

**Strategies to reduce Non-Communicable Diseases in the offspring: negative and positive
in utero programming.**

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Abstract

Non-communicable diseases (NCDs) are a major problem as they are the leading cause of death and represent a substantial economic cost. The “Developmental Origins of Health and Disease Hypothesis” proposes that adverse stimuli at different life stages can increase the predisposition to these diseases. In fact, adverse *in utero* programming is a major origin of these diseases due to the high malleability of embryonic development. This review provides a comprehensive analysis of the scientific literature on *in utero* programming and NCDs highlighting potential medical strategies to prevent these diseases based upon this programming. We fully address the concept and mechanisms involved in this programming (anatomical disruptions, epigenetic modifications and microbiota alterations). We also examine the negative role of *in utero* programming on the increased predisposition of NCDs in the offspring, which introduces the passive medical approach that consists of avoiding adverse stimuli including an unhealthy diet and environmental chemicals. Finally, we extensively discuss active medical approaches that target the causes of NCDs and have the potential to significantly and rapidly reduce the incidence of NCDs. These approaches can be classified as direct *in utero* programming modifications and personalised lifestyle pregnancy programs; they could potentially provide transgenerational NCDs protection. Active strategies against NCDs constitute a promising tool for the reduction NCDs.

Keywords: DOHaD, epigenetics, microbiota, preventive medicine.

1. Introduction

In the 1980s, Barker observed that in the 20th century, the highest rate of infant mortality and coronary heart disease-caused mortality were found in the same regions of England. The main cause of infant death at that time was low birthweight; therefore, the risk of developing coronary heart disease appeared to be higher in adults that had a low birthweight¹⁻³. These among other observations discussed below led to the “Barker hypothesis”. Testing this hypothesis established that nutritional restriction of the mother results in a low energy intrauterine environment that hinders foetal organ development and increases the offspring’s risk of developing non-communicable diseases (NCDs) later in life ^{4, 5}.

Non-communicable diseases are non-genetic non-infectious medical conditions which progress slowly and are present throughout life such as atherosclerosis or diabetes⁶. They constitute a major problem because they are the current leading cause of death around the world, according to the World Health Organisation. Approximately 36 million people die every year due to NCDs and around 9 million of these are premature deaths⁶. Moreover, NCDs pose a huge economic threat because they require expensive treatment and reduce the working population, either through incapacitation or premature death. For example, in 2005 India and Brazil lost \$9 and \$3 billion respectively due to diabetes, heart conditions and strokes⁶. As a consequence, identification of the causes and prevention of NCDs are of high importance.

An increasing amount of evidence supports the hypothesis that defective *in utero* programming is the origin of a broad range of NCDs (Table 1). In fact, multiple studies have corroborated the Barker hypothesis. For instance, people conceived during the Dutch famine of 1944 and 1955 were more likely to suffer excessive weight gain, hypertension and glucose

intolerance later in life⁷⁻⁹. In addition, nutritional and non-nutritional stimuli such as a caloric restricted diet and cigarette smoke could also affect offspring at multiple stages of development and increase NCDs risk in adulthood. As a result, the “Barker hypothesis” has developed into the “Developmental Origins of Health and Disease” (DOHaD). This theory proposes that stimuli at different life stages can augment predisposition to NCDs later in life and includes the concept of *in utero* programming by which programming of the embryo would promote offspring’s risk to NCDs later in life^{6, 10}.

Our aim is to provide a comprehensive review of the scientific literature on *in utero* programming and NCDs focusing on potential strategies utilising this programming to reduce the incidence of NCDs in the offspring. First, the concept and mechanisms involved in *in utero* programming are discussed. Second, the negative role of *in utero* programming on the increased predisposition of NCDs in the offspring is briefly assessed. This introduces the first line of defence against NCDs: a passive medical approach consisting of the avoidance or reduction of preventable common negative stimuli. Finally, active medical approaches to protect against these diseases later in life are examined; the majority of these strategies involve the novel induction of positive *in utero* programming.

2. Developmental Origins of Health and Disease and *in utero* programming

DOHaD stipulates that environmental stimuli at critical periods of development augment the risk of NCDs in adulthood due to subtle changes in biological functions⁶. These critical periods happen at different periods of the life of the individual, including *in utero*, neonatal and postnatal. This review focuses on the effect of *in utero* programming on the predisposition to NCDs. “Programming” is the process by which a stimulus at a critical period of development results in a lifelong effect. This critical period during *in utero*

programming corresponds to a window in which the biological system has a high degree of plasticity and is sensitive to the uterine environment provided by the mother. Once this window has passed the plasticity disappears and the functional capacity is stabilized^{5, 6, 11} – plasticity might reappear at later critical periods.

Embryonic development, which starts with the fertilisation of an egg and ends with a fully-formed organism, is an extremely complex process. This process involves modifications of DNA and histone proteins to control gene expression and facilitate precise cell-fate specifications in a spatio-temporal manner. Therefore, *in utero* programming might result in a broad range of lasting effects that can predispose foetuses to NCDs in adulthood. The basis of foetal programming remains largely unknown and further studies are needed. Nevertheless, current research has elucidated three potential main mechanisms by which stimuli can have an effect, these can be classified as anatomical disruptions, epigenetic modifications and microbiota alterations.

2.1. Anatomical disruptions

Embryonic development involves the proliferation and differentiation of cells to form all tissues and organs of the organism. Any stimulus during this process might affect the anatomy of the biological systems currently developing and for the ones developing later; it might lead to suboptimal organs and tissues of disproportionate size. The disruption of the normal structure might have a subtle effect on the physiology because it alters the number of cells, which can result in an inability to fulfill the biological needs reducing the net performance. Consequently, the physiological function of the affected organ or tissue might be hindered increasing the risk of NCDs later in life¹¹.

As an illustration, prolonged undernutrition of the embryo in late gestation diminishes the replication of nephrons in the kidney, which cannot proliferate after birth. Brenner proposed that this reduction of renal cells is associated with an increased pressure in the glomerular capillaries leading to glomerular sclerosis; a condition that causes further death of nephrons. This anatomical disruption could result in hypertension and extensive glomerular damage in later life¹². Consistent with this, low birthweight in humans is associated with low nephron number, increased glomerular volume and hypertension¹³⁻¹⁵. Loss of kidney mass due to accidents and resections is also associated with hypertension and further kidney damage^{14, 16}. Similarly, the transplantation of small kidneys to large humans is linked to hypertension^{14, 17}.

2.2. Epigenetic modifications

Epigenetics is the process that regulates gene expression without modifying the DNA sequence. This regulation is mainly achieved through four epigenetic modifications: non-coding RNAs, histone modifications, chromatin remodellers and DNA methylation. DNA methylation has been the most widely researched epigenetic modifications in *in utero* programming^{18, 19} (for more detailed information on the other epigenetic modifications see Vliet *et al.* 2007; Kaikkonen *et al.*, 2011 and Schmitz *et al.*, 2016²⁰⁻²²). In mammals, methylation of DNA takes place mainly on cytosines of CpG dinucleotides and represses the expression of the sequence. This repressive role is important during embryogenesis and is involved in a variety of events such as X-chromosome inactivation and genomic imprinting.

The epigenetic landscape changes considerably through embryonic development. There are two reprogramming events in which DNA is demethylated and progressively re-methylated. The first event, which occurs when the primordial cells migrate to the gonads, is sex-specific marking of the imprinting genes that indicates if the DNA of the gonads belong to a female or

a male. If the gametes contribute to a future pregnancy, these methylations will mark the maternal or paternal origin of the DNA. These imprinting genes control foetal and placental growth during *in utero* development of the offspring. The second reprogramming event takes place between the zygote and the blastocyst stage. DNA is actively demethylated at fertilisation to restore totipotency to all cells. This is followed by a passive demethylation until blastocyst stage when DNA *de novo* methylation patterns are restored to facilitate cell fates specifications. Imprinted genes escape this second event²³.

Evidence suggests that epigenetic modifications are sensitive to environmental stimuli. Ollikainen and colleagues demonstrated that the intrauterine environment alters the epigenetic profile by analysing the DNA methylation patterns of dizygotic (DZ) and monozygotic (MZ) twins²⁴. They observed that DZ and MZ twins exhibited 82% and 54% of methylation discordances at birth, respectively; MZ twins share the same genome and were expected to have the same epigenome, therefore, the epigenetic differences observed suggested *in utero* environment can modify the epigenome of the offspring²⁴. In addition, undernutrition changes the epigenetic profile of the offspring. For example, individuals conceived during the Dutch famine exhibited 5.6% less methylation of insulin-like growth factor II (*IGF2*) differentially methylated region (*DMR*) before exon 3 than their siblings conceived before or after the famine²⁵. Hypomethylation of this DMR leads to increased expression of IGF2. IGF2 is a growth factor that promotes embryonic and placental growth during *in utero* development; it is maternally imprinted and only the paternal allele is expressed. IGF2 overexpression leads to overgrowth, a phenotype observed in individuals conceived during the Dutch famine²⁵.

Due to the changing epigenetic landscape during embryonic development, any epigenetic alteration could have a broad range of effects on gene expression influencing the risk of NCDs in adulthood. *IGF2 DMR* hypomethylation increases the risk of colorectal cancer and NCDs associated with perinatal overgrowth such as prostate and breast cancer^{5, 25, 26}. Exposure to maternal tobacco *in utero* also induces epigenetic changes as reviewed in Suter *et al.* 2013²⁷. It is currently under study whether or not these modifications are associated with an increase in embryo growth restriction and associated NCDs.

2.3. Microbiota alterations

The human microbiota refers to the set of symbiotic microorganisms that cohabitate in the human body. The gut microbiota is the most studied subset. Microorganisms in the gut are essential for development since they are involved in the programming of metabolism and immune response in the offspring. Studies have demonstrated that prenatal disruption of the gut microbiota in animals is associated with NCDs later in life, including colorectal cancer and asthma^{28, 29}. In humans, a reduced allergy risk was observed in the offspring of mothers exposed to stables or barns during pregnancy³⁰ – this environment is rich in bacteria and most likely can affect the maternal microbiome composition. The role of microbiota alterations on *in utero* programming in humans remains largely unknown; studies have been mainly undertaken on postnatal programming (for the effect of the microbiome on NCDs development see Vuillermin *et al.*, 2017; Mulligan and Friedman, 2017 and Jenmalm, 2017³¹⁻³³).

Maternal microbes are transmitted to the offspring during childbirth. There is also some evidence that gut colonisation might start *in utero* due to the microbiota in the female reproductive tract however this has not been conclusively demonstrated^{34, 35}. Intrauterine

microbiota could arise from the gut microbiota or via the vaginal microbiota. Translocation of maternal gut bacteria to other body areas like the mammary gland in humans and mice has already been proven³⁶. Perturbations of maternal gut microbiota could disrupt offspring microbiota and hinder metabolic and immune programming. For example, in fly larvae, maternal G418 antibiotic (stimuli) eliminates *Acetobacter* species in the maternal gut leading to delayed development in the offspring. External administration of *Acetobacter* species restores normal offspring development³⁷. It is possible that modifications of the maternal microbiota could protect the offspring against diverse NCDs.

3. Passive strategy against NCDs: Negative *in utero* programming.

As previously introduced, negative stimuli in the maternal uterine environment can lead to detrimental *in utero* programming resulting in NCDs in adulthood. The first line of defence against these NCDs is to avoid or reduce the adverse *in utero* programming induced by common maternal lifestyle choices. The International Federation of Gynaecology and Obstetrics (FIGO) recognises the importance of supporting pregnant women to avoid defective *in utero* programming and reduce the incidence of NCDs. Consequently, FIGO with other agencies aims to improve prenatal care programmes ensuring early detection and treatment of maternal diseases as well as promoting proper education of mothers about the risks of environmental and nutritional exposures⁶. In this review we focus on basic scientific studies that gave rise to these FIGO approaches. We introduce the concept of negative *in utero* programming through two important maternal stimuli: diet and environmental chemicals

3.1. Diet

Undernutrition is related to an increased risk of hypertension, type 2 diabetes, obesity, and, immune and coronary heart diseases in the offspring. This association has been strongly demonstrated through a series of epidemiological studies summarised in Barker, 1998 and Ojha *et al.*, 2013^{11,38}. When the nutritional demand of the foetus is higher than the placental supply, the embryo initially starts catabolism. If the nutritional restriction is prolonged, the embryo first decreases its metabolic rate and substrate usage, thereby slowing foetal growth. Secondly, the embryo blood flow is redistributed by organ-specific vasodilatation to protect the most important organs like the brain and, finally, the foetal secretion of hormones and interaction with the placenta and mother are altered^{11,39}. The placenta is also modified to ease survival⁴⁰. Altered organ growth and maturation seems to affect the physiology of several biological components such as liver, pancreas, muscle, hypothalamic-pituitary-anterior axis, kidney, vasculature and heart. This leads to hyperlipidaemia, hypertension and diabetes increasing the risk of cardiovascular diseases reviewed in Boo & Harding, 2006⁵.

Slow foetal growth and blood redistribution in late gestation causes disruption of kidney anatomy leading to hypertension (section 2.1.)¹¹. Hypertension may also be caused by altered vascular function and renin-angiotensin-system homeostasis. Fleming's group demonstrated that maternal low protein diet during pregnancy or before implantation resulted in reduced vasodilatation in male mice. This diet also led to increased angiotensin-converting enzyme activity in the serum of female mice and lungs of male mice⁴¹. Angiotensin-converting enzyme produces angiotensin II a potent vasoconstrictor associated with hypertension.

The thrifty phenotype hypothesis proposed by Hales and Barker⁴² stipulates that the embryo reduces insulin secretion and increases insulin resistance during undernutrition to conserve more glucose, which is directed to the brain and heart instead of insulin-dependent tissues. It

has been shown that nutritional deficiency *in utero* in sheep is associated with an increase of insulin receptors (IR) but a decrease of downstream catalytic subunits in the skeletal muscle⁴³. The expression of the IR's downstream proteins phosphatidylinositol 3-kinase (PI3K) and protein kinase-B (AKT/PKB) was reduced by 40%. It has been proposed that later in life, the abundance of nutrients combined with this insulin intolerance could lead to diabetes⁴³. This study provided evidence of *in utero* alterations in the insulin metabolic pathway that, if conserved in adulthood, could lead to diabetes. Caution must be used when interpreting the results because the outcomes of animal studies might not be relevant for humans⁴⁴. Animal studies should be complemented with human epidemiological studies and *in vitro* studies using human tissues to more accurately determine the effect of stimuli on *in utero* programming and NCDs development in *Homo sapiens*, although *in vitro* studies have their own limitations. Furthermore, they have used a placental insufficiency intrauterine growth restriction (PI-IUGR) sheep model created by exposure to high temperature. The diet of the mothers was optimal for the development of the foetus. Thus, the study was not focused on the exclusive effects of undernutrition. Although placental insufficiency (PI) leads to reduce transport of nutrients including branched-chain aminoacids and this would mimic an undernutrition status, PI also provokes hypoxia that can also have negative effects on the foetus. To corroborate this study, the expression of IR, PI3K and AKT/PKB should be determined in ewes exposed exclusively to diet restriction. The expression levels of these proteins could also be analysed in humans known to have been exposed to undernutrition *in utero*. It would be interesting to follow up the sheep in this study and observe if the offspring develops diabetes in adulthood.

Undernutrition can alter the epigenome of the progeny. Male offspring (F1) of female mice subjected to caloric restriction exhibited hypomethylation of 17 regions in the sperm⁴⁵. This

suggests that the re-acquisition of methylation in progenitor cells may have been hindered *in utero*. Interestingly, the affected regions were close to genes whose expression patterns were also altered in F1 including *Sstr3*, *Tbc1d30*, *Sur1* and *Kcnj11*; these genes are known to be involved in metabolism and glucose tolerance. For instance, *Kcnj11* encodes two subunits of a β -cell pancreatic potassium receptor required for the regulation of insulin secretion; polymorphisms on this gene are linked to type 2 diabetes⁴⁵. This study demonstrates a transgenerational effect, *in utero* programming-induced modifications may extend to and affect several generations suggesting predisposition to NCDs might be transmitted through the family. It is thought that this effect could be caused by alteration of the paternal metabolic environment that would affect F2 or by epigenetic inheritance. In this study F1 offspring depicted low birthweight, impaired muscle stem cell population as well as pancreatic function, and glucose intolerance. F2 generation through the male line also exhibited the same alterations including glucose intolerance and low birthweight without having being exposed to the adverse stimulus. Consequently, the authors analysed the methylome of the F2 generation focusing on the DMRs found in F1 to identify the transgenerational mechanism involved⁴⁵. This might be seen counterintuitive since the second reprogramming event in the zygote is thought to demethylate the whole genome excepting the imprinted regions. However, recent studies suggest that other domains of the genome also resist demethylation at this reprogramming event⁴⁶⁻⁴⁸. Almost half of the DMRs analysed in this study were resistant. The methylome study of F2 offspring did not exhibit the previous hypomethylation suggesting that they might affect very early developmental processes before re-methylation with yet unknown outcome later in life⁴⁵. Future work should further investigate the observed transgenerational effect; the involvement of the hypomethylated genes on early embryonic development could be study with transgenic animals. Future work should also analyse if the DMRs identified in F1 are secondary to other alterations in the DNA. This study does not

demonstrate that undernutrition is directly inducing the demethylation, it might be affecting other chromatin elements such as transcription factors and histones.

Overnutrition is strongly associated with obesity, a non-communicable disease whose prevalence has skyrocketed in recent years³⁸. *In utero* overnutrition increases the risk of obesity, diabetes, hypertension, cardiovascular diseases and early adult death⁵. Children of obese women who lost weight show a different risk of being overweight in adulthood; children born when their mothers were at optimal weight have half the risk of becoming obese than their older siblings born when the mothers were obese⁴⁹. The developmental overnutrition hypothesis suggests that foetal overnutrition leads to different adaptations affecting control of the appetite, development of adipocytes, metabolism and endocrine axis. For example, obese ewes overfed during pregnancy resulted in offspring with increased expression of fatty acid transporters and increased rate of fatty acid synthesis in adipose tissue – these ewes were fed 150% of the national recommendations (USA) ⁵⁰. Bitter taste receptors have recently been proved to be in the heart and agonists of these receptors cause relaxation of heart muscles, reduced ventricular pressure and increased pressure of the aorta⁵¹. Interestingly, maternal obesity due to high fat diet during pregnancy and lactation results in a decrease of bitter taste receptors in hearts of rats⁵². The decrease of these receptors might increase offspring risk of cardiovascular diseases in adulthood. In addition, maternal overnutrition induces an epigenetic modification of the *hypothalamic anorexigenic neuropeptide proopiomelanocortin (POMC)* gene⁵³. *POMC* encodes α -melanocyte-stimulating hormone that suppress the appetite. The offspring of rats fed with high fat diets exhibited increased levels of 5-methylcytosine (5mC) in *Pomc* promoter, which was associated with high bodyweight (Marco *et al.*, 2016). On the contrary, offspring from control rats presented high levels of 5-hydroxymethylcytosine but low 5mC in this promoter.

Increased 5mC allows binding of methyl binding domain 1 (MBD1) to *Pomc* promoter. MBD1 then binds to SETDB1 methyltransferase that bi-methylates histones (H3K9me2); this histone modification decreases *Pomc* expression. Therefore, maternal obesity modifies the methylation pattern of *Pomc* promoter in the offspring leading to histone modifications that decrease the expression of this gene. The resulted *Pomc* downregulation could hinder the suppression of the appetite contributing to the obese phenotype in the offspring⁵³.

3.2. Environmental chemicals

People are exposed to environmental chemicals from common sources such as cosmetic products and pharmaceutical treatments. These chemicals might influence *in utero* programming increasing the risk of NCDs in the offspring. A good example is bisphenol A (BPA) that increase offspring risk of infertility⁵⁴⁻⁵⁶. Certain analgesics like paracetamol, aspirin and indomethacin also augment offspring risk of NCDs (Table 1).

BPA is an endocrine disrupting chemical found in plastic and has been found in air, water, dust and in some biological samples such as hair, breast milk and placenta⁵⁷. In sheep, BPA exposure *in utero*, at doses relevant to humans, causes shortening of the time between the rise in estradiol and the LH surge as well as changes in ovarian follicular dynamics; this results in variable occurrence of follicular waves and alteration of follicular counts⁵⁶. Shortening of the estradiol-LH surge interval suggests that the positive feedback of estradiol to generate the LH surge and promote ovulation is altered. This seems to be caused by an increased sensitivity of the hypothalamic-pituitary axis to oestrogen and an earlier upregulation of gonadotropin-releasing hormone receptor in the pituitary. BPA increases expression of oestrogen receptor 1 and reduces expression of oestrogen receptor 2 in the hypothalamus, which promotes and inhibits LH surge, respectively⁵⁶. It would be interesting to expose human pituitary cells to

BPA *in vitro* at concentrations relevant to humans. This experiment could be done using a human pituitary adenoma cell line and would determine if the expressions of oestrogen receptors 1 and 2 are altered by BPA.

The presence of BPA in multiple human reproductive fluids and tissues such as ovarian follicular fluid, foetal plasma and placental tissue may result in a transgenerational effect of infertility. BPA exposure to pregnant mice modified the reproductive capacity of offspring multiple generations later⁵⁸. Pregnant mice (F0) were exposed to BPA concentrations relevant to environmental doses. Their female offspring (F1) generated the F2 generation and F2 females gave birth to the F3 generation. F3 depicted delayed pubertal onset and first oestrus, and reduced fertility. Moreover, F1, F2 and F3 exhibited a decreased ability to maintain pregnancy with increasing age⁵⁸. This transgenerational effect could be study in humans; an epidemiological study could investigate associations between subfertile women and previous BPA exposure in the family. Moreover, *in utero* BPA exposure had a dimorphic effect in mice livers, increasing oestrogen receptors and 17 α -hydrolase in females but reducing them in males. On the contrary, cyclooxygenase 1 was decreased in the liver of female mice but increased in male mice⁵⁹.

The underlying mechanisms of BPA's negative effect on *in utero* programming and NCDs development are controversial. One study showed that BPA exposure *in utero* in mice did not affect DNA methylation of the liver in offspring⁶⁰. On the contrary, BPA exposure *in utero* has also been shown to reduce global DNA methylation and to increase DNA methylation at *Gck* promoter in liver and sperm of rats^{61,62}. The differences between these studies' outcomes might be due to the windows of exposure, the species studied and the doses of BPA administered. It is important to use doses relevant to human exposure in studies about

environmental chemicals since different doses might have notably different effects on *in utero* programming. However, the relevant doses might be difficult to determine if the substance has a rapid clearance ratio or if its exposure concentration is highly variable between populations.

4. Active strategies against NCDs: Positive *in utero* programming.

In utero programming could potentially be used to prevent NCDs in later life. The increasing knowledge about NCDs' mechanisms and defective *in utero* programming origin allows to actively target against the causes of these diseases. These active approaches will not only decrease the rise in NCDs, like the passive approach, but could potentially reduce notably and faster their incidence. Importantly, the characteristic transgenerational effect of *in utero* programming suggests that these active approaches could induce NCDs protection through multiple generations. These active approaches are new and the majority of the information available is based upon animal studies – although there are some nutritional modification studies undertaken in humans. As mentioned earlier, animal studies cannot be extrapolated to humans and future *in vitro* studies with human tissue need to be performed to determine if these approaches could protect against NCDs. If they show a protective effect, extensive clinical trials should be undertaken to corroborate their effect in human beings and to discard any potential side effects. This section provides a comprehensive summary of the current literature on positive *in utero* programming, it does not suggest these treatments should be recommended to pregnant women because of the above reasons. The aim is to provide a starting point for the research of active *in utero* programming in the fight against NCDs. These active approaches can be divided into direct *in utero* programming modifications and personalised lifestyle pregnancy programs (PLPP).

4.1. Direct *in utero* programming modifications

The defective *in utero* programming origin of NCDs allows the development of treatments that preclude this negative programming. In addition, understanding NCD's mechanisms, it is possible to positively manipulate *in utero* programming to protect against them in the future.

4.1.1. Preclusion of negative *in utero* programming

This active strategy hinders *in utero* programming by adverse stimuli to which the mother could be exposed. For instance, it is well known that maternal hypercholesterolemia predispose offspring to atherosclerosis in later life due to increased lipid peroxidation and foetal fatty streak generation; in animal models maternal treatment with cholesterol lowering agents such as cholestyramine as well as with antioxidants such as vitamin E reduces offspring risk of developing this disease⁶³.

Recently, maternal prebiotic treatment was shown to protect against the increased risk of offspring obesity induced by maternal overnutrition⁶⁴. Treatment of rats with oligofructose altered the composition of the maternal gut flora decreasing gestational weight gain and adiposity as well as reducing maternal energy intake. Bacterial fermentation of oligofructose induces the secretion of satiety hormones in the intestine inhibiting appetite⁶⁴. In addition, resveratrol, an anti-inflammatory phytochemical, protects against lipopolysaccharide (LPS) caused-adverse *in utero* programming in the developing brain of rats⁶⁵. LPS is an endotoxin found on gram-negative bacteria that activate astrocytes and microglia in the developing brain generating an inflammatory response. This leads to a deficit of dopamine; 3,4-dihydroxyphenylacetic acid, homovanillic acid and tyrosine hydroxylase; feeding the pregnant rats with a resveratrol-supplemented diet maintained normal expression levels of these proteins after LPS injection. This is important because dopamine deficit and chronic

inflammation are markers of neurological NCDs like Parkinson's disease⁶⁵. Maternal supplementation with germinated brown rice also protected the offspring against insulin resistance due to maternal high fat diet in rats⁶⁶.

Continuous maternal exposure to light during pregnancy induces hypertension in male offspring in rats⁶⁷. Recently, maternal melatonin or agomelatine supplementation during pregnancy and lactation was observed to prevent offspring programmed hypertension. Interestingly, melatonin and agomelatine stopped upregulation of sodium transporters and the renin-angiotensin system genes, such as *Slc9a3*, *Slc12a3*, *Agtr2* and *Mas1*. They also downregulated core clock genes like *Bmal1* and *Clock*. All these genes are important in programmed hypertension⁶⁷. Since the dams were supplemented with melatonin or agomelatine during pregnancy and lactation, it is unknown if the protective effect is due to *in utero* programming, postnatal programming or both. Future studies could further investigate the role of these programmings.

4.1.2. Positive direct manipulation of *in utero* programming

Positive *in utero* programming treatments can potentially prevent the development of NCDs risk factors through life like high blood LDL levels.

In 2006 maternal immunization against oxidized LDL (oxLDL) was shown to prevent atherosclerosis in the offspring of hypercholesterolemic and normocholesterolemic rabbits and mice⁶⁸. Increased numbers of IgM against oxLDL epitopes and IgM-LDL immune complexes were found in the offspring of the immunized mothers. Moreover, atherosclerosis was reduced by 56% in the offspring of oxLDL immunized females compared to control. Even a single immune challenge with oxLDL stimulated a significant rise in IgM and IgM-

LDL complexes in naive rabbits never exposed to hypercholesterolemic diets. The results suggested that oxLDL maternal immunization prevents atherosclerosis by three mechanisms: reducing the exposure of the foetus to maternal hypercholesterolemic, reducing the amount of oxLDL in maternal circulation and by programming the immune response in the offspring. OxLDL maternal immunization positively changed postnatal humoral response to reduce this NCD⁶⁸.

The concept of *in utero* immune programming might be quite controversial since, up until now, the only known mechanisms to transmit immunological protection to the offspring consists of the transfer of maternal IgG antibodies through the placenta and the transmission of enterocytes coated with antibodies during lactation; the latter does not exist in humans. Thus, it is completely unknown if this therapy would be applicable to humans. Nevertheless, if *in utero* immune programming is possible, the development of multiple immune-related disorders could be prevented. A recent study showed that immunisation of hypercholesterolemic mice with oxLDL induces antibody-mediated immune response against a broad range of oxidation-specific epitopes in the offspring, which decreases risk to atherosclerosis and also insulin resistance and type 2 diabetes⁶⁹. Therefore, it would reduce the offspring's risk of cardiovascular diseases later in life.

Low doses of formaldehyde during pregnancy in rats were observed to protect offspring against asthma by interfering with inflammation of the lung⁷⁰. Maternal inhalation of formaldehyde in rats, at doses deemed harmless for human health, resulted in decreased recruitment of immune cells in the offsprings' lungs, blood and bone marrow after sensitisation with ovalbumin. The synthesis of anaphylactic antibodies was also reduced as well as tracheal hyperresponsiveness⁷⁰. Despite the protective effect of formaldehyde against

asthma, intake of this substance during pregnancy is not recommended. Formaldehyde is a dangerous chemical used to fix tissues and cells. It is associated with multiple side effects in adults such as bronchitis, dermatitis and chest pain. Furthermore, in the previous study the offspring of formaldehyde treated rats depicted low birthweight. However, a new synthetic chemical could be engineered based upon formaldehyde structure to maintain the beneficial effects but eliminate the adverse ones.

Interestingly, manipulation of the maternal microbiota during pregnancy can protect against the development of autoimmune diabetes in the offspring⁷¹. Type I diabetes is a genetic disease in which the pancreatic islets are destroyed by T-cells. Maternal treatment with neomycin modified the microorganism population of the mother increasing the number of *Bacteroidetes* and *Cyanobacteria* in the intestine of non-obese diabetic mice. These changes in the maternal gut microbiota resulted in variations in the offspring microbiota that could influence the immune programming. The antigen presenting cells of the neomycin treated mice's offspring exhibited weaker stimulation of CD8⁺ and CD4⁺ T cells. In addition, neomycin perinatal exposure reduced the incidence of autoimmune diabetes in the offspring including the second generation of mice⁷¹. This study suggests that positive *in utero* programming could be used to treat genetic diseases of offspring which with soaring levels of diabetes and treatment costs escalating, is well worth exploring further.

4.2. Personalised lifestyle pregnancy programs (PLPP)

Healthy life habits of the mother can also induce positive *in utero* programming. PLPP programs could be developed for pregnant women to protect their offspring against common NCDs and high-risk ones, based upon family history.

4.2.1. Maternal diet

Certain diets contain bioactive compounds that reduce offspring's risk of developing certain NCDs such as cancer or cardiovascular diseases. They might affect epigenetics processes and the maternal/foetus microbiota. Identification of these biological products and consumption of them at the developmental stages when *in utero* programming is more susceptible to their action might become an efficient preventive measure against NCDs⁷². An important strength of this approach is that specific diet recommendations are easily applicable.

Illustrations of bioactive compounds are genistein, sulforaphane and resveratrol⁷². Genistein is a phytoestrogen found in soybean products that seems to modify the hypermethylated phenotype of the agouti mice decreasing obesity and protecting against mammary gland cancer later in life. Sulforaphane is an isothiocyanate found in certain vegetables, such as broccoli and cauliflower, which can reduce the risk of some cancers. A maternal sulforaphane enriched diet prevents the offspring of a mouse model of spontaneous mammary gland cancer to develop this disease⁷². Resveratrol is a polyphenol found in plants such as cranberries and blueberries. As previously mentioned (section 4.1.1), it can be used to directly suppress the adverse *in utero* programming of LPS in the brain. In addition, resveratrol regulates histone methylation and acetylation modifying the epigenome⁷³. For example, resveratrol activates sirtuin 1 (SIRT1), a histone deacetylase involved in healthy aging that promotes renewal of cells in the retina and protects retinal neurons. Abnormal localisation of SIRT1 increases the apoptosis of retinal photoreceptors, as reviewed in Pennington & DeAngelis, 2015⁷⁴. Consequently, maternal supplementation with resveratrol could also promote acute vision in the offspring. The regulatory role of dietary bioactive substances is extensively reviewed in Vahid *et al.*, 2015⁷³.

Maternal diets rich in fruits and vegetables during pregnancy are associated with reduced risk of certain cancers in the offspring including childhood brain tumours, lymphoblastic leukaemia and germ cell tumours⁷⁵⁻⁷⁷. An epidemiological study showed maternal diet rich in fruits but low in fried foods and cured meats decreased offsprings' risk of unilateral retinoblastoma. The latter disease was also prevented by maternal high intake of vegetables and fruits but low consumption of fried foods, sweets, and red and cured meats. The main weaknesses of this epidemiological study were that food questionnaires were performed 13 years after pregnancy and that the responses could be biased depending on the mothers' perception of a healthy diet⁷⁸.

Consumption of fats might also be beneficial to the offspring. Although, different types of fats appear to have different effects. Oils containing high amounts of n-6 fatty acids appear to increase the risk of mammary tumorigenesis in the offspring^{79, 80} while oils with high levels of n-3 fatty acids seems to reduce it⁸¹⁻⁸³. A lard-based high fat diet (37% saturated, 38% monounsaturated, 24% polyunsaturated fatty acids) during pregnancy or pregnancy and lactation decreased risk of mammary cancer in rats. The consumption of this diet during pregnancy in rats resulted in reduced incidence and multiplicity of mammary cancer, as well as longer tumour latency. The lard-based high fat diet also induced an increase in H3K9me3 methylation and expression of p21 as well as p27, which are involved in G1-checkpoint of mitosis⁸⁴. Interestingly, maternal consumption of conjugated linoleic acid (CLA) – a n-6 fatty acid- during pregnancy and lactation prevented increased adiposity in offspring from high fat diet dams⁸⁵. The optimal combinations of fatty acids in the pregnant maternal diet to prevent the development of NCDs in the offspring should be elucidated as this has far reaching effects for women's health.

Taurine supplementation during pregnancy and lactation protects against type II diabetes in foetal undernutrition⁸⁶⁻⁸⁹. Taurine is a sulphur-containing non-essential amino acid that works as an antioxidant and a neurotransmitter; its concentration in foetal blood is 1.5 fold higher than in the mother. In rats maternal supplementation with taurine promotes proliferation and decreases apoptosis of β -cells in pancreatic islet. It also increases glucose metabolism and insulin sensitivity. Interestingly, taurine consumption during pregnancy leads to certain gender-specific differences. It improves β -cells function more in males than in females and improves insulin sensitivity in peripheral tissues more in females than in males⁸⁹. Type II diabetes and foetal low protein nutrition are also associated with altered mitochondrial DNA (mtDNA). mtDNA is reduced in type II diabetes and insulin resistance⁸⁸. Moreover, gestational protein restriction in mice was shown to induce significant changes in gene expression in liver and skeletal muscle; the most abundant changes were in mitochondrial genes especially those involved in oxidative phosphorylation⁸⁷. In fact, mitochondria with abnormal shapes and reduced number of secretory granules were observed in β -cells of *in utero* malnourish rats. These mitochondria also exhibited decreased *cytochrome c oxidase subunit I (COX I)* expression. COX I is a protein encoded in the mitochondrial DNA that belongs to complex II of the oxidative phosphorylation. Maternal taurine supplementation restored all the above mitochondrial alterations⁸⁸. In rats, maternal taurine supplementation exclusively in late pregnancy increases insulin resistance and obesity in the offspring⁹⁰. Therefore, these data indicate the importance of taurine during pregnancy. It would be advantageous to determine if supplementation in early pregnancy has negative or positive effects to develop accurate taurine supplementation guidelines during pregnancy. Modifications of mtDNA through positive *in utero* programming present a great potential since mtDNA is transmitted maternally and beneficial modifications are likely to be transmitted to several generations.

It is worth mentioning that diabetes often leads to diabetic retinopathy that causes loss of vision. This loss of vision is due to the destruction of retinal vasculature, neo-vascularisation and rupture of the blood-retinal barrier. Low-salt maternal diets protect against these effects reducing the risk of diabetic retinopathy and related diseases like retinopathy of prematurity⁹¹. It would be interesting to assess the beneficial effect of maternal low-salt diets in human offspring.

As previously mentioned, prebiotics prevent against the adverse *in utero* programming induced by maternal obesity (section 4.1.1). Prebiotic diets can also reduce the risk of allergies later in life⁹²⁻⁹⁶. In wheat-immunised mice, a maternal diet containing galacto-oligosaccharide and inulin prevented wheat allergies in the offspring⁹⁶. Similarly, maternal intake of short-chain galacto- and long-chain fructo-oligosaccharides protected against egg allergies in ova-immunised mice^{93, 94}. Prebiotic diet during pregnancy also prevented asthma in house dust mite-immunised mice offspring⁹⁵. These beneficial effects of prebiotics is due to reduced induction of immunoglobulins E and histamine, damped Th2 immune response and increased number of T regulatory cells. This immune regulatory effect of prebiotics could be used to protect against immune-related NCDs in the offspring. It would be very interesting to test if maternal prebiotic consumption can protect against certain immunological reproductive diseases. Maternal immune tolerance is essential for a successful pregnancy and its failure leads to rejection of the embryo preventing implantation or damaging the embryo through gestation. Prebiotic diets might increase maternal and offspring immune tolerance which might reduce the risk of infertility due to recurrent implantation failure or miscarriage. A detailed study of three bacterial strains showed that, in mice, perinatal consumption of *Bifidobacterium longum* significantly increased the numbers

of Foxp3⁺ T regulatory cells, involved in immune regulation⁹². Direct administration of these bacteria during pregnancy might protect offspring against immune-related NCDs.

Importantly, clinical studies showed a potential benefit of maternal nutritional supplementation to prevent NCDs in humans. A reduction in low birthweight was observed after maternal supplementation with a cocktail of multiple micronutrients⁹⁷ and supplementation with iron and folate^{98,99}. A balanced protein-energy supplementation during pregnancy is also associated with increased birthweight in the offspring of undernourished women, reducing the risk of low birthweight¹⁰⁰. Given the importance of birthweight on NCDs development in the offspring, maternal supplementation could be a good approach to prevent them. In addition, folate supplementation is associated with reduced levels of microalbuminuria in the offspring¹⁰¹ – this is considered to protect against neuronal damage.

4.2.2. Maternal exercise

Maternal exercise is widely recommended for a healthy pregnancy. The American College of Obstetricians and Gynaecologists recommends daily half-hour of moderate exercise during pregnancy. Water exercises are desirable to help regulate body temperature and blood flow. In animals, maternal physical activity has long-term benefits in the offspring. Increased spatial memory and number of hippocampus cells was observed in the offspring of pregnant rats that swam. Maternal swimming also enhanced neurogenesis and short-memory, and augmented antioxidants activity and mitochondria biogenesis in the brain of offspring rats; these mechanisms may underlie the neuroprotection¹⁰². Therefore maternal exercise during pregnancy might protect against neuronal damage later in life, this would reduce the incidence of neurodegenerative NCDs such as Alzheimer and Glaucoma. Until now the positive *in utero* programming of exercise has only been studied in animals. Human

epidemiological studies could assess the association between maternal sport habits before and during pregnancy with the incidence of neurodegenerative diseases later in life.

The mechanisms underlying the positive *in utero* programming of exercise also remain to be elucidated. Interestingly, regular physical exercise in adult rats is associated with increased brain expression of neurotrophic and growth factors as well as epigenetic modifications¹⁰³. For instance, the expression of vascular endothelial growth factor A (*VegfA*) gene is increased in the hippocampus of male rats after 2 weeks of voluntary running; this is associated with CpG hypomethylation in *VegfA* promoters and exon 1¹⁰³. Therefore, the neurogenesis in offspring enhanced by maternal exercise during pregnancy in rats might be the results of epigenetic modifications during *in utero* programming.

5. Conclusion

In utero programming origin of NCDs allows the development of several medical strategies to reduce their incidence. This programming is based upon the great plasticity of embryonic development in which stimuli can lead to anatomical disruptions, epigenetic modifications and microbiota alterations.

Adverse stimuli lead to negative *in utero* programming causing alterations with subtle detrimental effects on tissue physiology that increase offspring risk of NCDs later in life. Unhealthy diet and environmental chemicals increase the risk of cardiovascular diseases, atherosclerosis and infertility in the offspring. They represent maternal lifestyle choices that could be avoided or reduced as a passive defence against NCDs. Consequently, FIGO plans to improve maternal education to prevent pregnant women from getting in contact with those risk stimuli for NCDs. Nevertheless, many unknowns remain to be elucidated to optimise this passive approach. Future studies should determine whether preventable stimuli other than those mentioned herein might negatively affect *in utero* programming. Most importantly, future research should investigate if *in utero* programming has more, the same or less relevance than postnatal programming for the development of NCDs

The increase in knowledge about *in utero* programming and NCDs development gives rise to the opportunity to actively target NCDs, which has the potential to significantly and rapidly reduce NCD incidence. Furthermore, active strategies might provide transgenerational protection. These strategies can be divided into direct *in utero* programming modifications and PLPP. The former might involve the preclusion of negative *in utero* programming by adverse stimuli or the induction of positive *in utero* programming that prevents the development of NCDs risk factors later in life. PLPP programs include beneficial maternal

647 diet and exercise that protect offspring against these diseases. The active medical approach is
648 promising since it could be used to prevent common NCDs and high risk ones, according to
649 family history. However, research on the active use of *in utero* programming to protect
650 against NCDs is at a very early stage and future studies needs to determine if this approach is
651 effective in humans and any potential side effects before being considered for clinical
652 practice.

653

654

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665 None

666

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References

1. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet*. 1986;1(8489), 1077-1081.
2. Barker DJ, Osmond C, Simmonds SJ, Wield GA. The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. *BMJ*. 1993;306(6875), 422-426.
3. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet*. 1989;2(8663), 577-580.
4. Ellison PT. Evolutionary perspectives on the fetal origins hypothesis. *Am J Hum Biol*. 2005;17(1), 113-118.
5. de Boo HA, Harding JE. The developmental origins of adult disease (Barker) hypothesis. *Aust N Z J Obstet Gynaecol*. 2006;46(1), 4-14.
6. Roura LC, Arulkumaran SS. Facing the noncommunicable disease (NCD) global epidemic - The battle of prevention starts in utero - The FIGO challenge. *Best Practice & Research Clinical Obstetrics & Gynaecology*. 2015;29(1), 5-14.
7. Roseboom TJ, van der Meulen JH, Osmond C, et al. Coronary heart disease after prenatal exposure to the Dutch famine, 1944-45. *Heart*. 2000;84(6), 595-598.
8. Painter RC, Roseboom TJ, Bleker OP. Prenatal exposure to the Dutch famine and disease in later life: an overview. *Reprod Toxicol*. 2005;20(3), 345-352.
9. de Rooij SR, Painter RC, Phillips DI, et al. Impaired insulin secretion after prenatal exposure to the Dutch famine. *Diabetes Care*. 2006;29(8), 1897-1901.
10. Ramirez-Velez R. [In utero fetal programming and its impact on health in adulthood]. *Endocrinol Nutr*. 2012;59(6), 383-393.
11. Barker DJ. In utero programming of chronic disease. *Clin Sci (Lond)*. 1998;95(2), 115-128.

- 696 12. Mackenzie HS, Brenner BM. Fewer nephrons at birth: a missing link in the
697 etiology of essential hypertension? *Am J Kidney Dis.* 1995;26(1), 91-98.
- 698 13. Hughson M, Farris AB, 3rd, Douglas-Denton R, Hoy WE, Bertram JF. Glomerular
699 number and size in autopsy kidneys: the relationship to birth weight. *Kidney Int.*
700 2003;63(6), 2113-2122.
- 701 14. Hoy WE, Hughson MD, Bertram JF, Douglas-Denton R, Amann K. Nephron
702 number, hypertension, renal disease, and renal failure. *J Am Soc Nephrol.*
703 2005;16(9), 2557-2564.
- 704 15. Hoy WE, Douglas-Denton RN, Hughson MD, Cass A, Johnson K, Bertram JF. A
705 stereological study of glomerular number and volume: preliminary findings in a
706 multiracial study of kidneys at autopsy. *Kidney Int Suppl.* 2003; doi:
707 10.1046/j.1523-1755.63.s83.8.x(83), S31-37.
- 708 16. Mei-Zahav M, Korzets Z, Cohen I, et al. Ambulatory blood pressure monitoring in
709 children with a solitary kidney - a comparison between unilateral renal agenesis
710 and uninephrectomy. *Blood Press Monit.* 2001;6(5), 263-267.
- 711 17. Brenner BM, Milford EL. Nephron underdosing: a programmed cause of chronic
712 renal allograft failure. *Am J Kidney Dis.* 1993;21(5 Suppl 2), 66-72.
- 713 18. Maccani MA, Knopik VS. Cigarette smoke exposure-associated alterations to non-
714 coding RNA. *Front Genet.* 2012;3, 53.
- 715 19. Babenko O, Kovalchuk I, Metz GA. Stress-induced perinatal and
716 transgenerational epigenetic programming of brain development and mental
717 health. *Neurosci Biobehav Rev.* 2015;48, 70-91.
- 718 20. van Vliet J, Oates NA, Whitelaw E. Epigenetic mechanisms in the context of
719 complex diseases. *Cell Mol Life Sci.* 2007;64(12), 1531-1538.

21. Kaikkonen MU, Lam MT, Glass CK. Non-coding RNAs as regulators of gene expression and epigenetics. *Cardiovasc Res.* 2011;90(3), 430-440.
22. Schmitz SU, Grote P, Herrmann BG. Mechanisms of long noncoding RNA function in development and disease. *Cell Mol Life Sci.* 2016;73(13), 2491-2509.
23. Santos F, Dean W. Epigenetic reprogramming during early development in mammals. *Reproduction.* 2004;127(6), 643-651.
24. Ollikainen M, Smith KR, Joo EJ, et al. DNA methylation analysis of multiple tissues from newborn twins reveals both genetic and intrauterine components to variation in the human neonatal epigenome. *Hum Mol Genet.* 2010;19(21), 4176-4188.
25. Heijmans BT, Tobi EW, Stein AD, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci U S A.* 2008;105(44), 17046-17049.
26. Cui H, Onyango P, Brandenburg S, Wu Y, Hsieh CL, Feinberg AP. Loss of imprinting in colorectal cancer linked to hypomethylation of H19 and IGF2. *Cancer Res.* 2002;62(22), 6442-6446.
27. Suter MA, Anders AM, Aagaard KM. Maternal smoking as a model for environmental epigenetic changes affecting birthweight and fetal programming. *Mol Hum Reprod.* 2013;19(1), 1-6.
28. Couturier-Maillard A, Secher T, Rehman A, et al. NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *J Clin Invest.* 2013;123(2), 700-711.
29. Russell SL, Gold MJ, Willing BP, Thorson L, McNagny KM, Finlay BB. Perinatal antibiotic treatment affects murine microbiota, immune responses and allergic asthma. *Gut Microbes.* 2013;4(2), 158-164.

- 745 30. Ege MJ, Bieli C, Frei R, et al. Prenatal farm exposure is related to the expression of
746 receptors of the innate immunity and to atopic sensitization in school-age
747 children. *J Allergy Clin Immunol*. 2006;117(4), 817-823.
- 748 31. Vuillermine PJ, Macia L, Nanan R, Tang ML, Collier F, Brix S. The maternal
749 microbiome during pregnancy and allergic disease in the offspring. *Semin*
750 *Immunopathol*. 2017;39(6), 669-675.
- 751 32. Mulligan CM, Friedman JE. Maternal modifiers of the infant gut microbiota:
752 metabolic consequences. *J Endocrinol*. 2017;235(1), R1-R12.
- 753 33. Jenmalm MC. The mother-offspring dyad: microbial transmission, immune
754 interactions and allergy development. *J Intern Med*. 2017;282(6), 484-495.
- 755 34. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation
756 may be initiated in utero by distinct microbial communities in the placenta and
757 amniotic fluid. *Sci Rep*. 2016;6, 23129.
- 758 35. Chen C, Song X, Wei W, et al. The microbiota continuum along the female
759 reproductive tract and its relation to uterine-related diseases. *Nat Commun*.
760 2017;8(1), 875.
- 761 36. Perez PF, Dore J, Leclerc M, et al. Bacterial imprinting of the neonatal immune
762 system: lessons from maternal cells? *Pediatrics*. 2007;119(3), e724-732.
- 763 37. Fridmann-Sirkis Y, Stern S, Elgart M, et al. Delayed development induced by
764 toxicity to the host can be inherited by a bacterial-dependent, transgenerational
765 effect. *Front Genet*. 2014;5, 27.
- 766 38. Ojha S, Robinson L, Symonds ME, Budge H. Suboptimal maternal nutrition affects
767 offspring health in adult life. *Early Hum Dev*. 2013;89(11), 909-913.

39. Yoshimura S, Masuzaki H, Miura K, Gotoh H, Ishimaru T. Fetal blood flow redistribution in term intrauterine growth retardation (IUGR) and post-natal growth. *Int J Gynaecol Obstet*. 1998;60(1), 3-8.
40. Sandovici I, Hoelle K, Angiolini E, Constancia M. Placental adaptations to the maternal-fetal environment: implications for fetal growth and developmental programming. *Reprod Biomed Online*. 2012;25(1), 68-89.
41. Watkins AJ, Lucas ES, Torrens C, et al. Maternal low-protein diet during mouse pre-implantation development induces vascular dysfunction and altered renin-angiotensin-system homeostasis in the offspring. *Br J Nutr*. 2010;103(12), 1762-1770.
42. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. 1992. *Int J Epidemiol*. 2013;42(5), 1215-1222.
43. Thorn SR, Regnault TR, Brown LD, et al. Intrauterine growth restriction increases fetal hepatic gluconeogenic capacity and reduces messenger ribonucleic acid translation initiation and nutrient sensing in fetal liver and skeletal muscle. *Endocrinology*. 2009;150(7), 3021-3030.
44. Barry JS, Anthony RV. The pregnant sheep as a model for human pregnancy. *Theriogenology*. 2008;69(1), 55-67.
45. Radford EJ, Ito M, Shi H, et al. In utero effects. In utero undernourishment perturbs the adult sperm methylome and intergenerational metabolism. *Science*. 2014;345(6198), 1255903.
46. Borgel J, Guibert S, Li Y, et al. Targets and dynamics of promoter DNA methylation during early mouse development. *Nat Genet*. 2010;42(12), 1093-1100.

- 792 47. Smallwood SA, Tomizawa S, Krueger F, et al. Dynamic CpG island methylation
793 landscape in oocytes and preimplantation embryos. *Nat Genet.* 2011;43(8), 811-
794 814.
- 795 48. Smith ZD, Chan MM, Mikkelsen TS, et al. A unique regulatory phase of DNA
796 methylation in the early mammalian embryo. *Nature.* 2012;484(7394), 339-344.
- 797 49. Smith J, Cianflone K, Biron S, et al. Effects of maternal surgical weight loss in
798 mothers on intergenerational transmission of obesity. *J Clin Endocrinol Metab.*
799 2009;94(11), 4275-4283.
- 800 50. Long NM, Rule DC, Tuersunjiang N, Nathanielsz PW, Ford SP. Maternal obesity in
801 sheep increases fatty acid synthesis, upregulates nutrient transporters, and
802 increases adiposity in adult male offspring after a feeding challenge. *PLoS One.*
803 2015;10(4), e0122152.
- 804 51. Foster SR, Blank K, See Hoe LE, et al. Bitter taste receptor agonists elicit G-
805 protein-dependent negative inotropy in the murine heart. *FASEB J.* 2014;28(10),
806 4497-4508.
- 807 52. Raipuria M, Hardy GO, Bahari H, Morris MJ. Maternal obesity regulates gene
808 expression in the hearts of offspring. *Nutr Metab Cardiovasc Dis.* 2015;25(9),
809 881-888.
- 810 53. Marco A, Kisliouk T, Tabachnik T, Weller A, Meiri N. DNA CpG Methylation (5-
811 Methylcytosine) and Its Derivative (5-Hydroxymethylcytosine) Alter Histone
812 Posttranslational Modifications at the Pomc Promoter, Affecting the Impact of
813 Perinatal Diet on Leanness and Obesity of the Offspring. *Diabetes.* 2016;65(8),
814 2258-2267.
- 815 54. Savabieasfahani M, Kannan K, Astapova O, Evans NP, Padmanabhan V.
816 Developmental programming: differential effects of prenatal exposure to

- bisphenol-A or methoxychlor on reproductive function. *Endocrinology*. 2006;147(12), 5956-5966.
55. Veiga-Lopez A, Luense LJ, Christenson LK, Padmanabhan V. Developmental programming: gestational bisphenol-A treatment alters trajectory of fetal ovarian gene expression. *Endocrinology*. 2013;154(5), 1873-1884.
56. Veiga-Lopez A, Beckett EM, Abi Salloum B, Ye W, Padmanabhan V. Developmental programming: prenatal BPA treatment disrupts timing of LH surge and ovarian follicular wave dynamics in adult sheep. *Toxicol Appl Pharmacol*. 2014;279(2), 119-128.
57. Liao C, Kannan K. A survey of alkylphenols, bisphenols, and triclosan in personal care products from China and the United States. *Arch Environ Contam Toxicol*. 2014;67(1), 50-59.
58. Ziv-Gal A, Wang W, Zhou C, Flaws JA. The effects of in utero bisphenol A exposure on reproductive capacity in several generations of mice. *Toxicol Appl Pharmacol*. 2015;284(3), 354-362.
59. Ilagan Y, Mamillapalli R, Goetz LG, Kayani J, Taylor HS. Bisphenol-A exposure in utero programs a sexually dimorphic estrogenic state of hepatic metabolic gene expression. *Reprod Toxicol*. 2017;71, 84-94.
60. van Esterik JC, Vitins AP, Hodemaekers HM, et al. Liver DNA methylation analysis in adult female C57BL/6JxFVB mice following perinatal exposure to bisphenol A. *Toxicol Lett*. 2015;232(1), 293-300.
61. Ma Y, Xia W, Wang DQ, et al. Hepatic DNA methylation modifications in early development of rats resulting from perinatal BPA exposure contribute to insulin resistance in adulthood. *Diabetologia*. 2013;56(9), 2059-2067.

- 841 62. Li G, Chang H, Xia W, Mao Z, Li Y, Xu S. F0 maternal BPA exposure induced
842 glucose intolerance of F2 generation through DNA methylation change in Gck.
843 *Toxicol Lett.* 2014;228(3), 192-199.
- 844 63. Palinski W, Napoli C. The fetal origins of atherosclerosis: maternal
845 hypercholesterolemia, and cholesterol-lowering or antioxidant treatment during
846 pregnancy influence in utero programming and postnatal susceptibility to
847 atherogenesis. *FASEB J.* 2002;16(11), 1348-1360.
- 848 64. Paul HA, Bomhof MR, Vogel HJ, Reimer RA. Diet-induced changes in maternal gut
849 microbiota and metabolomic profiles influence programming of offspring obesity
850 risk in rats. *Sci Rep.* 2016;6, 20683.
- 851 65. Rose KM, Parmar MS, Cavanaugh JE. Dietary supplementation with resveratrol
852 protects against striatal dopaminergic deficits produced by in utero LPS
853 exposure. *Brain Res.* 2014;1573, 37-43.
- 854 66. Adamu HA, Imam MU, Ooi DJ, Esa NM, Rosli R, Ismail M. In utero exposure to
855 germinated brown rice and its oryzanol-rich extract attenuated high fat diet-
856 induced insulin resistance in F1 generation of rats. *BMC Complement Altern Med.*
857 2017;17(1), 67.
- 858 67. Tain YL, Lin YJ, Chan JYH, Lee CT, Hsu CN. Maternal melatonin or agomelatine
859 therapy prevents programmed hypertension in male offspring of mother
860 exposed to continuous light. *Biol Reprod.* 2017;97(4), 636-643.
- 861 68. Yamashita T, Freigang S, Eberle C, et al. Maternal immunization programs
862 postnatal immune responses and reduces atherosclerosis in offspring. *Circ Res.*
863 2006;99(7), e51-64.

- 864 69. Eberle C, Merki E, Yamashita T, et al. Maternal immunization affects in utero
865 programming of insulin resistance and type 2 diabetes. *PLoS One*. 2012;7(9),
866 e45361.
- 867 70. Maiellaro M, Correa-Costa M, Vitoretti LB, et al. Exposure to low doses of
868 formaldehyde during pregnancy suppresses the development of allergic lung
869 inflammation in offspring. *Toxicol Appl Pharmacol*. 2014;278(3), 266-274.
- 870 71. Hu Y, Jin P, Peng J, Zhang X, Wong FS, Wen L. Different immunological responses
871 to early-life antibiotic exposure affecting autoimmune diabetes development in
872 NOD mice. *J Autoimmun*. 2016;72, 47-56.
- 873 72. Li Y, Saldanha SN, Tollefsbol TO. Impact of epigenetic dietary compounds on
874 transgenerational prevention of human diseases. *AAPS J*. 2014;16(1), 27-36.
- 875 73. Vahid F, Zand H, Nosrat-Mirshekarlou E, Najafi R, Hekmatdoost A. The role
876 dietary of bioactive compounds on the regulation of histone acetylases and
877 deacetylases: a review. *Gene*. 2015;562(1), 8-15.
- 878 74. Pennington KL, DeAngelis MM. Epigenetic Mechanisms of the Aging Human
879 Retina. *J Exp Neurosci*. 2015;9(Suppl 2), 51-79.
- 880 75. Jensen CD, Block G, Buffler P, Ma X, Selvin S, Month S. Maternal dietary risk
881 factors in childhood acute lymphoblastic leukemia (United States). *Cancer Causes*
882 *Control*. 2004;15(6), 559-570.
- 883 76. Musselman JR, Jurek AM, Johnson KJ, et al. Maternal dietary patterns during early
884 pregnancy and the odds of childhood germ cell tumors: A Children's Oncology
885 Group study. *Am J Epidemiol*. 2011;173(3), 282-291.
- 886 77. Pogoda JM, Preston-Martin S, Howe G, et al. An international case-control study
887 of maternal diet during pregnancy and childhood brain tumor risk: a histology-
888 specific analysis by food group. *Ann Epidemiol*. 2009;19(3), 148-160.

- 889 78. Lombardi C, Ganguly A, Bunin GR, et al. Maternal diet during pregnancy and
890 unilateral retinoblastoma. *Cancer Causes Control*. 2015;26(3), 387-397.
- 891 79. Hilakivi-Clarke L, Clarke R, Onojafe I, Raygada M, Cho E, Lippman M. A maternal
892 diet high in n - 6 polyunsaturated fats alters mammary gland development,
893 puberty onset, and breast cancer risk among female rat offspring. *Proc Natl Acad*
894 *Sci U S A*. 1997;94(17), 9372-9377.
- 895 80. de Assis S, Khan G, Hilakivi-Clarke L. High birth weight increases mammary
896 tumorigenesis in rats. *Int J Cancer*. 2006;119(7), 1537-1546.
- 897 81. Stark AH, Kossoy G, Zusman I, Yarden G, Madar Z. Olive oil consumption during
898 pregnancy and lactation in rats influences mammary cancer development in
899 female offspring. *Nutr Cancer*. 2003;46(1), 59-65.
- 900 82. Olivo SE, Hilakivi-Clarke L. Opposing effects of prepubertal low- and high-fat n-3
901 polyunsaturated fatty acid diets on rat mammary tumorigenesis. *Carcinogenesis*.
902 2005;26(9), 1563-1572.
- 903 83. Ion G, Akinsete JA, Hardman WE. Maternal consumption of canola oil suppressed
904 mammary gland tumorigenesis in C3(1) TAg mice offspring. *BMC Cancer*.
905 2010;10, 81.
- 906 84. de Oliveira Andrade F, Fontelles CC, Rosim MP, et al. Exposure to lard-based
907 high-fat diet during fetal and lactation periods modifies breast cancer
908 susceptibility in adulthood in rats. *J Nutr Biochem*. 2014;25(6), 613-622.
- 909 85. Segovia SA, Vickers MH, Gray C, Zhang XD, Reynolds CM. Conjugated Linoleic
910 Acid Supplementation Improves Maternal High Fat Diet-Induced Programming
911 of Metabolic Dysfunction in Adult Male Rat Offspring. *Sci Rep*. 2017;7(1), 6663.

- 912 86. Arany E, Strutt B, Romanus P, Remacle C, Reusens B, Hill DJ. Taurine supplement
913 in early life altered islet morphology, decreased insulinitis and delayed the onset of
914 diabetes in non-obese diabetic mice. *Diabetologia*. 2004;47(10), 1831-1837.
- 915 87. Mortensen OH, Olsen HL, Frandsen L, et al. A maternal low protein diet has
916 pronounced effects on mitochondrial gene expression in offspring liver and
917 skeletal muscle; protective effect of taurine. *J Biomed Sci*. 2010;17 Suppl 1, S38.
- 918 88. Lee YY, Lee HJ, Lee SS, et al. Taurine supplementation restored the changes in
919 pancreatic islet mitochondria in the fetal protein-malnourished rat. *Br J Nutr*.
920 2011;106(8), 1198-1206.
- 921 89. Tang C, Marchand K, Lam L, et al. Maternal taurine supplementation in rats
922 partially prevents the adverse effects of early-life protein deprivation on beta-
923 cell function and insulin sensitivity. *Reproduction*. 2013;145(6), 609-620.
- 924 90. Hultman K, Alexanderson C, Manneras L, Sandberg M, Holmang A, Jansson T.
925 Maternal taurine supplementation in the late pregnant rat stimulates postnatal
926 growth and induces obesity and insulin resistance in adult offspring. *J Physiol*.
927 2007;579(Pt 3), 823-833.
- 928 91. Deliyanti D, Armani R, Casely D, Figgett WA, Agrotis A, Wilkinson-Berka JL.
929 Retinal vasculopathy is reduced by dietary salt restriction: involvement of Glia,
930 ENaC α , and the renin-angiotensin-aldosterone system. *Arterioscler Thromb*
931 *Vasc Biol*. 2014;34(9), 2033-2041.
- 932 92. Lyons A, O'Mahony D, O'Brien F, et al. Bacterial strain-specific induction of
933 Foxp3⁺ T regulatory cells is protective in murine allergy models. *Clin Exp Allergy*.
934 2010;40(5), 811-819.
- 935 93. Hogenkamp A, Knippels LM, Garssen J, van Esch BC. Supplementation of Mice
936 with Specific Nondigestible Oligosaccharides during Pregnancy or Lactation

- 937 Leads to Diminished Sensitization and Allergy in the Female Offspring. *J Nutr.*
938 2015;145(5), 996-1002.
- 939 94. Hogenkamp A, Thijssen S, van Vlies N, Garssen J. Supplementing pregnant mice
940 with a specific mixture of nondigestible oligosaccharides reduces symptoms of
941 allergic asthma in male offspring. *J Nutr.* 2015;145(3), 640-646.
- 942 95. Verheijden KA, Willemsen LE, Braber S, et al. Dietary galacto-oligosaccharides
943 prevent airway eosinophilia and hyperresponsiveness in a murine house dust
944 mite-induced asthma model. *Respir Res.* 2015;16, 17.
- 945 96. Bouchaud G, Castan L, Chesne J, et al. Maternal exposure to GOS/inulin mixture
946 prevents food allergies and promotes tolerance in offspring in mice. *Allergy.*
947 2016;71(1), 68-76.
- 948 97. Vaidya A, Saville N, Shrestha BP, Costello AM, Manandhar DS, Osrin D. Effects of
949 antenatal multiple micronutrient supplementation on children's weight and size
950 at 2 years of age in Nepal: follow-up of a double-blind randomised controlled
951 trial. *Lancet.* 2008;371(9611), 492-499.
- 952 98. Pena-Rosas JP, De-Regil LM, Dowswell T, Viteri FE. Daily oral iron
953 supplementation during pregnancy. *Cochrane Database Syst Rev.* 2012;12,
954 CD004736.
- 955 99. Pena-Rosas JP, De-Regil LM, Dowswell T, Viteri FE. Intermittent oral iron
956 supplementation during pregnancy. *Cochrane Database Syst Rev.* 2012; doi:
957 10.1002/14651858.CD009997(7), CD009997.
- 958 100. Imdad A, Bhutta ZA. Maternal nutrition and birth outcomes: effect of balanced
959 protein-energy supplementation. *Paediatr Perinat Epidemiol.* 2012;26 Suppl 1,
960 178-190.

- 961 101. Stewart CP, Christian P, Schulze KJ, Leclercq SC, West KP, Jr., Khatry SK. Antenatal
962 micronutrient supplementation reduces metabolic syndrome in 6- to 8-year-old
963 children in rural Nepal. *J Nutr.* 2009;139(8), 1575-1581.
- 964 102. Marcelino TB, Longoni A, Kudo KY, et al. Evidences that maternal swimming
965 exercise improves antioxidant defenses and induces mitochondrial biogenesis in
966 the brain of young Wistar rats. *Neuroscience.* 2013;246, 28-39.
- 967 103. Solvsten CAE, de Paoli F, Christensen JH, Nielsen AL. Voluntary Physical Exercise
968 Induces Expression and Epigenetic Remodeling of VegfA in the Rat
969 Hippocampus. *Mol Neurobiol.* 2018;55(1), 567-582.
- 970 104. Ng SP, Conklin DJ, Bhatnagar A, Bolanowski DD, Lyon J, Zelikoff JT. Prenatal
971 exposure to cigarette smoke induces diet- and sex-dependent dyslipidemia and
972 weight gain in adult murine offspring. *Environ Health Perspect.* 2009;117(7),
973 1042-1048.
- 974 105. Napoli C, Infante T, Casamassimi A. Maternal-foetal epigenetic interactions in the
975 beginning of cardiovascular damage. *Cardiovasc Res.* 2011;92(3), 367-374.
- 976 106. Davis EF, Lazdam M, Lewandowski AJ, et al. Cardiovascular risk factors in
977 children and young adults born to preeclamptic pregnancies: a systematic
978 review. *Pediatrics.* 2012;129(6), e1552-1561.
- 979 107. Christensen S, Jaffar Z, Cole E, et al. Prenatal environmental tobacco smoke
980 exposure increases allergic asthma risk with methylation changes in mice.
981 *Environ Mol Mutagen.* 2017;58(6), 423-433.
- 982 108. Bousquet J, Jacot W, Yssel H, Vignola AM, Humbert M. Epigenetic inheritance of
983 fetal genes in allergic asthma. *Allergy.* 2004;59(2), 138-147.

- 984 109. Govindarajah V, Leung YK, Ying J, et al. In utero exposure of rats to high-fat diets
985 perturbs gene expression profiles and cancer susceptibility of prepubertal
986 mammary glands. *J Nutr Biochem*. 2016;29, 73-82.
- 987 110. Baik I, Becker PS, DeVito WJ, et al. Stem cells and prenatal origin of breast cancer.
988 *Cancer Causes Control*. 2004;15(5), 517-530.
- 989 111. Grotmol T, Weiderpass E, Tretli S. Conditions in utero and cancer risk. *Eur J*
990 *Epidemiol*. 2006;21(8), 561-570.
- 991 112. Mazaud-Guittot S, Nicolas Nicolaz C, Desdoits-Lethimonier C, et al. Paracetamol,
992 aspirin, and indomethacin induce endocrine disturbances in the human fetal
993 testis capable of interfering with testicular descent. *J Clin Endocrinol Metab*.
994 2013;98(11), E1757-1767.
- 995 113. Holm JB, Mazaud-Guittot S, Danneskiold-Samsoe NB, et al. Intrauterine Exposure
996 to Paracetamol and Aniline Impairs Female Reproductive Development by
997 Reducing Follicle Reserves and Fertility. *Toxicol Sci*. 2016;150(1), 178-189.
- 998 114. Palmer JR, Hatch EE, Rosenberg CL, et al. Risk of breast cancer in women exposed
999 to diethylstilbestrol in utero: preliminary results (United States). *Cancer Causes*
1000 *Control*. 2002;13(8), 753-758.
- 1001 115. Markham JA, Taylor AR, Taylor SB, Bell DB, Koenig JL. Characterization of the
1002 cognitive impairments induced by prenatal exposure to stress in the rat. *Front*
1003 *Behav Neurosci*. 2010;4, 173.
- 1004 116. Booij L, Benkelfat C, Leyton M, et al. Perinatal effects on in vivo measures of
1005 human brain serotonin synthesis in adulthood: a 27-year longitudinal study. *Eur*
1006 *Neuropsychopharmacol*. 2012;22(6), 419-423.

117. Holloway T, Moreno JL, Umali A, et al. Prenatal stress induces schizophrenia-like alterations of serotonin 2A and metabotropic glutamate 2 receptors in the adult offspring: role of maternal immune system. *J Neurosci*. 2013;33(3), 1088-1098.
118. Huttunen MO, Niskanen P. Prenatal loss of father and psychiatric disorders. *Arch Gen Psychiatry*. 1978;35(4), 429-431.
119. Myhrman A, Rantakallio P, Isohanni M, Jones P, Partanen U. Unwantedness of a pregnancy and schizophrenia in the child. *Br J Psychiatry*. 1996;169(5), 637-640.
120. van Os J, Selten JP. Prenatal exposure to maternal stress and subsequent schizophrenia. The May 1940 invasion of The Netherlands. *Br J Psychiatry*. 1998;172, 324-326.
121. Khashan AS, Abel KM, McNamee R, et al. Higher risk of offspring schizophrenia following antenatal maternal exposure to severe adverse life events. *Arch Gen Psychiatry*. 2008;65(2), 146-152.
122. Khashan AS, McNamee R, Henriksen TB, et al. Risk of affective disorders following prenatal exposure to severe life events: a Danish population-based cohort study. *J Psychiatr Res*. 2011;45(7), 879-885.

1026 Table 1. Examples of NCDs caused by defective *in utero* programming.

NCDs	Stimuli	<i>In utero</i> programming mechanisms	Articles	Reviews
Cardiovascular diseases	<ul style="list-style-type: none"> • Overnutrition • Undernutrition • Cigarette smoke • Altitude • Preeclampsia • Hypercholesterolemic diet 	<ul style="list-style-type: none"> • Alteration of oxidation-sensitive signalling pathways in the arterial wall. • Enhance fatty streak formation • Imprinting and epigenetic alterations. • Downregulation of placental glucose transporter 	104	40, 63, 105, 106
Asthma	<ul style="list-style-type: none"> • Non-specific environmental factors • Cigarette smoke 	<ul style="list-style-type: none"> • Defective epigenetic gene silencing • Altered DNA methylation 	107	108
Breast Cancer	<ul style="list-style-type: none"> • High oestrogen levels • Low progesterone levels. • High-fat diet • Alcohol • Overnutrition 	<ul style="list-style-type: none"> • Altered gene expression • Increased <i>IGF-1</i> expression. • Reduced <i>IGFBP-3</i> expression. 	109	110, 111
Testicular Cancer	<ul style="list-style-type: none"> • High oestrogen levels. • High anti-androgens levels • Analgesics 	<ul style="list-style-type: none"> • Disturbed <i>in utero</i> development of gonads • Reduce secretion of insulin-like factor 3 		111, 112
Female subfertility	<ul style="list-style-type: none"> • Paracetamol 	<ul style="list-style-type: none"> • Reduced anogenital distance and follicle reserve 	113	
Vaginal adenocarcinoma	<ul style="list-style-type: none"> • Diethylstilbestrol (DES) 	<ul style="list-style-type: none"> • Endocrine abnormalities 	114	
Psychological disorders	<ul style="list-style-type: none"> • Stress • Cigarette smoke 	<ul style="list-style-type: none"> • Epigenetic alterations • Decreased serotonin secretion • Defective cortex maturation • Altered expression of neuroreceptors 	115-122	19
Type 2 Diabetes	<ul style="list-style-type: none"> • Undernutrition • Overnutrition 	<ul style="list-style-type: none"> • Increased insulin receptors (IR) • Decreased IR catalytic subunits: PI3K, AKT/ PKB • Altered DNA methylation 	43, 45	5, 11, 42
Renal (Kidney) diseases & associated Hypertension	<ul style="list-style-type: none"> • Undernutrition in late gestation 	<ul style="list-style-type: none"> • Low number of nephrons 	13, 15	12, 14

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