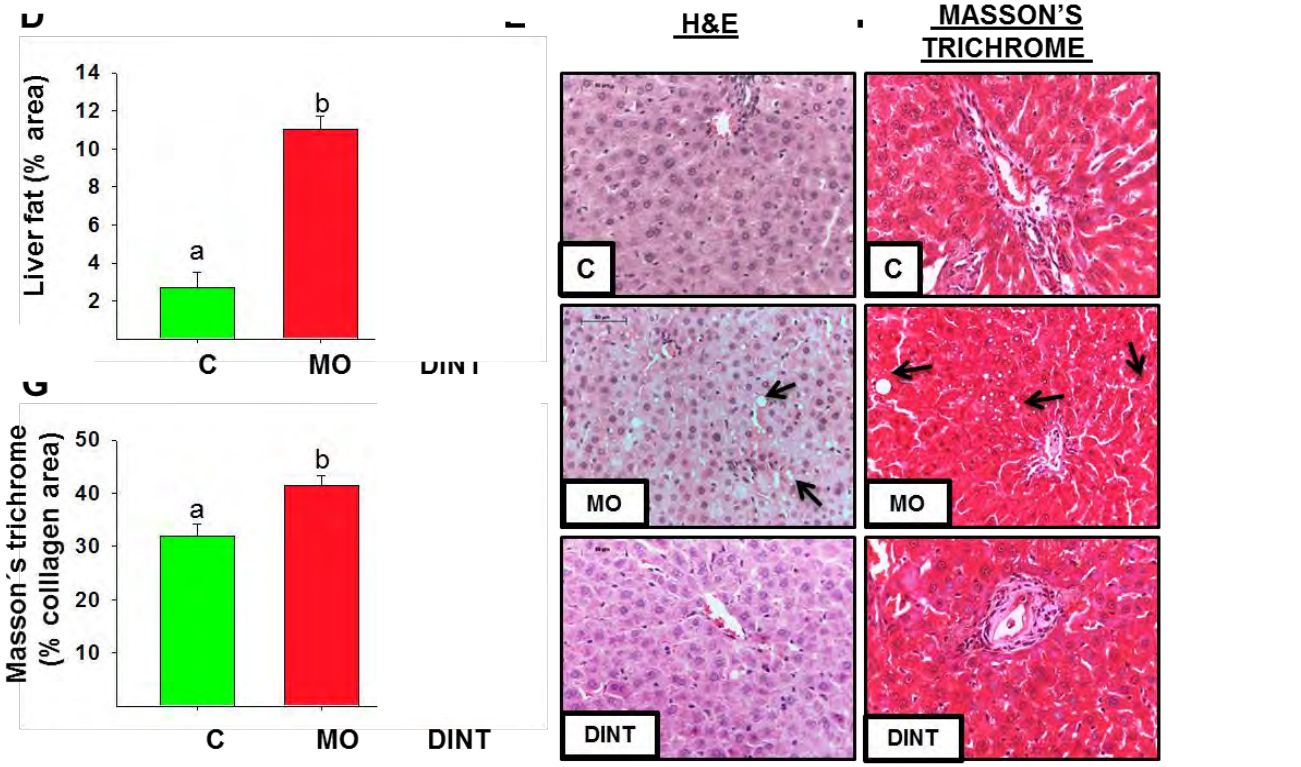
FATTY LIVER
metabolic alterations

Increase *de novo* lipogenesis
Reduce FA oxidation

MALE F1 LIVER MEASUREMENTS AND STAINING – EFFECT OF MO ONLY AT 110 DAYS







Data M ± SEM, *n = 5-8* rats per group. *P* <0.05 for different letters. Arrows indicates lipid droplets.

Hypothesis

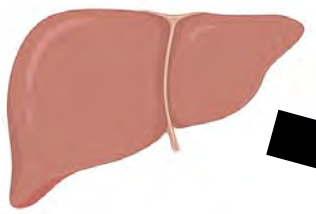
Dietary intervention (DINT) by transferring MO rats back to normal chow one month before pregnancy would prevent adverse F1 male outcomes. This type of intervention is of great clinical importance.

MALE F1 OUTCOME MEASUREMENTS AT 110 DAYS POSTNATAL LIFE

CONTROL

MATERNAL OBESITY

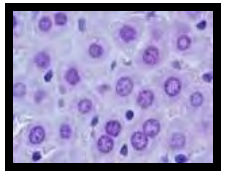
DINT



FOLCH

Liver content of fat
Liver triglycerides

Histology



H&E staining
Masson staining

FROZEN TISSUE (-70 °C)

Isolate RNAs

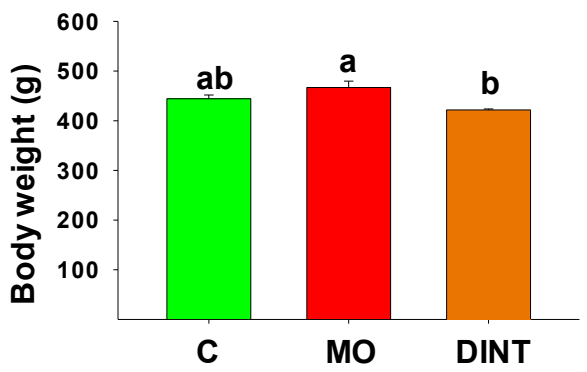
RNAseq

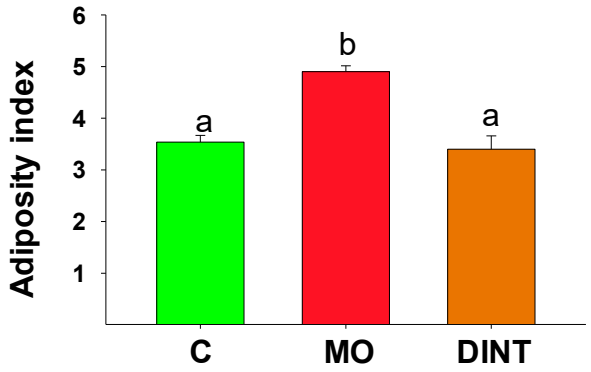
Expression profile

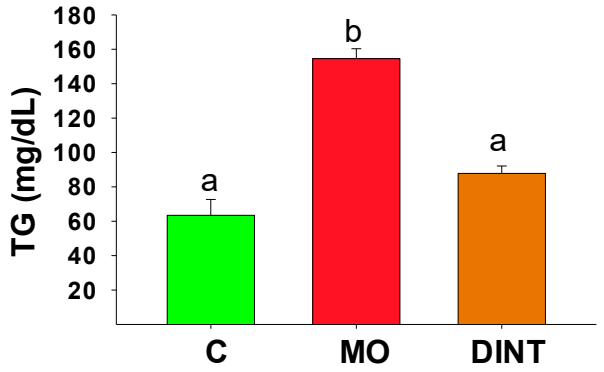
Differentially expressed transcripts

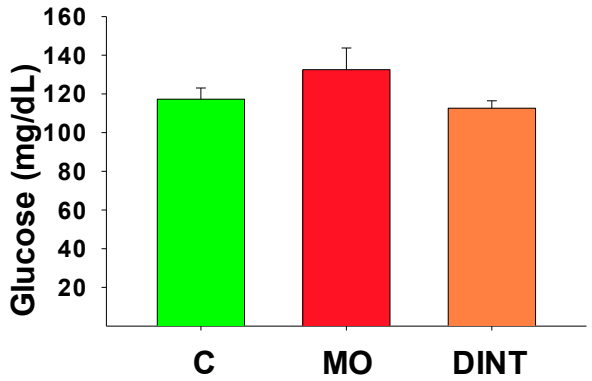
Cellular functional/pathway analysis

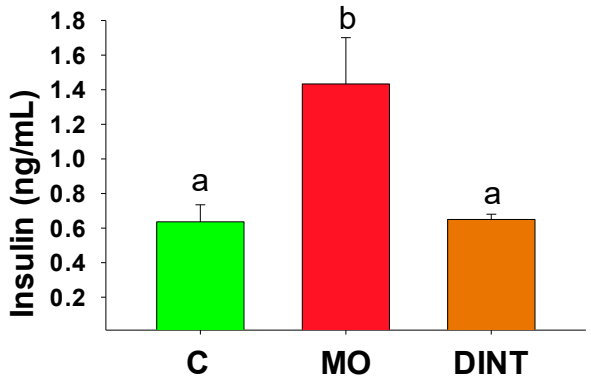
F1 MALE METABOLISM AT 110 DAYS POSTNATAL LIFE

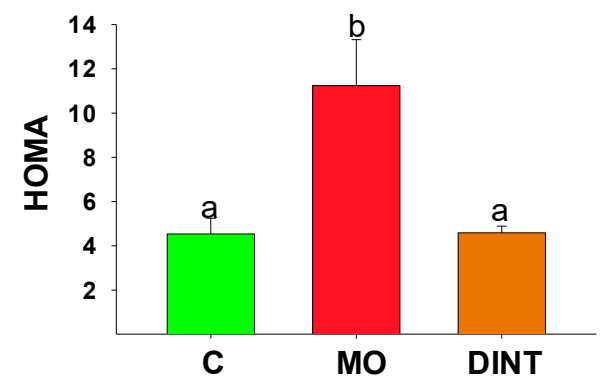












Data are mean ± SEM. $p < 0.05$ for different letters within sex, n=6-8.

MALE F1 liver differentially expressed genes at 110 days postnatal life

COMPARISON	# DOWN	# UP
* MO 110 vs C 110 d	1,317	48
DINT 110 vs MO 110 d	50	94
DINT 110 vs C 110 d	111	66

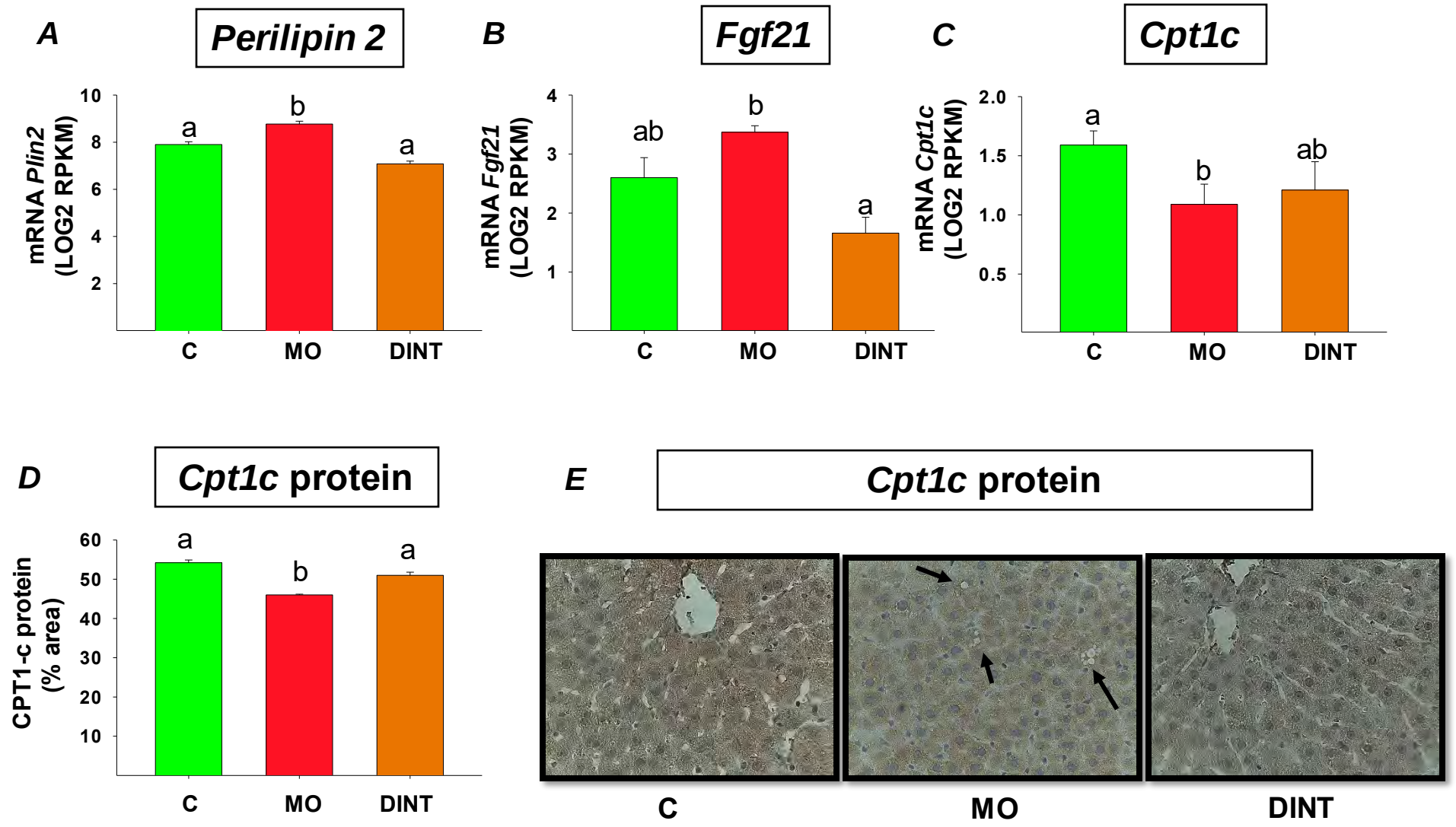
CONTROL n=6; MO n=5; DINT n=5.

* Lomas-Soria, C. PMID: 29972240.

MALE KEGG PATHWAYS IN THE COMPARISON DINT vs MO

KEGG pathway	P Value	DOWN	UP
Biosynthesis of unsaturated fatty acids	0.002	3	0
Fatty acid elongation	0.002	3	0
Thyroid cancer	0.002	1	2
PPAR signaling pathway	0.003	4	0
Bladder cancer	0.003	1	2
Fatty acid degradation	0.004	3	0
Vascular smooth muscle contraction	0.012	1	3
Pl3K-Akt signaling pathway	0.012	3	4
Focal adhesion	0.015	2	3
Maturity onset diabetes of the young	0.016	1	1
Apelin signaling pathway	0.018	0	5
Circadian rhythm	0.019	1	1
Hypertrophic cardiomyopathy (HCM)	0.023	0	3
Dilated cardiomyopathy (DCM)	0.027	0	3
Tight junction	0.033	0	4
Pathways in cancer	0.034	3	5
Cell adhesion molecules (CAMs)	0.036	0	4

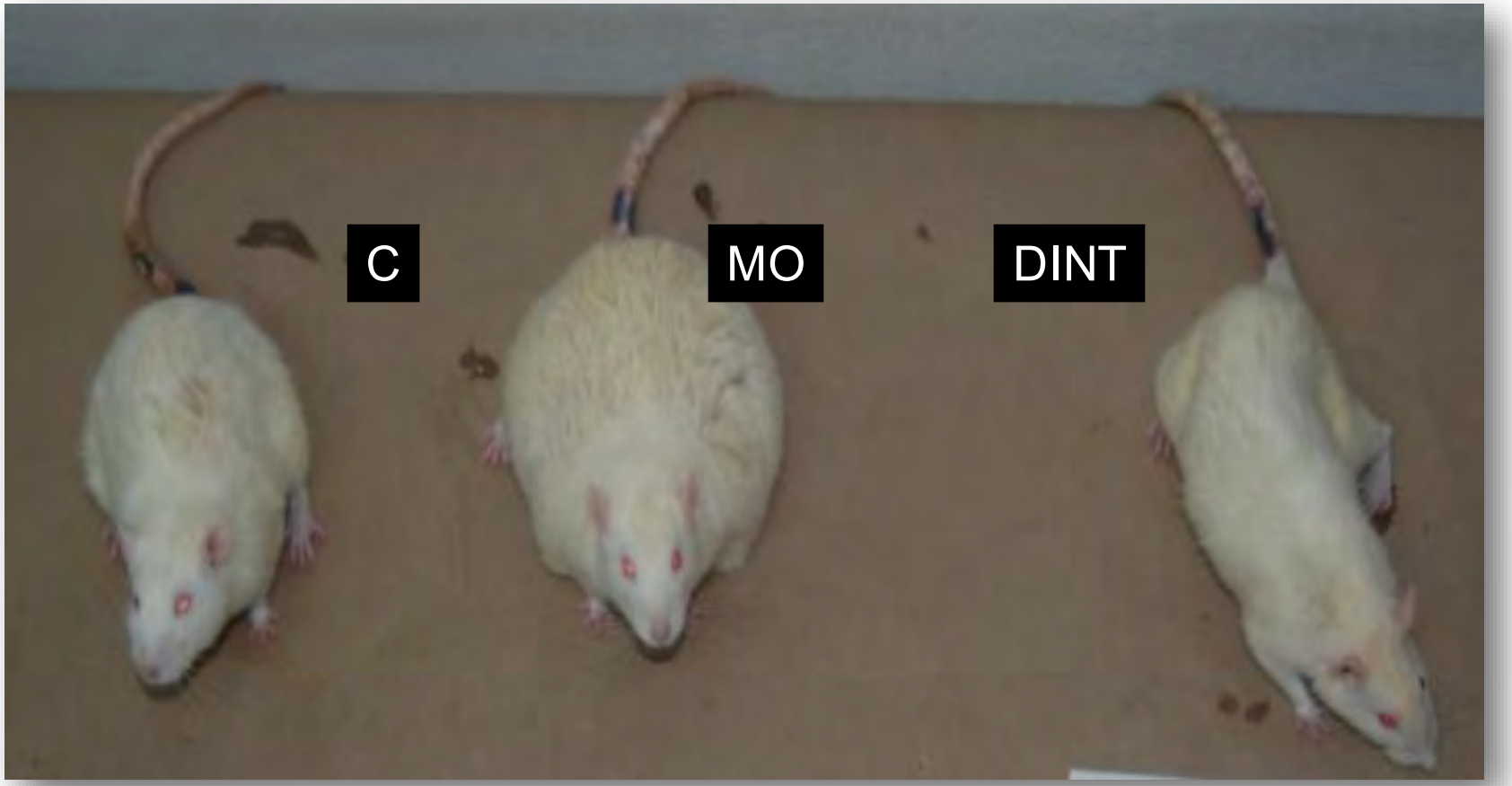
Male F1 liver differentially expressed genes at 110 days postnatal life

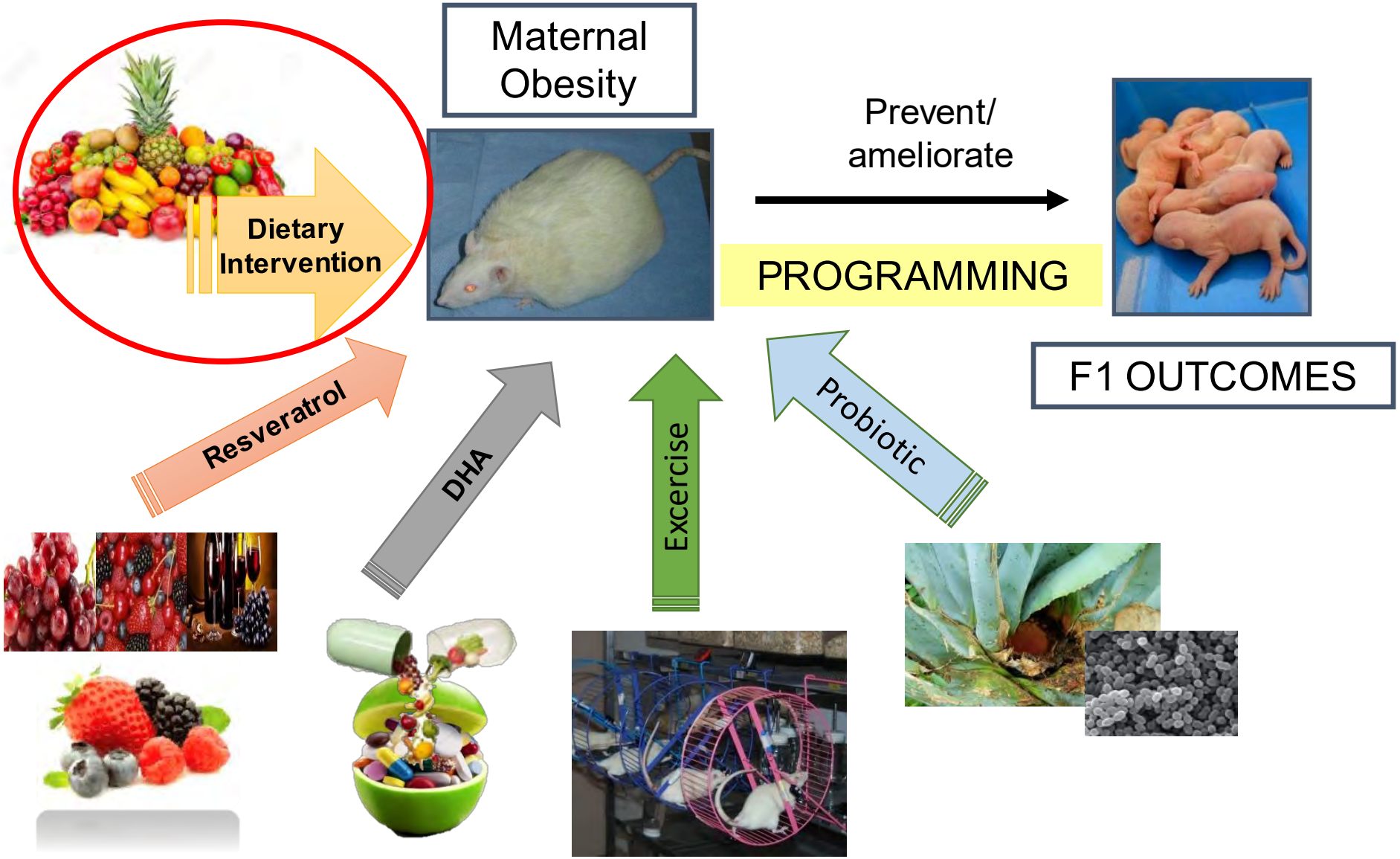


A, perilipin 2, ***B***, Fibroblast growth factor 21 and ***C***, Carnitine Palmitoyltransferase 1C, gene expression and ***D***, Cpt1-c protein, ***E*** representative immunohistochemical images. $P < 0.05$ for data not sharing a letter. n=5-6 rats from different litter.

CONCLUSIONS RELATING TO EFFECTS OF MATERNAL DIETARY INTERVENTION

Maternal obesity programs offspring liver fat accumulation, changes in hepatic gene expression levels, which leads to a negative impact on metabolism (PMID:29972240). DINT prevents these outcomes, mainly through pathways associated with lipid metabolism and improvement of mitochondrial function.





DIFFERENT DIETARY INTERVENTIONS

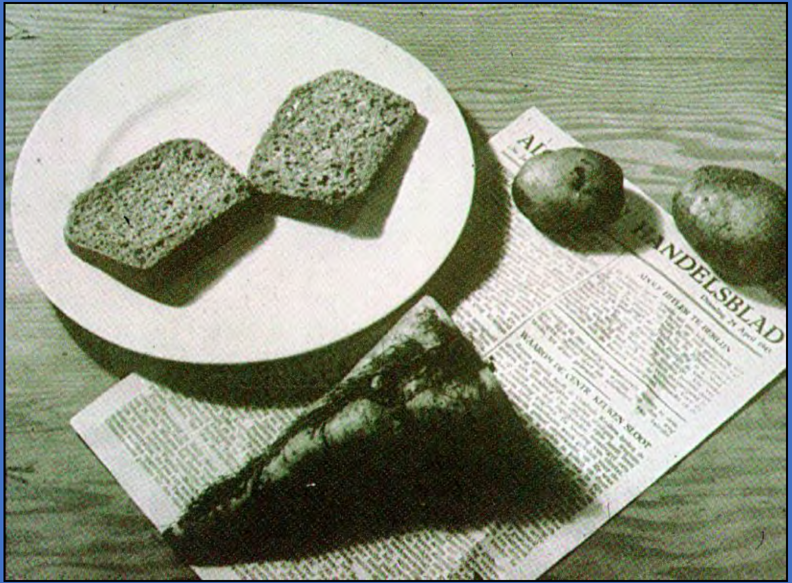
Zambrano E., PMID: 20351043; *Nathanielsz P.*, PMID: 24147928

Programming by Maternal undernutrition.

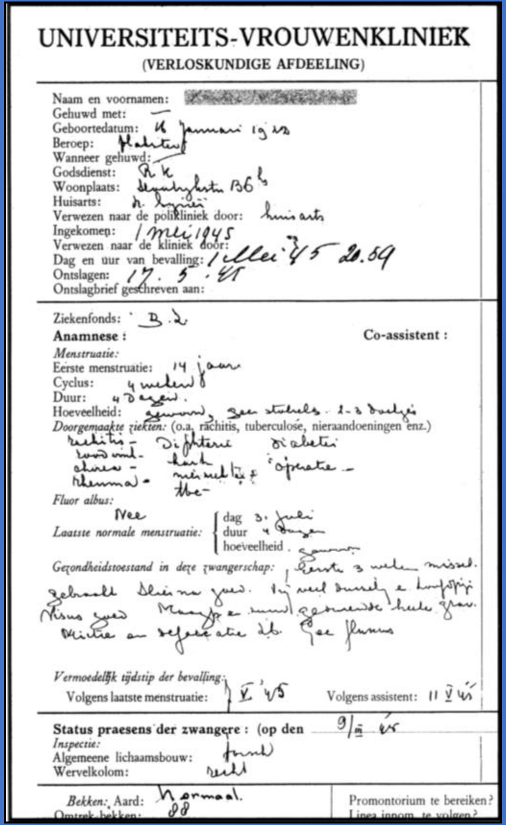
The Dutch Famine, often called the Dutch Hunger Winter, is an important human historical famine event that occurred at the end of the Second World War 1944. It resulted in life-course programming of health of affected Dutch women and men as a result of reduced maternal nutrition.

The Dutch kept good records of pregnancies and details of the life-course health of the babies born around the time f the famine.

Average diet was 400 calories per day.



Daughters of the Dutch Hunger Winter



UNIVERSITEITS-VROUWENKLINIEK
(VERLOSKUNDIGE AFDEELING)

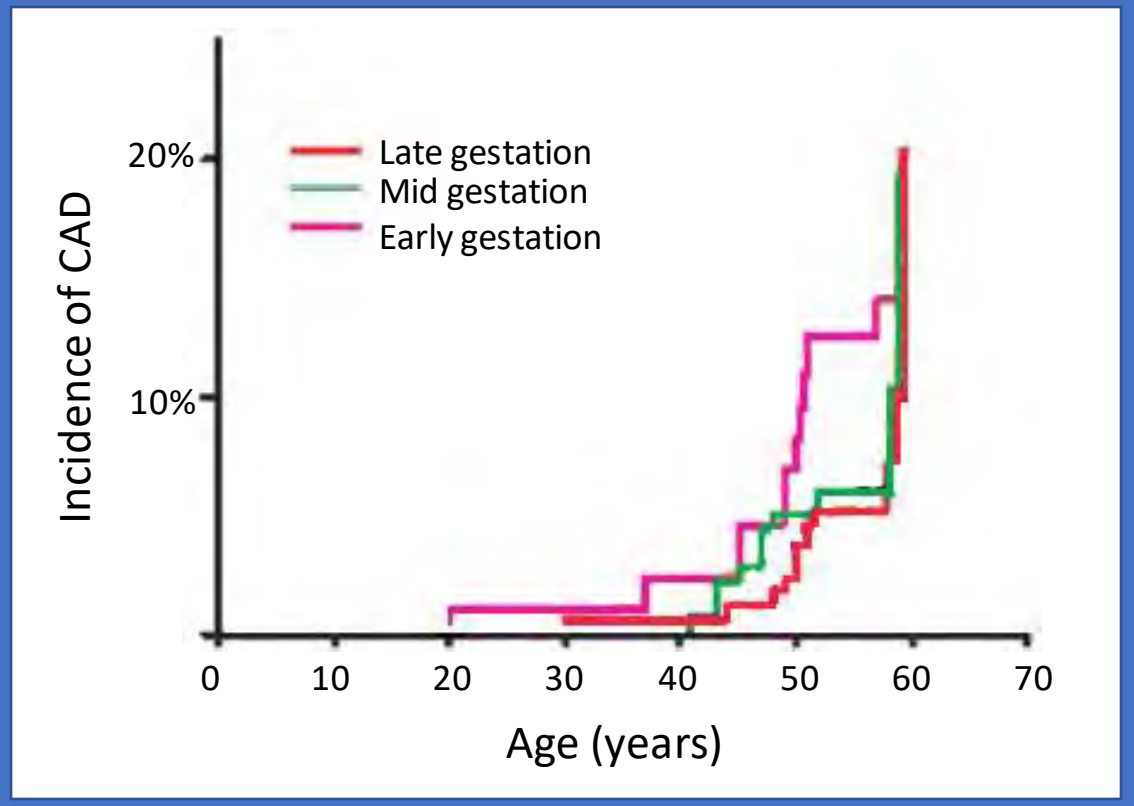
Naam en voornamen: [redacted]
Gehuwd met:
Geboortedatum:
Beroep:
Wanneer gehuwd:
Godsdienst:
Woonplaats:
Huisarts:
Verwezen naar de polikliniek door:
Ingekomen:
Verwezen naar de kliniek door:
Dag en uur van bevalling:
Ontslagen:
Ontslagbrief geschreven aan:

Ziekenfonds:
Anamnese: Co-assistent:
Menstruatie:
Eerste menstruatie:
Cyclus:
Duur:
Hoeveelheid:
Doorgemaakte ziekten: (o.a. rachitis, tuberculose, nieraandoeningen enz.)
Fluor albus:
Laatste normale menstruatie: dag / duur / hoeveelheid
Gezondheidstoestand in deze zwangerschap:
Vermoedelijk tijdstip der bevalling:
Volgens laatste menstruatie: Volgens assistent:

Status praesens der zwangere: (op den
Inspectie:
Algemeene lichaamsbouw:
Wervelkolom:
Bekken: Aard: Promontorium te bereiken?
Omtrek bekken: Linea innom. te volgen?

These are studies by the Dutch Group that has extensively studied the children of the Dutch Hunger Winter, especially Rebecca Painter and Tessa Roseboom.

Premature incidence of Coronary Artery Disease (CAD) in the offspring of the Dutch Hunger Winter.



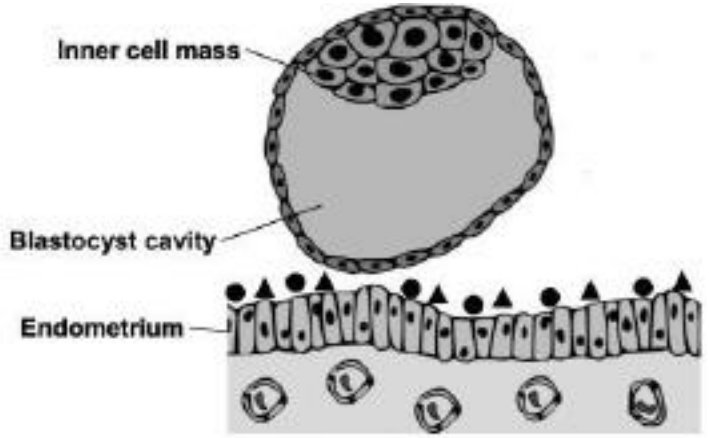
Painter RC et al Am J Clin Nutr 2006;84:322–7.

Cumulative incidence of CAD in persons exposed to famine in late, mid, or early gestation. The cumulative incidence of CAD was greater following early gestation famine exposure.

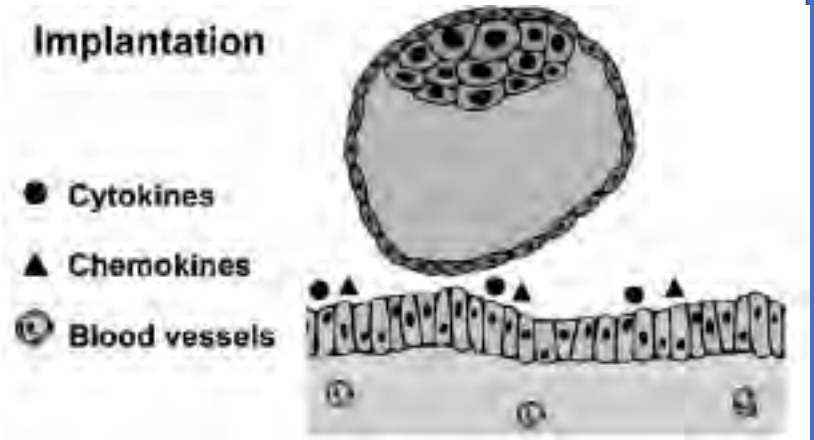
Programming of fetal development does not only occur in response to maternal challenges. We can observe unwanted changes in the uterus of female mice mated with protein deficient male mice. Offspring of protein deficient male mice suffer from a higher incidence of cardiovascular disease and diabetes than offspring of well-fed male mice.

Uterus

- **Decreased area of uterine blood vessels**
- **Proinflammatory cytokines and chemokines reduced**



Control father



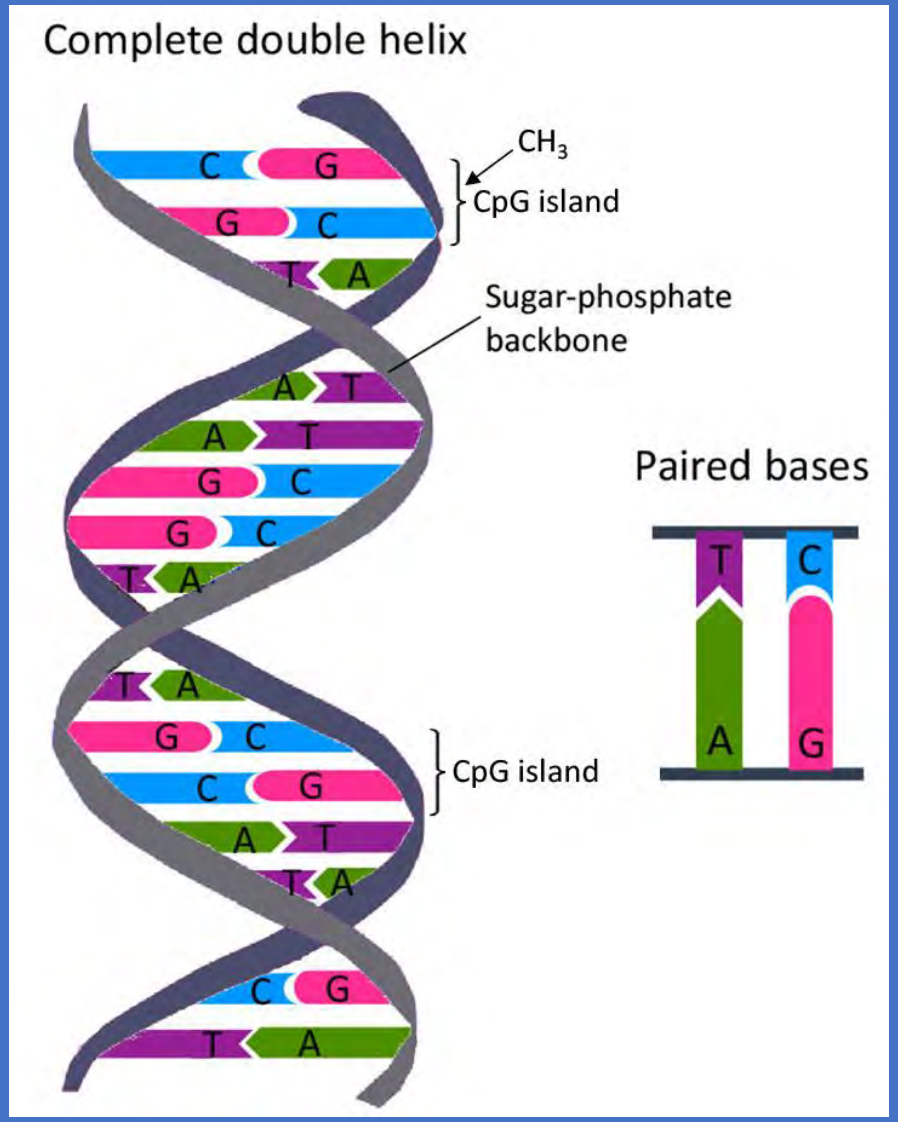
Protein deficient father

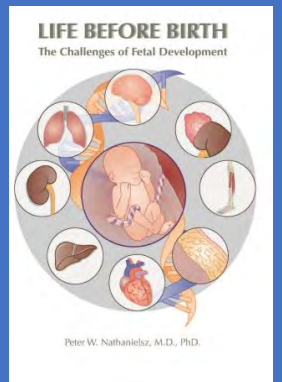
NATURE AND NURTURE -
WE ARE THE PRODUCT OF A COMBINATION OF BOTH GENETICS AND EPIGENETICS

The environment in which the fetus develops modifies the fetal genetic plan by gene-environment interactions to convert our genome into our epigenome.

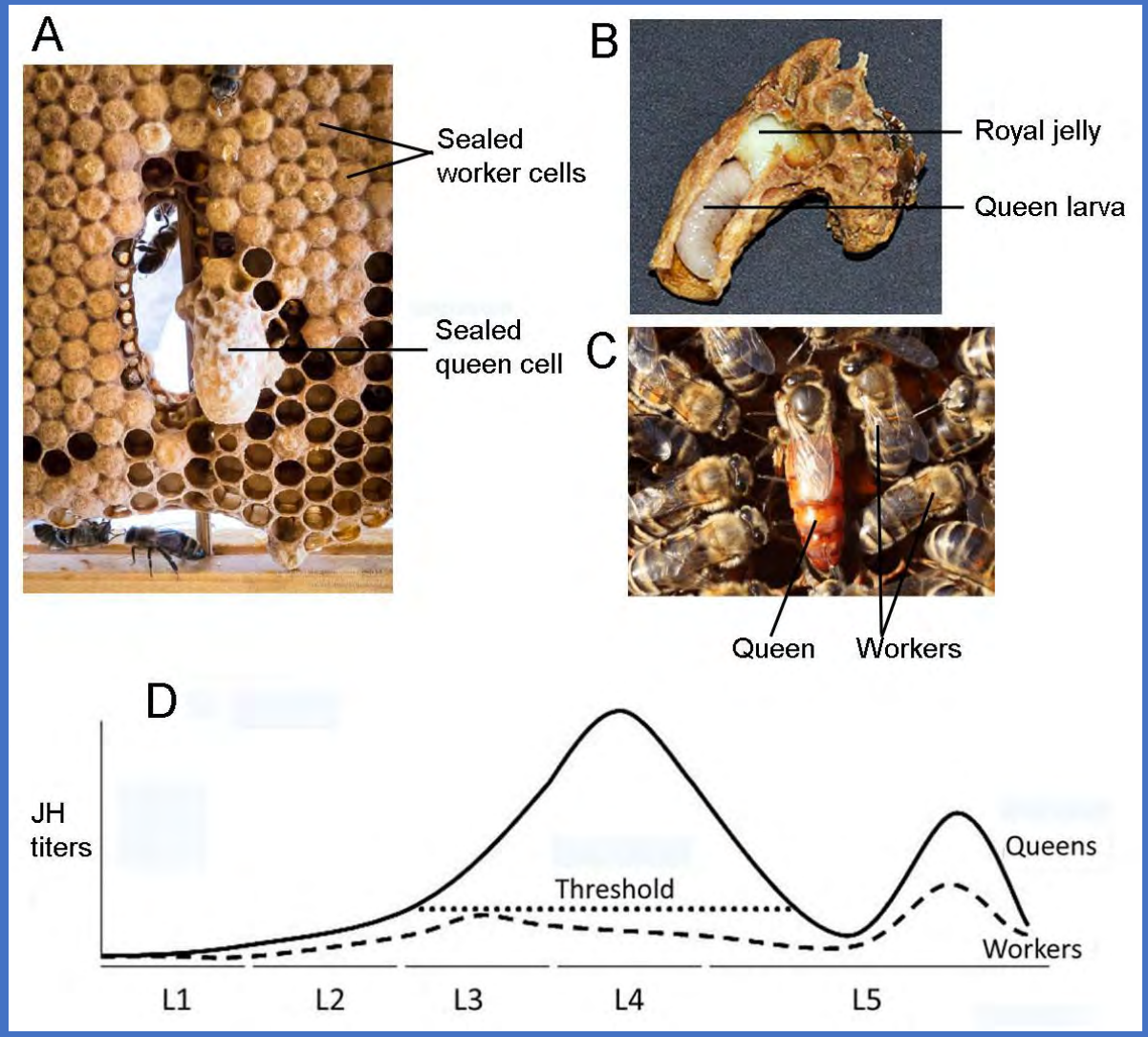
Feeding extra folic acid in a pregnant mouse' diet increases gene methylation and changes the coat colour of offspring in her litter

Methylation of genes alters their activity – usually decreasing activity.



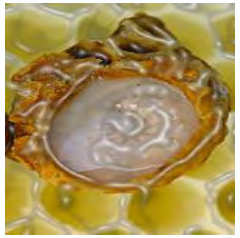


Nutrigenomics in the honeybee



Programming of longevity by royal jelly in honeybee larvae

Larvae



Royal jelly:
Amino acids
Vitamins
Lipids
12% sugar

Life span

Queen

Both jellies have different kind of protein

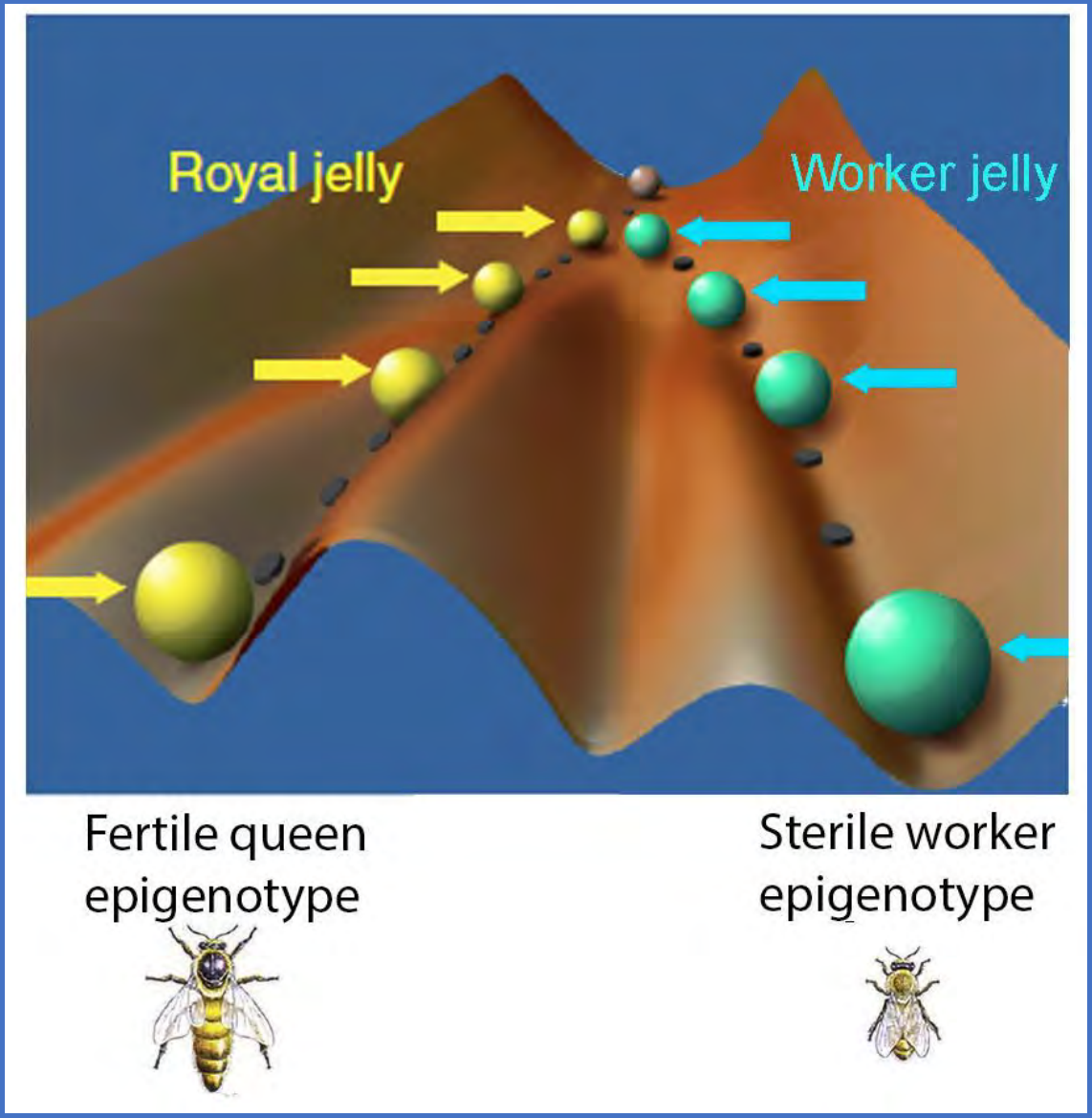
Worker

Worker jelly:
Glandular secretions
Honey
Pollen
4% sugar

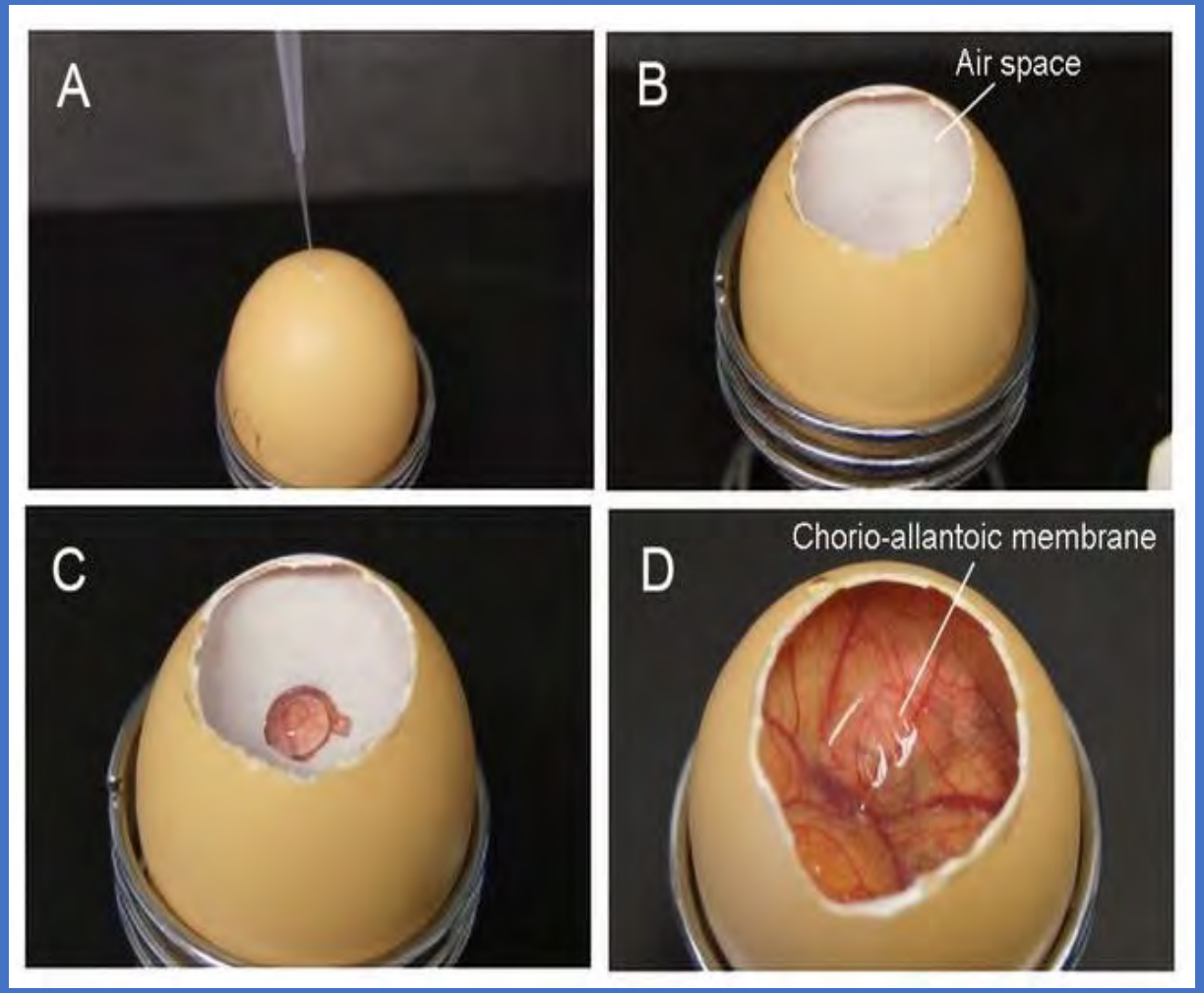
Life span

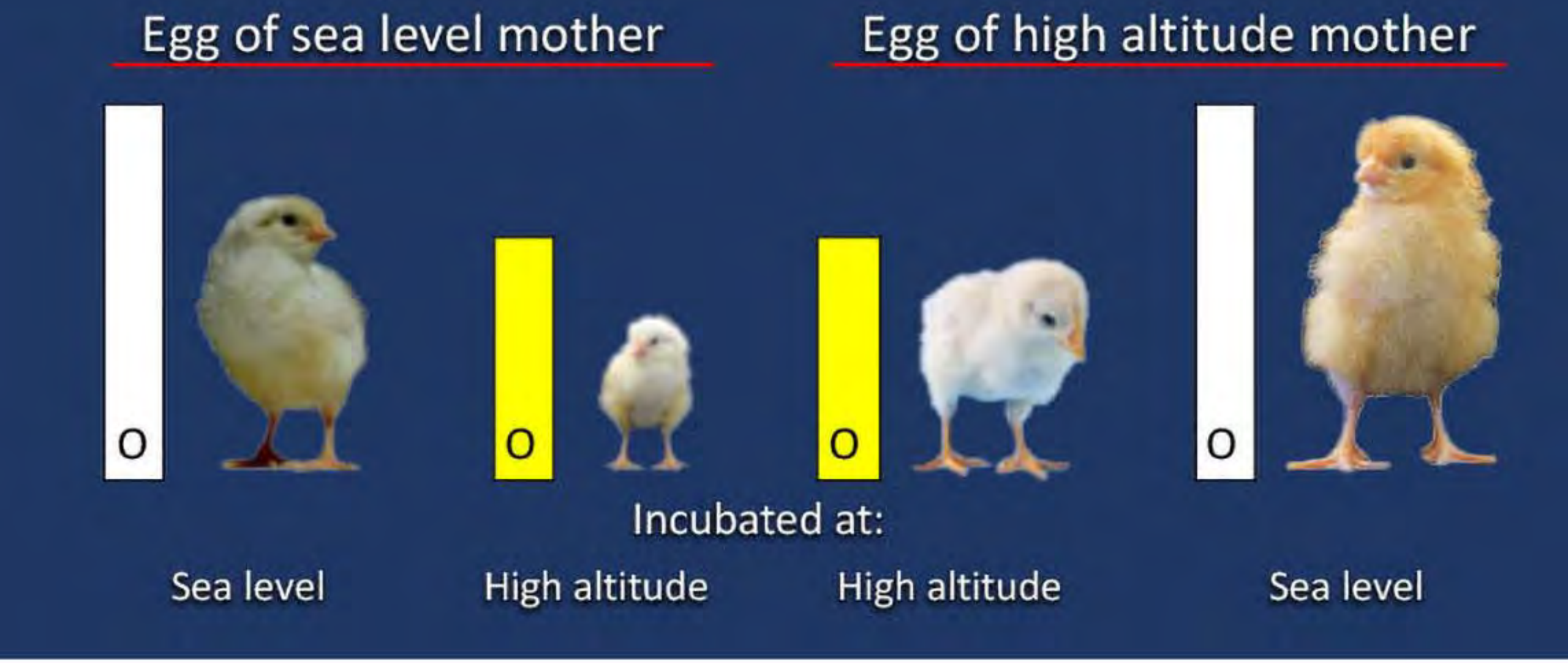
Larvae eating Royal Jelly become Queen honeybees who live longer than worker bees who do not receive royal jelly during development an excellent example of *Programming Aging interactions* by a nutritional stimulus

Waddington’s epigenetic landscape



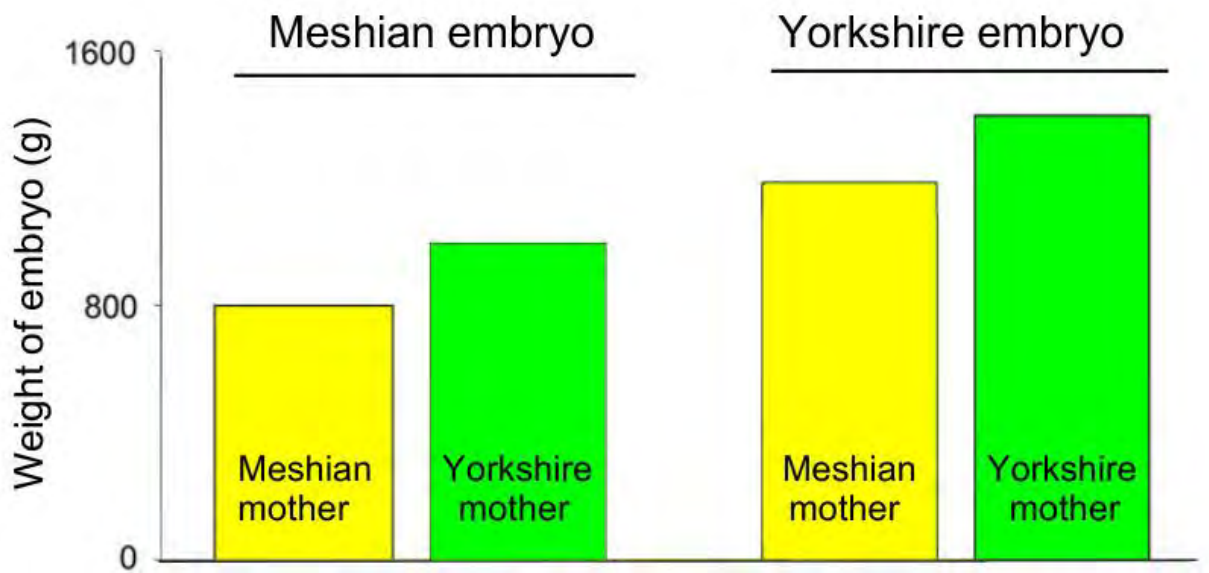
Fetal Hypoxia and Developmental Programming: **Oxygenation of the chicken embryo inside the egg**

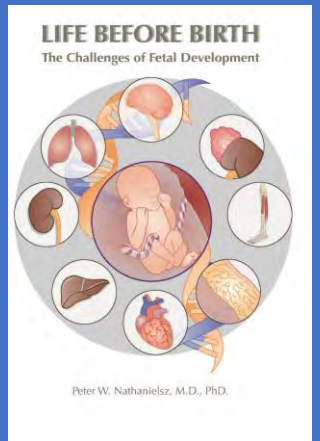




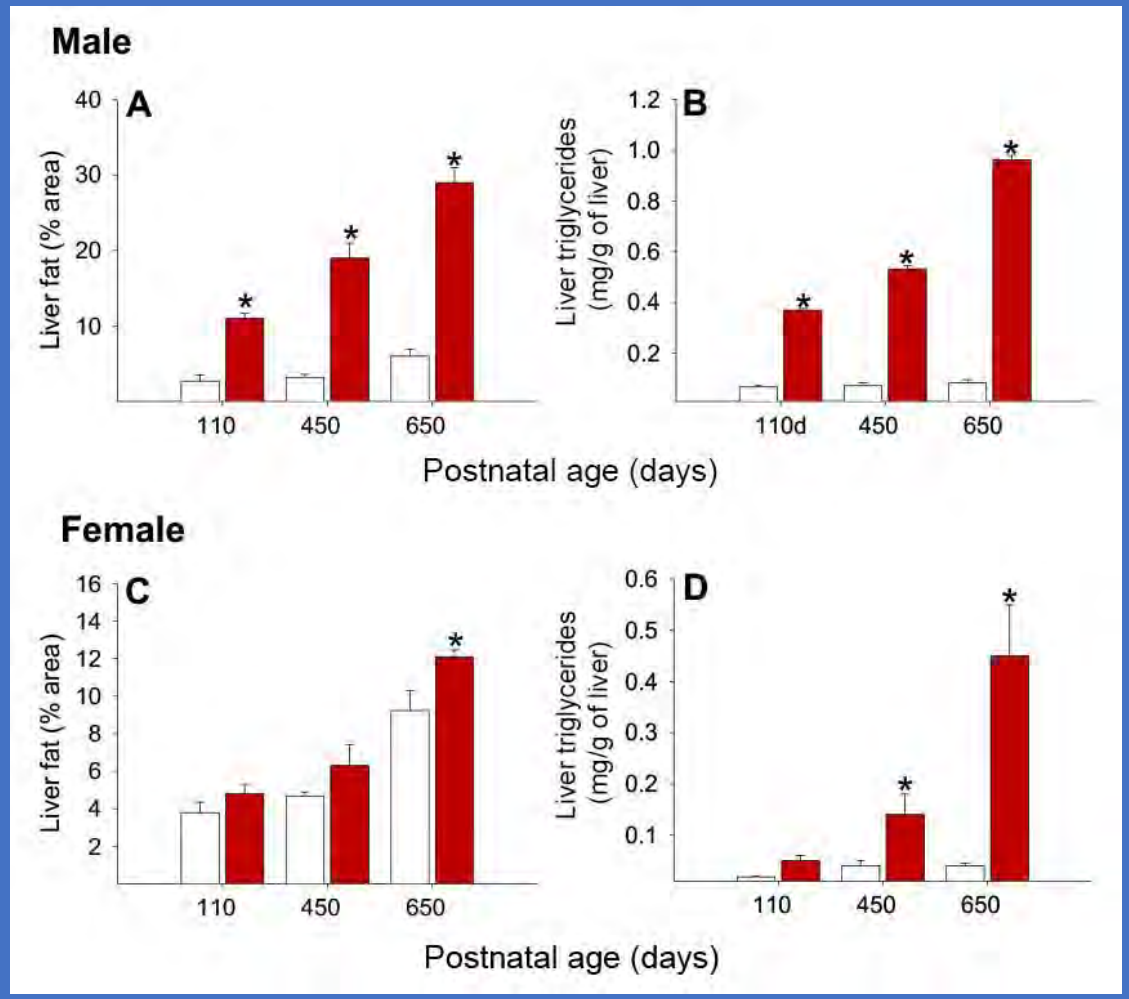
Effect of high-altitude hypoxia on embryo growth. White and yellow columns show level of oxygen (O) in the chicken embryo blood at sea level and high altitude respectively.

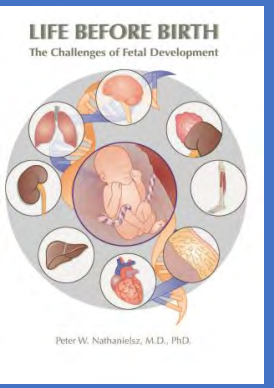
Both local uterine environment and embryo's genetic complement affect fetal growth. Embryo transfer study by Stephen Ford on birthweight of large Yorkshire pigs and small Chinese Meshian pigs.





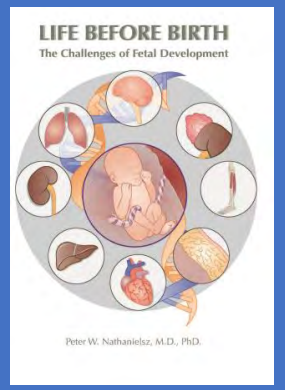
The age-related rate of rise of and female liver fat and triglycerides across the life course is increased by maternal obesity.

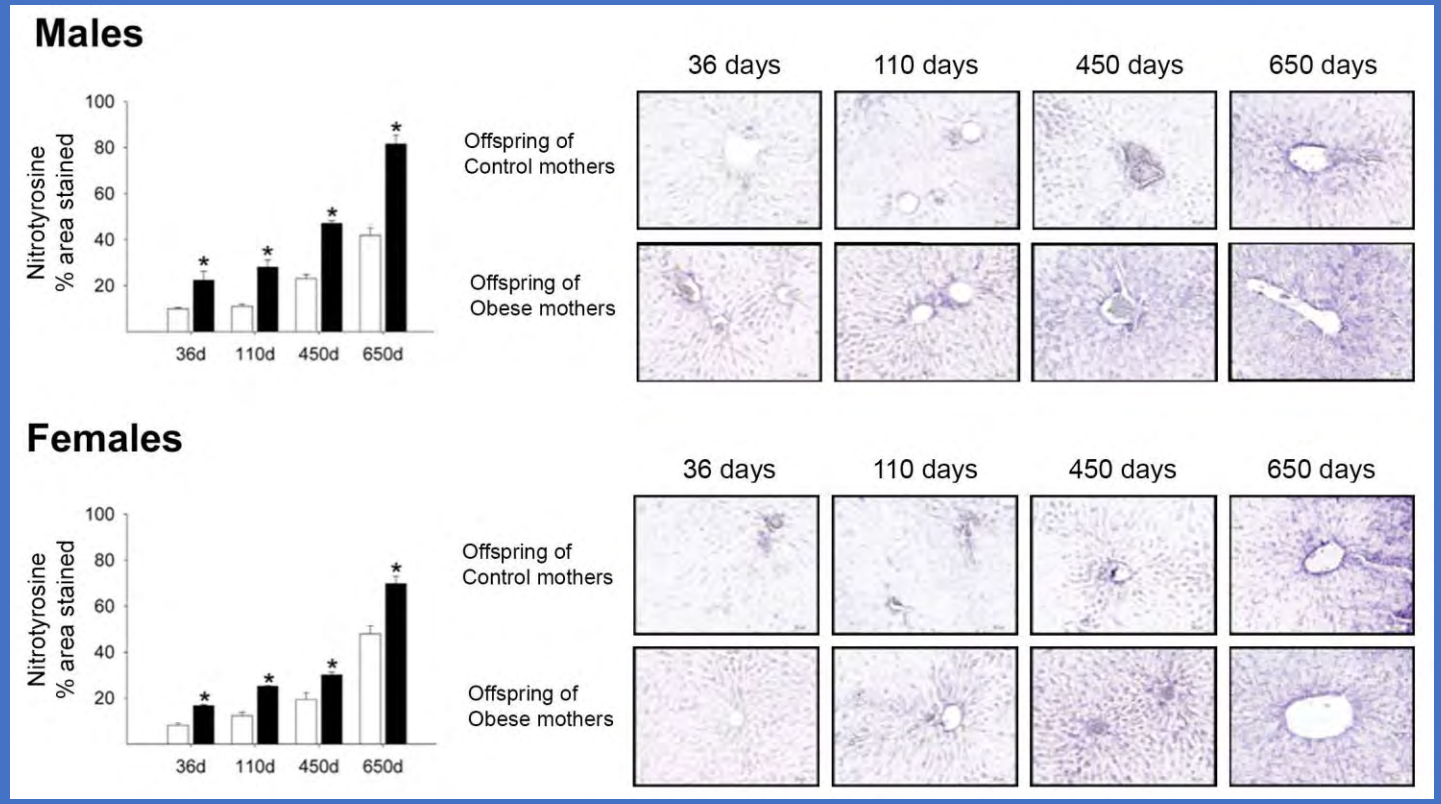




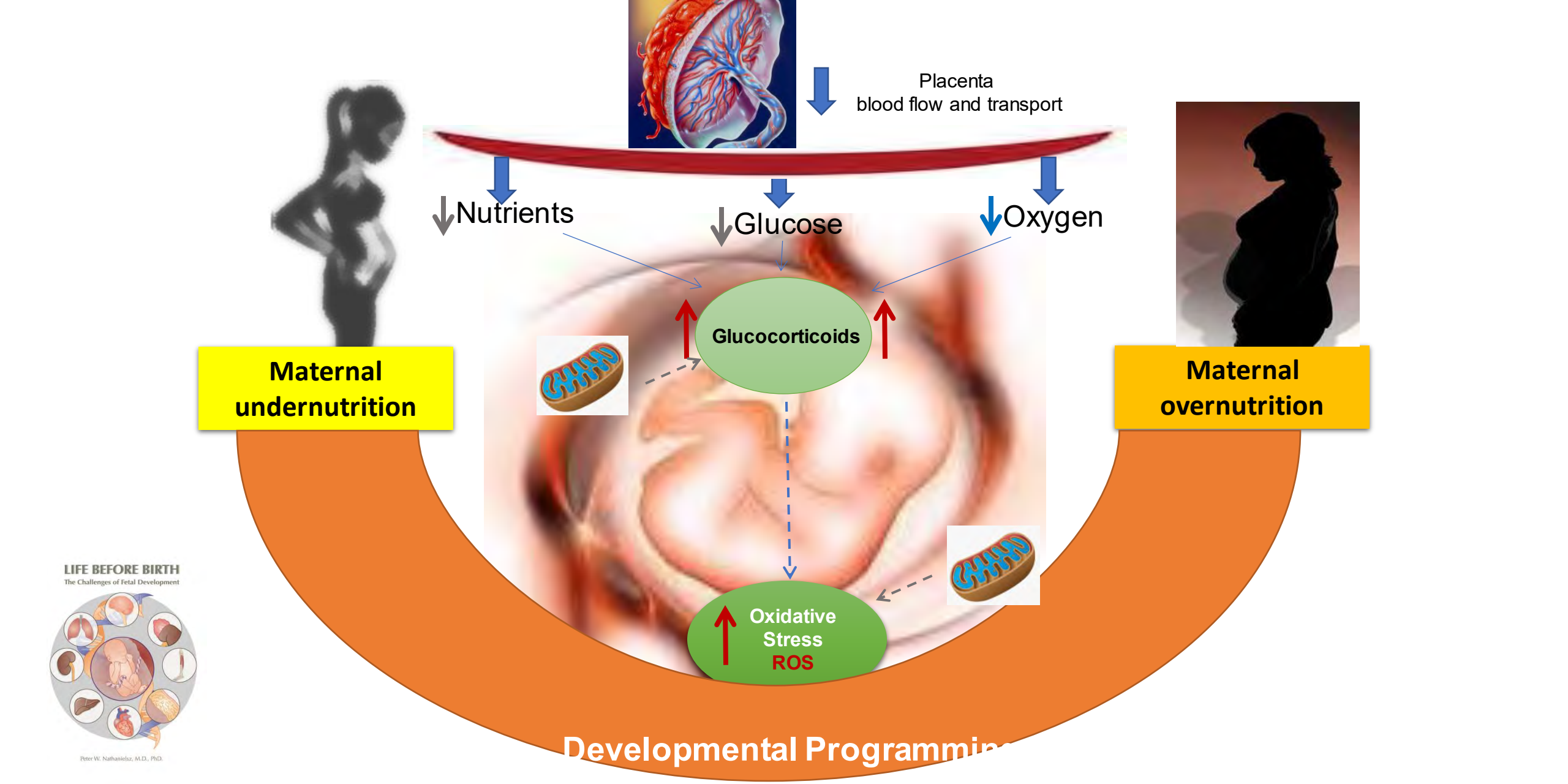
Offspring of control mothers fed normally in pregnancy and lactation (open) and pobese mothers fed a high fat diet in pregnancy and lactation (red). Values mean + SEM. * ($p < 0.05$) showing programming acceleration and accentuation of normal metabolic aging of lipid metabolism .

The age-related rate of rise of male and female liver nitrotyrosine across the life course is increased by maternal obesity (MO).



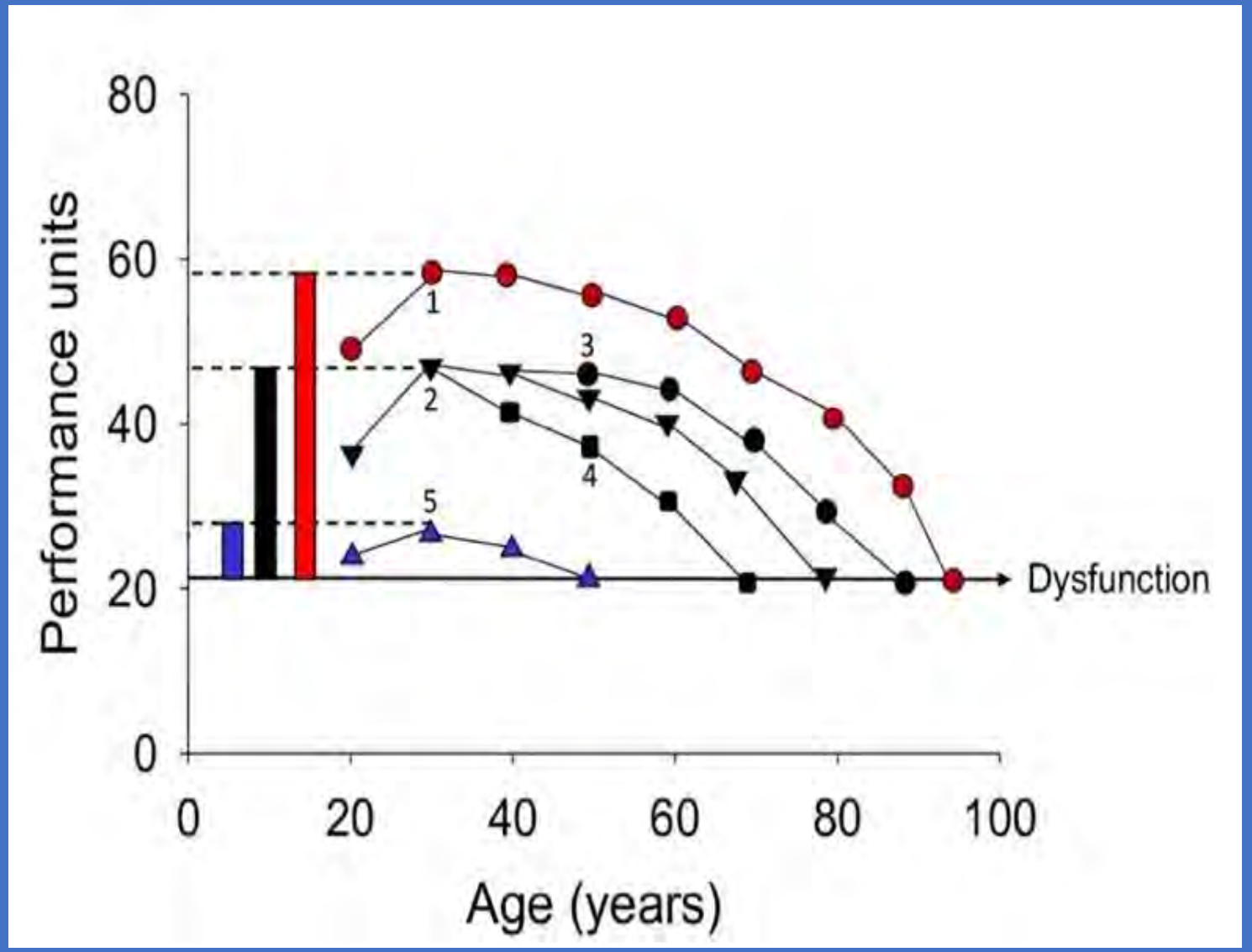


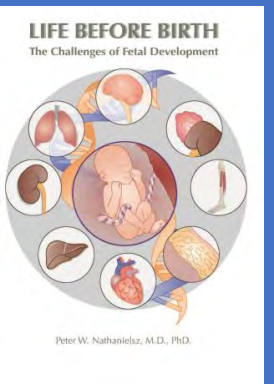
Liver nitrotyrosine rises faster and higher in male and female offspring of MO mothers than control well fed mothers. These results support the view that reactive oxygen stress is associated with earlier aging in MO programmed offspring. As shown previously with fats, programming outcomes are more pronounced in males.



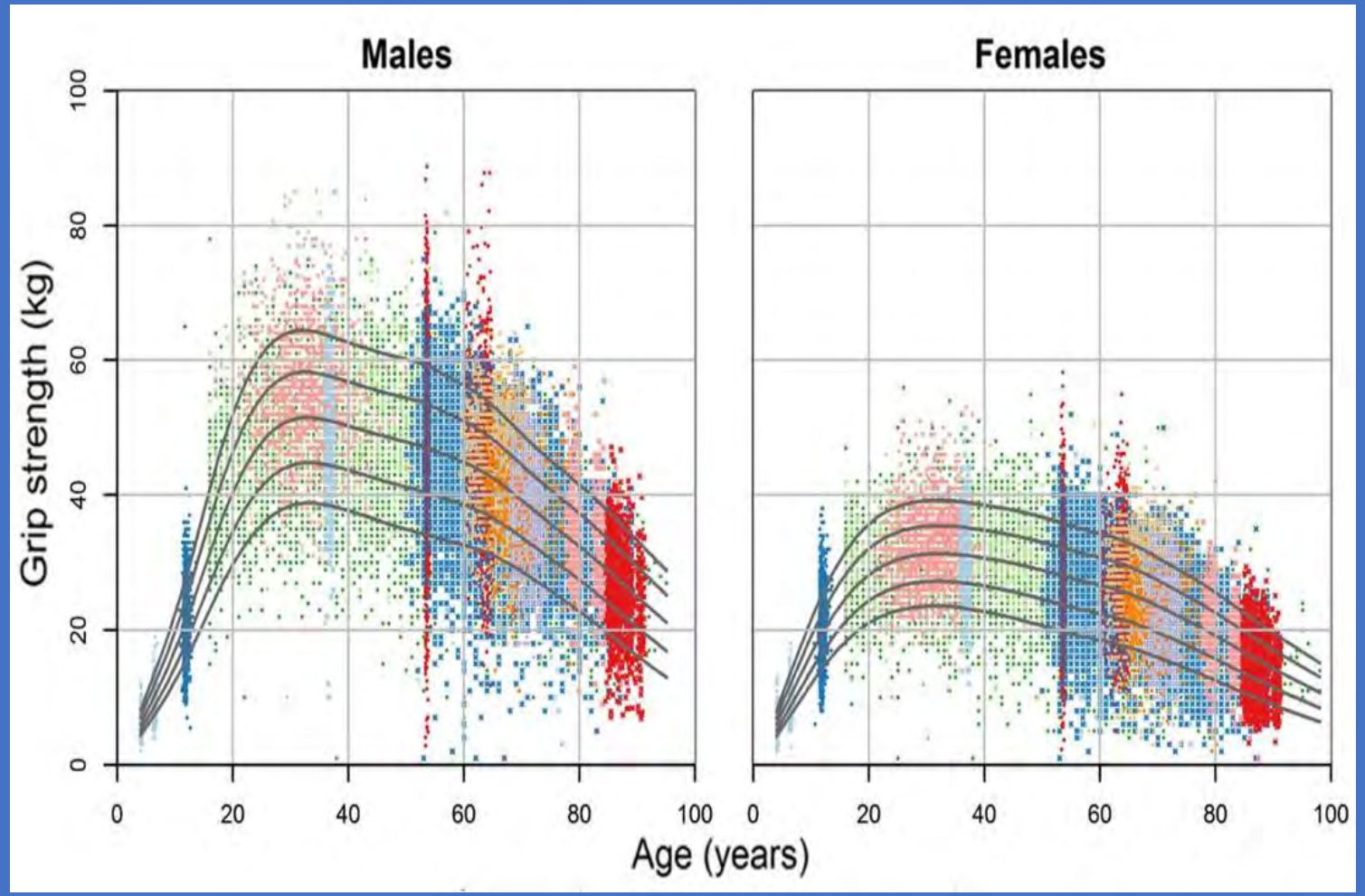
Common mechanisms of programming by over and undernutrition

PROGRAMMING AND RESILIENCE





Grip strength, a human aging marker, declines from as early as thirty years of



Impact of Maternal Exercise during Pregnancy on Offspring Chronic Disease Susceptibility

A. Nicole Blaize, Kevin J. Pearson, and Sean Newcomer,

Exerc Sport Sci Rev. 2015 October ; 43: 198.

Maternal behaviors during pregnancy have been reported to impact offspring health in adulthood. In this review we explore the novel hypothesis that exercise during pregnancy can protect against chronic disease susceptibility in offspring. To date research has demonstrated that improvements in metabolic outcomes, cardiovascular risk, and cancer can occur in response to maternal exercise during pregnancy.

Programming of adiposity and Maternal and Offspring Exercise.

There are several very important papers from your University I would like to mention as an introduction.

Exercise Improves Glucose Disposal and Insulin Signaling in Pregnant Mice Fed a High Fat Diet.

Lindsay G Carter, Sara Y Ngo Tenlep, Laura A Woollett, and Kevin J Pearson *J Diabetes Metab*. 2015 December ; 6(12): ***2015 December: doi:10.4172/2155-156.1000634.***

This study determined the effects of voluntary maternal exercise on glucose tolerance and body composition in pregnant high fat diet fed mice.

Methods—Female mice were put on a standard diet or high fat diet for two weeks. The mice were then split into 4 groups; control standard diet fed, exercise standard diet fed, control high fat diet fed, and exercise high fat diet fed. Exercise mice had voluntary access to a running wheel in their home cage one week prior to mating, during mating, and throughout pregnancy. Glucose tolerance and body composition were measured during pregnancy. Akt was quantified in skeletal muscle and adipose tissue isolated from saline or insulin injected pregnant dams as a marker for insulin signaling.

Conclusion—The use of voluntary exercise improves glucose homeostasis and body composition in pregnant female mice.

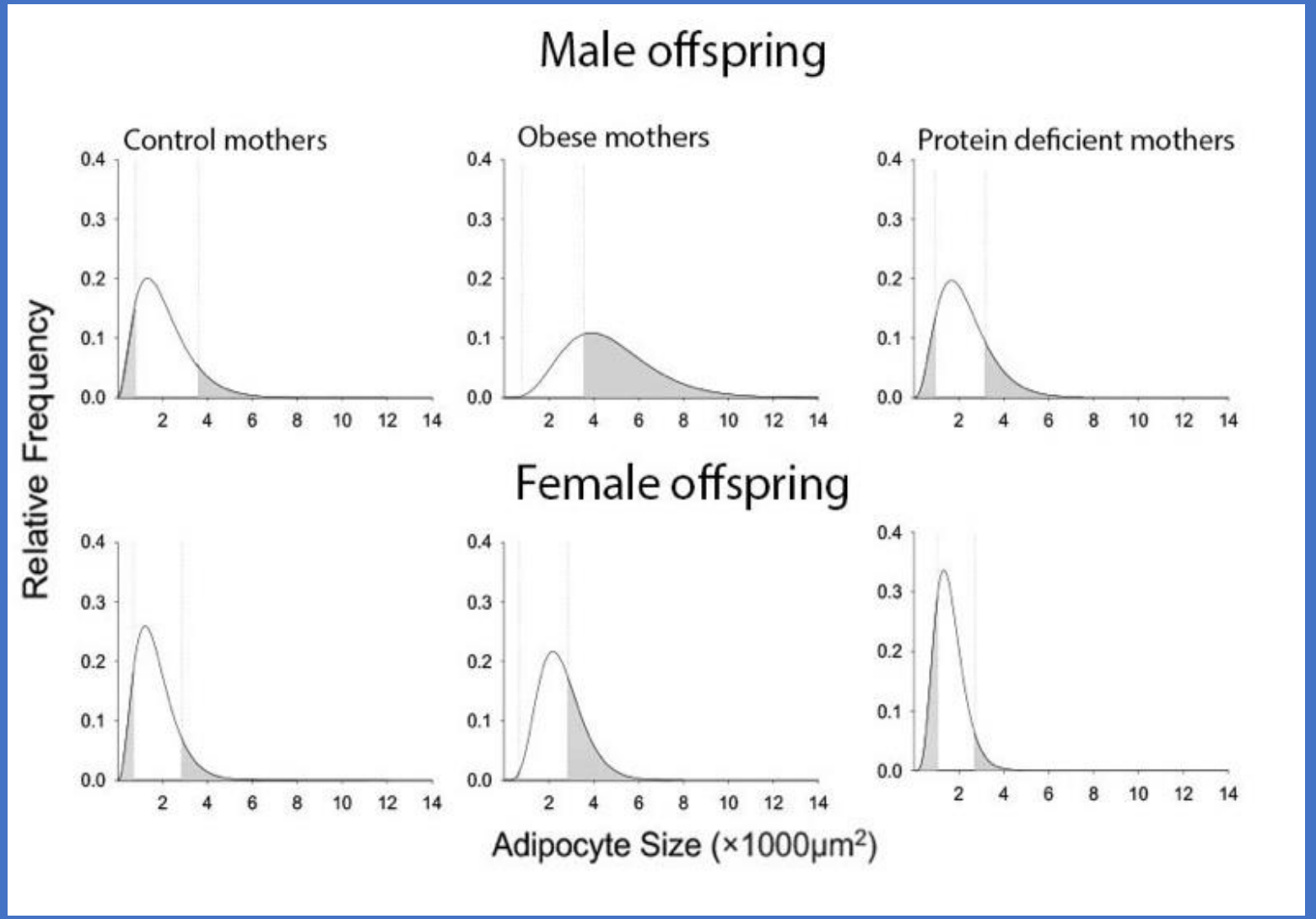
Gestational exercise protects adult male offspring from high-fat diet induced hepatic steatosis. *Ryan D. Sheldon, A. Nicole Blaize, Justin A. Fletcher, Kevin J. Pearson, Shawn Donkin, Sean C. Newcomer, and R. Scott Rector. J Hepatol*. 2016 January ; 64(1): 171–178. doi:10.1016/j.jhep.2015.08.022.

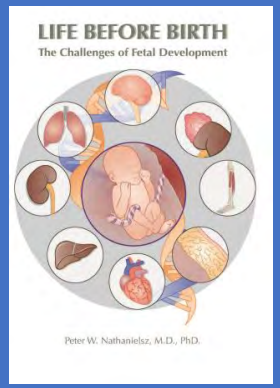
Maternal exercise regimen undertaken was running exercise or wheel-locked sedentary groups throughout gestation (days 4-21).

Outcomes: male offspring from exercised dams were protected against high fat diet-induced hepatic steatosis associated with increased markers of hepatic mitochondrial biogenesis , autophagic potential and hepatic triacylglycerol secretion.

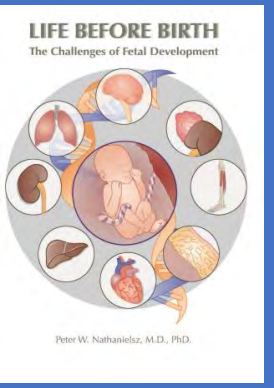
Programming of fat cell size in fat depots

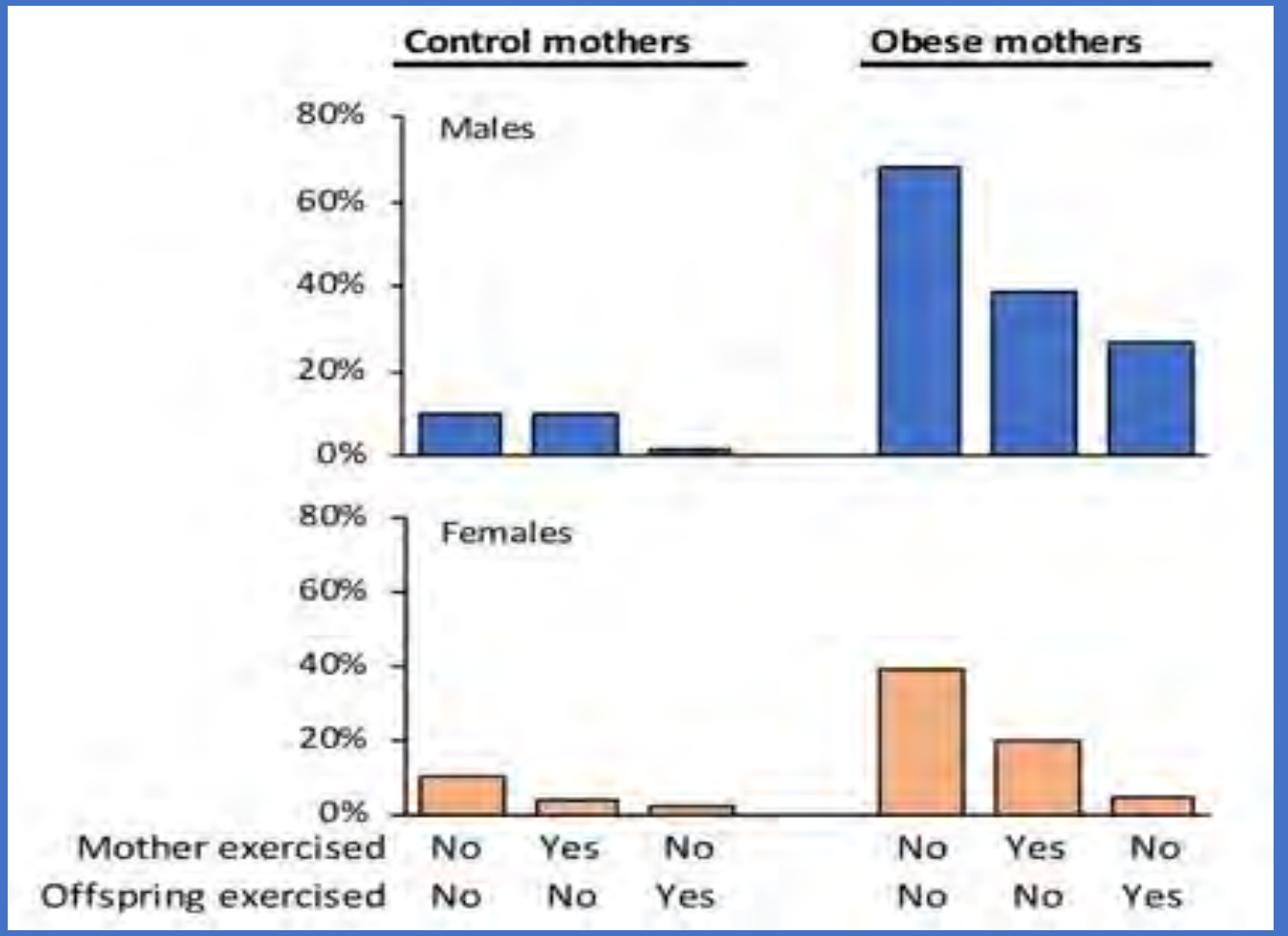
Programming of distribution of fat cell sizes in rat abdominal fat depots at 110 days of life – mid-adulthood. Shaded areas in all results represent the smallest ten percent and largest ten percent of fat cell sizes in offspring of control mothers.





Effects of maternal and fetal exercise on percentages of large adipocytes in intrabdominal fat in offspring of control-fed and high-fat-diet-fed obese mothers.

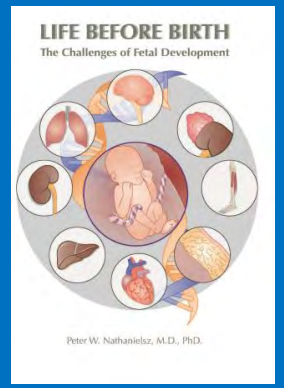


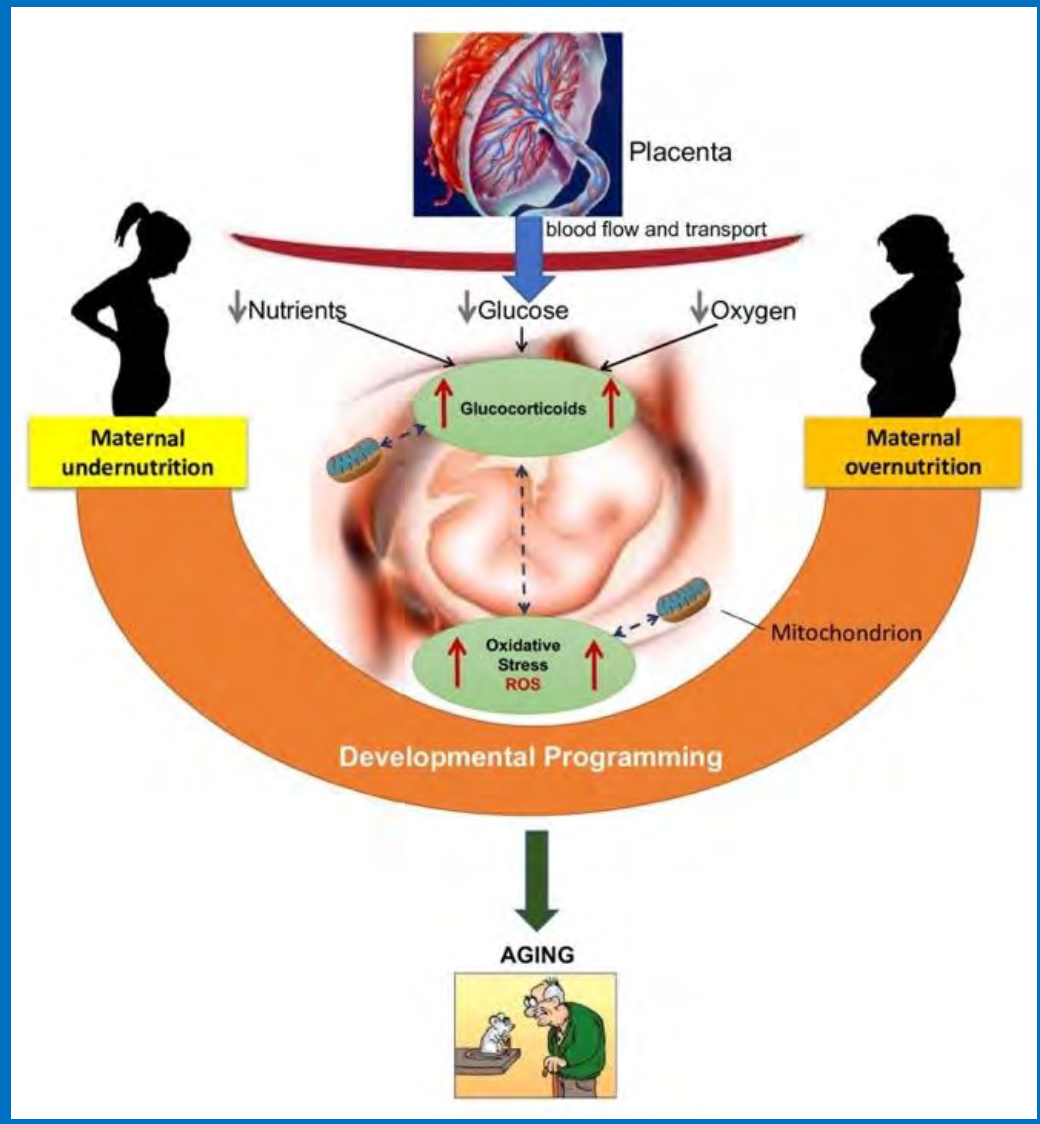


Three offspring groups: Controls in which neither mother or t offspring exercised and two experimental groups, either mother was exercised during pregnancy, or the offspring exercised, never both. The groupings are shown beneath the figure.

SUMMARY.

Programming and aging can both involve elevated glucocorticoids, metabolic dysfunction and increased OS (Fig. 1). The smaller female adverse response to many programming challenges is probably one of many causes of the female aging advantage. Increased susceptibility to programmed acceleration of aging is likely further increased by a second hit in life will also.



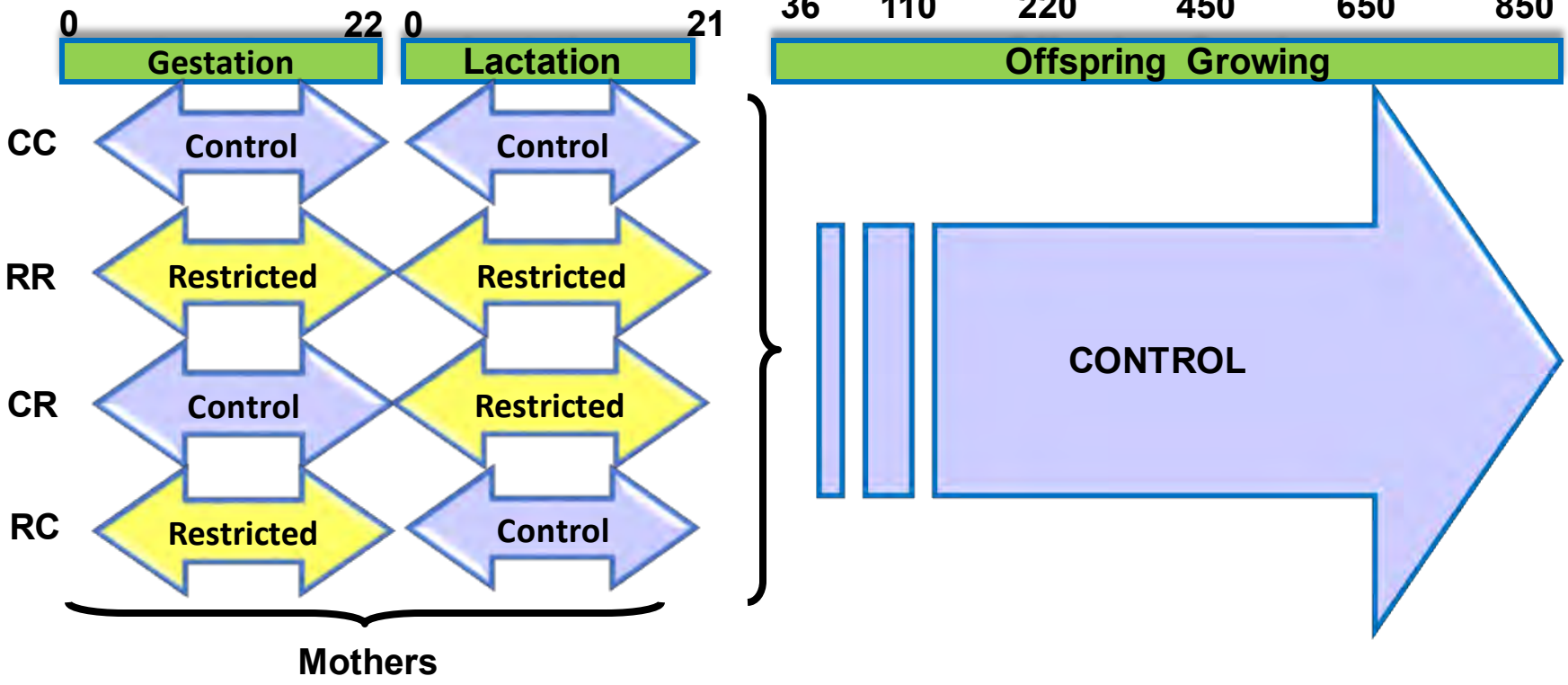


Similarity in programming and aging mechanisms.

Reproduced with permission from
Life Before Birth: The challenges of fetal life
Peter W. Nathanielsz 2021.

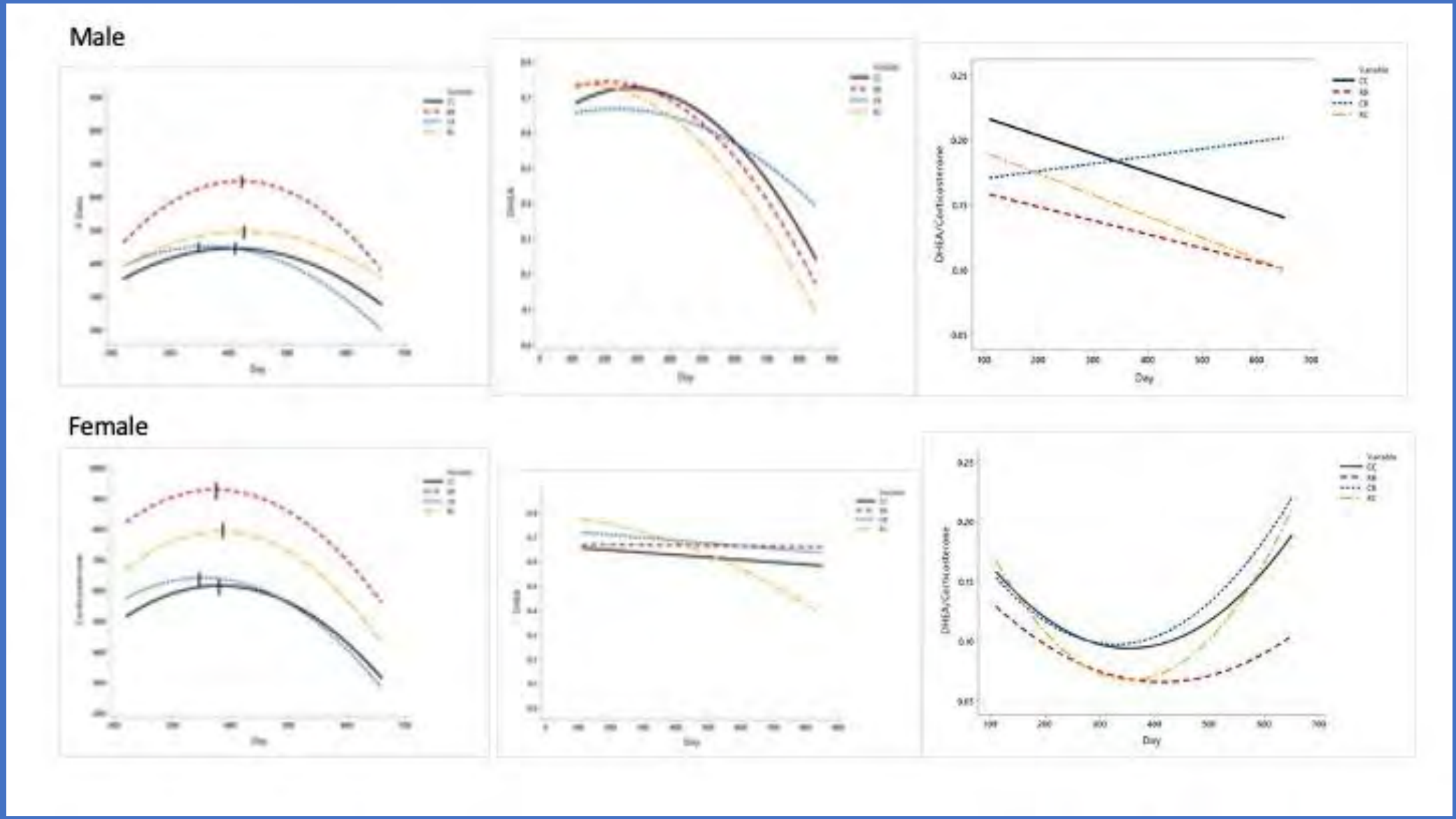
Programming of glucocorticoid function

Rat maternal protein restricted diet model



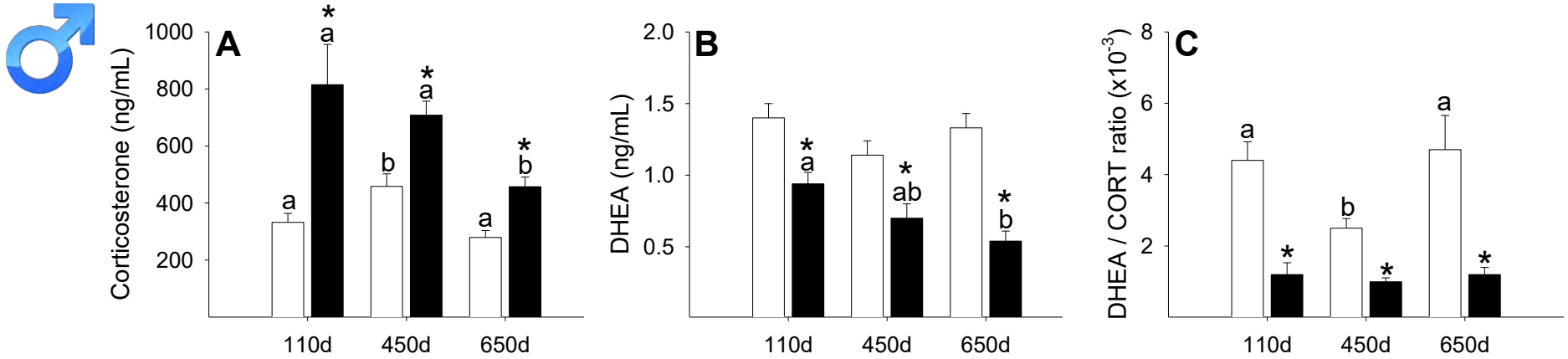
Isocaloric Diet 3.85 kcal/g
Control (20 % casein)
Restricted (10% casein)

Life-course changes in rat plasma corticosterone and dehydroepiandrosterone (DHEA).

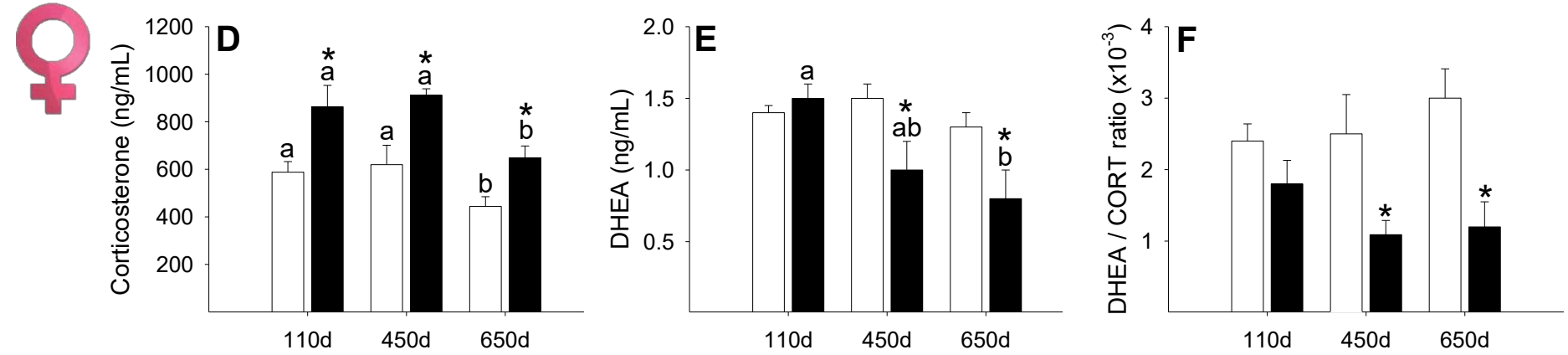


MO result in increased corticosterone in adult life. A fall in corticosterone begins around PND 450 in controls and MO

Male



Female

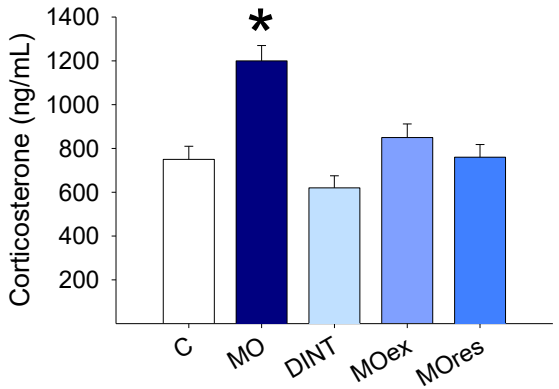


DHEA is protective against aging, the later fall in the female ratio may represent one of the mechanisms of the so called ”female aging advantage” (Cheng 2019).

M ± SEM, n=5-14. * P<0.05 vs C: different letters for the same group at different ages

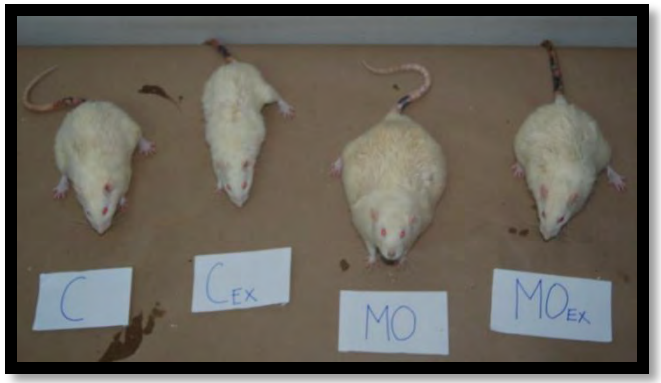
Rodríguez-González GL. J Physiol (2019). 597(23):5549-5563

Outcomes of maternal interventions in obese mothers



Mothers
end of lactation

650 days offspring



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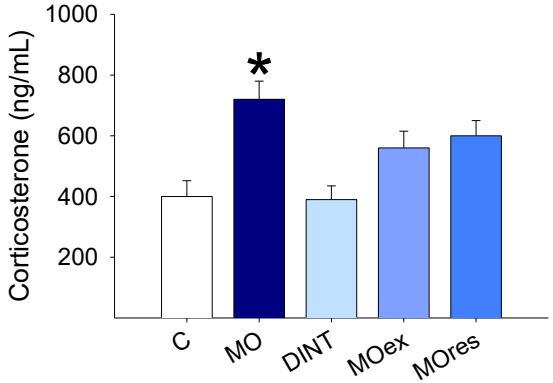
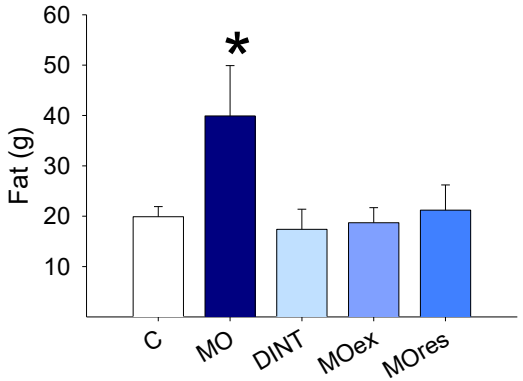


Male offspring
PND 650

♂



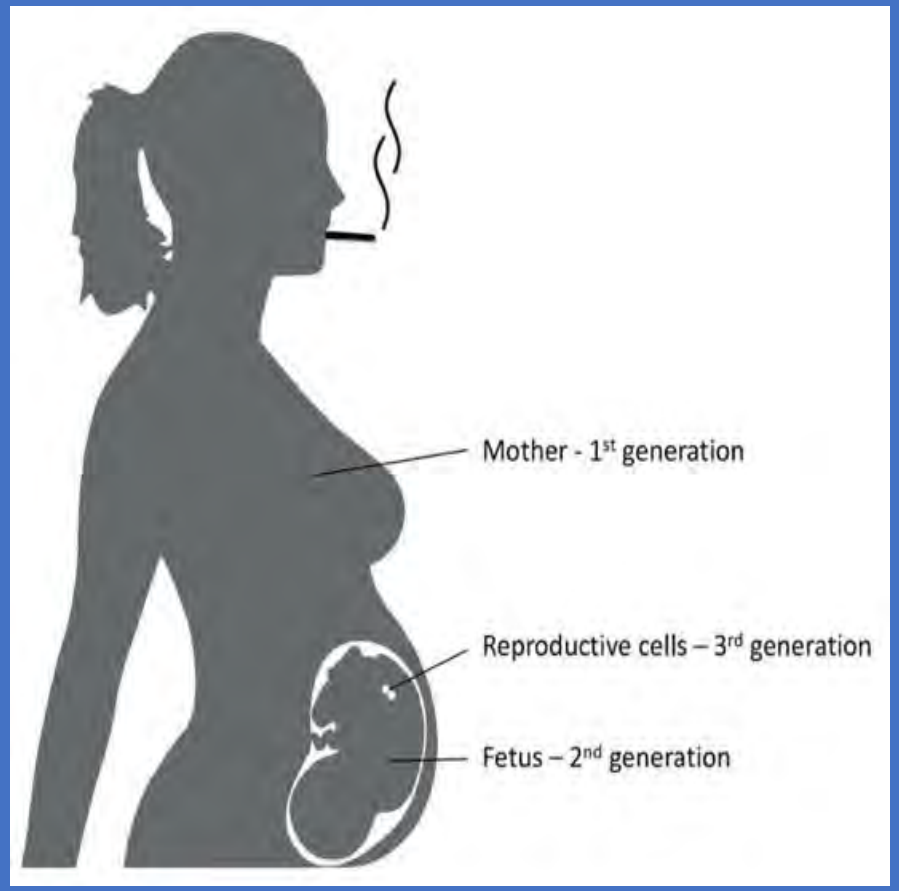
Male offspring
PND 650

M ± SEM, $p<0.05$ * vs C; n = 6 to 8 from different litters

International Journal of Obesity (2015) 39, 712–719
Nutrition Review (2013) . 71 (Suppl.1): S78-S87.

Pollution:

Smoking in pregnancy affects the health of three generations.



Perspective, Pollution

Particulate matter and disrupted fetal development

As an intrauterine toxin and strong oxidant, maternal exposure to particulate matter in pregnancy is associated with birth complications and long-term offspring health problems , including abnormal organogenesis, preterm birth, small for gestational age, impairment in newborn lung and immune function, and increased risk of brain developmental disorders and cognitive disorders after birth ***However, this topic is still understudied, considering that there is no evidence of a safe exposure threshold of any of the air pollutants.***

The ability of PM2.5 to cross the blood–placental barrier suggests that PM2.5 can circulate in fetal blood. Therefore, PM may directly induce oxidative stress and inflammatory responses in the growing fetus and affect fetal development. This theory has been supported by studies on umbilical blood in newborns with prenatal PM exposure, in which reduced endogenous antioxidant Superoxide Dismutase 2 and DNA oxidative stress damage are discovered consistently in mother–baby pairs.

I do have a list of papers. Will send them to Dr. Pearson and Kara Richardson and they can perhaps circulate with the slides.

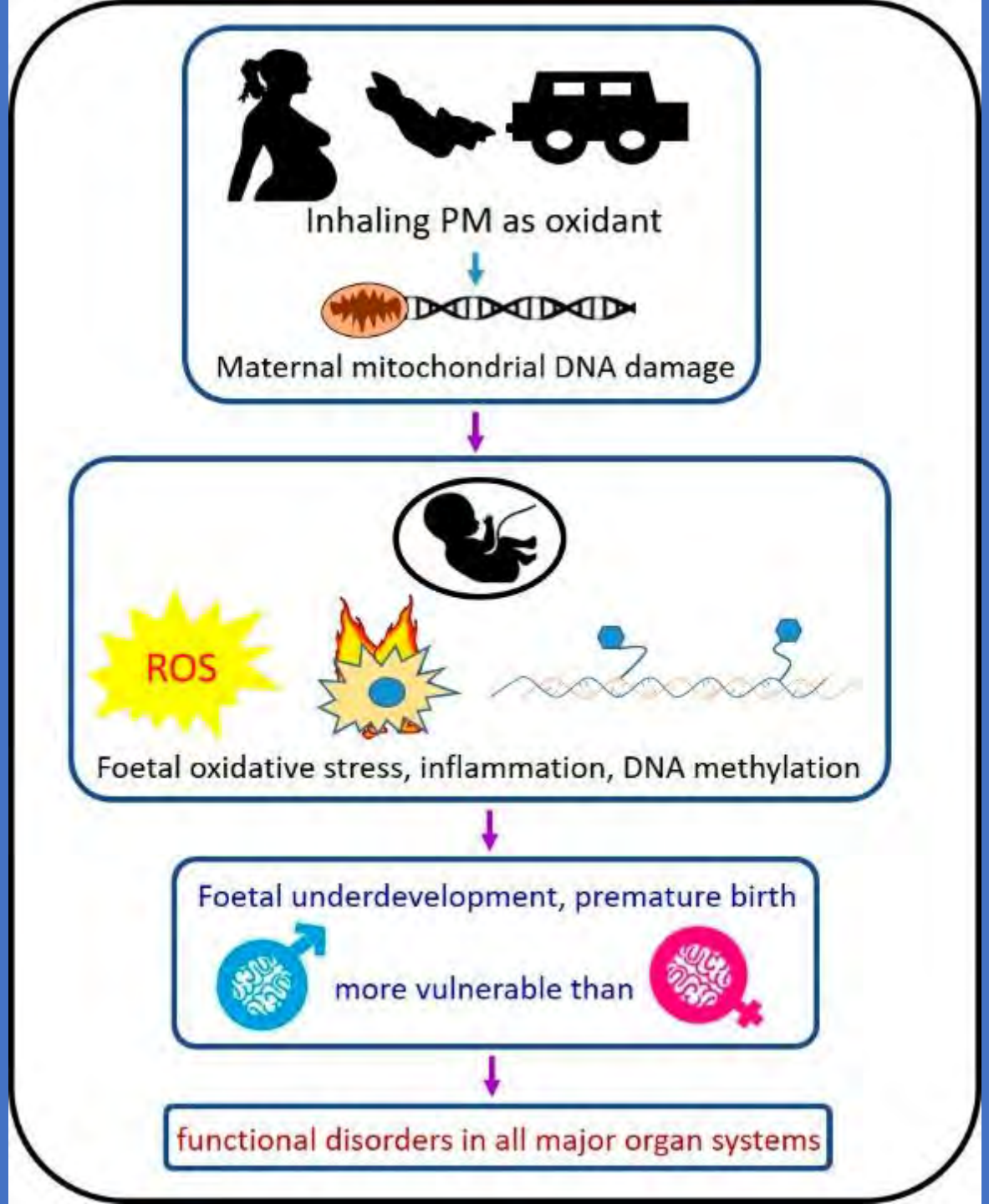
In vitro studies using embryonic cells or trophoblast cells have discovered dose-dependent toxicities of PMs on cell cycle and viability. PM exposure affects several pathways, including heightened oxidative stress, inflammatory response and endoplasmic reticulum stress, resulting in ROS-JNK/ERK-apoptosis and G0/G1 arrest pathways. The cellular powerhouse mitochondria are sensitive to oxidative stress induced damage; however, mitochondrial function and integrity are not affected by PM exposure in one in vitro study.

Changes in mitochondrial DNA copy number and methylation changes have been found in the cord blood of babies born to mothers exposed to PM during pregnancy. This may be inherited from mothers, instead of caused by in utero PM exposure. In addition, in utero exposure to fine ambient PM correlates with heightened placental oxidative stress and inflammatory responses with decreased placental mass and gene expression responsible for placental angiogenesis. This may impair nutrient delivery to the fetus, leading to intrauterine underdevelopment.

Pollution and Programming References

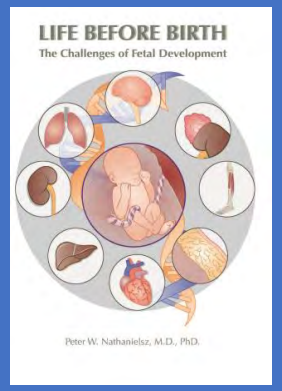
1. Jarvis, I. *et al.* Assessing the association between lifetime exposure to greenspace and early childhood development and the mediation effects of air pollution and noise in Canada: a population-based birth cohort study. *Lancet. Planet. Health.* **5**, e709–e717 (2021).

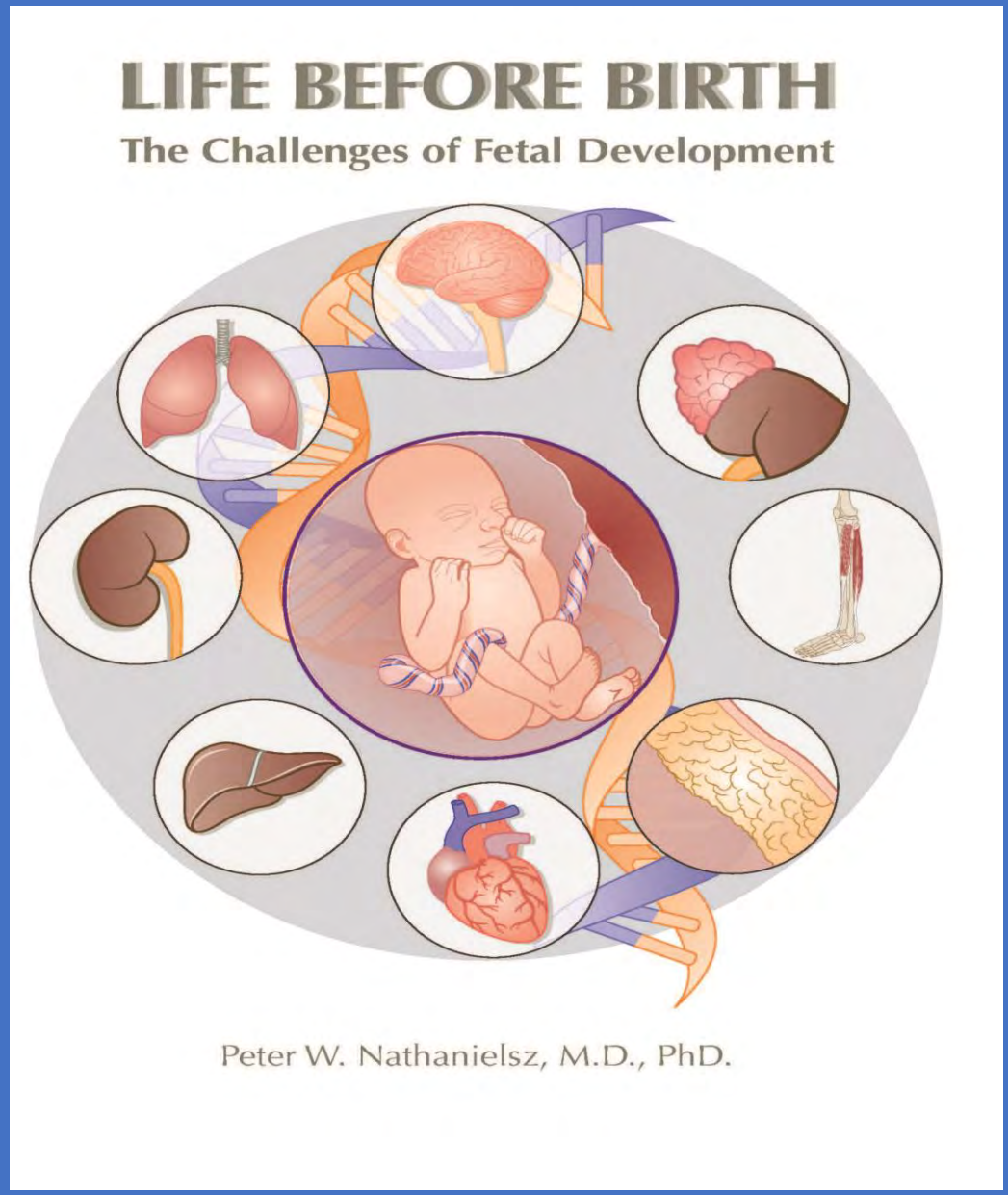
2. Bergstra, A. D., Brunekreef, B. & Burdorf, A. The influence of industry-related air pollution on birth outcomes in an industrialized area. *Environ. Pollut.* **269**, 115741 (2021).


Inhaling PM as oxidant
Maternal mitochondrial DNA damage
ROS
Foetal oxidative stress, inflammation, DNA methylation
Foetal underdevelopment, premature birth
more vulnerable than
functional disorders in all major organ systems

Some other stories in Life Before Birth

- The most Important Journey we will ever make - Chapter 1
- The male and female placenta - Chapter 2
- Fetal body movements and uterine hugs - Chapter 6
- Fetal response to a Lack of oxygen or nutrients - Chapter 6
- Fetal growth - Chapter 12
- Mother's 24-Hour clock precisely times delivery - Chapter 15
- Ten principles of programming - Chapter 17
- Offspring programming by maternal obesity - Chapter 18
- Programming of the appetite negative feedback control loop - Chapter 18
- Multiomics - Chapter 19
- The fetus is NOT a small version of the adult - Chapter 20
- Sex differences in programming - Chapter 21
- Programming-aging interactions - Chapter 21
- Putting knowledge of fetal development into practice Lucilla Poston - Chapter 22





All illustrations showing the cover of Life Before Birth: The challenges of fetal development are free for use by anyone as long as the origin from the book is acknowledged.

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