Fetal Epigenetics: How In Utero and Early-Life Environmental Inputs Program Life-Long Changes

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Introduction

The seeds of adult and childhood disease appear to be sown in utero and in infancy. The health (or lack thereof) that one is experiencing in the present moment may have more to do with the first nine months of that individual's life than any other single factor. It may seem fairly straightforward that healthy mothers give rise to healthy offspring. However, it is becoming increasingly evident that the intra-uterine environment may have a unique and far greater impact on *lifelong* health than was previously understood. In fact, these in utero signals (or insults, depending upon the definition) can permanently alter gene function in ways that may last a lifetime.[1] Increasing our understanding of this process is clinically important because as more is learned about the origins of adult disease, there are more opportunities to make simple and effective changes that have untold positive influences on the life-long health of the mother and child. It is important to consider the causes of disease throughout the whole life-cycle so that proper interventions may be offered that can prevent disease from ever gaining a foothold. Much emphasis has been placed on preventive medicine for the postnatal life of the adult and addressing factors such as diet and lifestyle. However, only recently has it become clear that prevention of disease means addressing the health of an individual even *before* that individual is conceived.

The mechanisms behind this critical window of human development have become elucidated with the current understanding of **epigenetics** – the science of how given a single genotype (inherited genes), many different phenotypes (function and expression of those genes) are possible without any change to the inherited gene sequence itself. To put it another way, when the exact same set of genes is nourished in two different environments, the result will be two very different sets of offspring characteristics. The gene set we inherit supplies a fixed number of *possible* phenotypes, whereas the environment within which those genes function determines which of these phenotypes will be expressed. These differing phenotypes have permanent consequences for the child's lifetime endocrine, metabolic and cellular repair mechanisms. This process is termed developmental plasticity,[2] which explains how a single genotype can give rise to a range of physiological and structural changes persisting throughout the lifetime.

The totality of environmental influences on the child's genetic and phenotypic make-up has recently been termed the fetal **exposome**.[3] Not only do individual factors affect fetal development, but the maternal-fetal exposome acts synergistically to influence long-term health outcomes. For example,

when a mother has a vitamin A deficiency, the effects of that deficiency are made worse by exposure to PDBEs (flame retardants, which are ubiquitous in our environment and exposure is universal). It also works the other way, where the detrimental effects to the fetus of exposure to pollutants can be alleviated if maternal nutrient status is improved.[4,5]

The development of an individual's phenotype happens first and foremost in the womb. It makes sense from an evolutionary standpoint that potent modifications in our genome happen in the uterine environment so as to give the child the best possible chance of survival given certain environmental conditions. Many of these plastic responses are beneficial to the offspring. However, many end up being detrimental because the phenotype-induced changes do not match the post-natal environment.[2,6,7] This "mismatch" will be explored later and may be one of the most important causes of detrimental outcomes to offspring. The most well-studied example of this mismatch involves a restricted fetal environment, as is the case of many pregnancies, which causes the baby to be born low birth weight. Subsequently, when the post-natal environment involves excess macronutrients, this leads to rapid catch-up growth in the first few months or years of life. This mismatch leaves undeniable scars on the lifelong metabolism of the child.

Environmental factors such as low oxygen levels produce changes in the uterine environment which drive *non-genetic* organ development, such as brain, heart, lungs, mitochondria and kidney.[8,9,10,11] These gene-independent influences involve alterations in cell organization, cell differentiation, and alter the number of cells in a particular organ (Figure 1). These gene-independent responses are also part of our developmental plasticity,[6,7] and have lifelong consequences including elevated blood pressure and increased risk of cardiovascular disease.[12] Even merely suboptimal levels of nutrition or minor perturbations in maternal physiology can have heretofore unknown detrimental effects on the lifetime health and susceptibility to disease of the child by changing the structure, function and future reserve of a given organ. Together, these genetic and epigenetic influences on the developing fetus are collectively known as the "developmental origins of health and disease (DOHD)." [13]

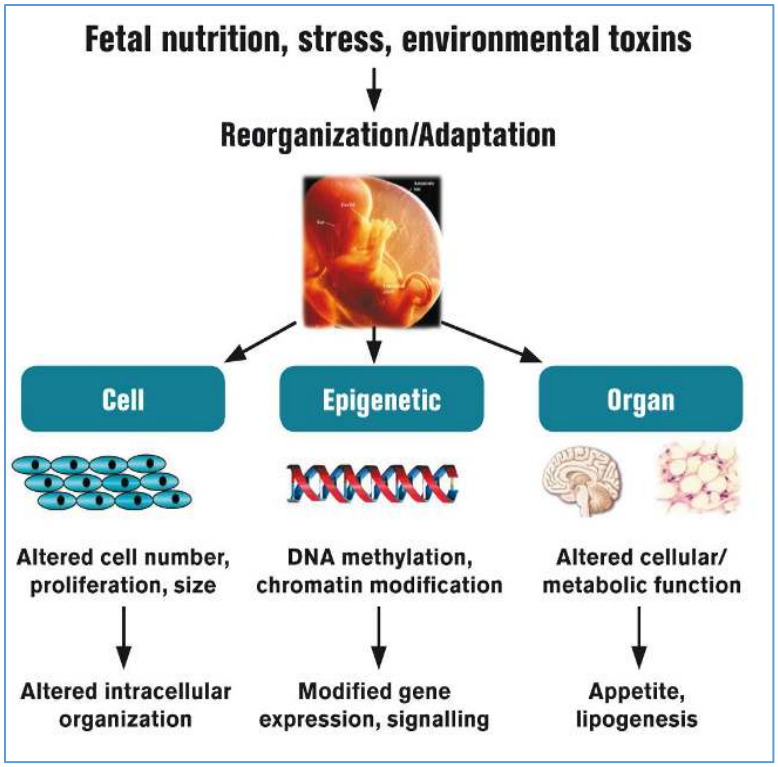

Figure 1. Fetal plasticity. Maternal stress, exposure to environmental toxins, and nutritional deficiencies make up a significant portion of the fetal exposome and drive epigenetic expression and organogenesis. Fetal plasticity is modified through a) altered cell number and size given nutrient or oxygen deprivation, b) changes in gene expression through altered methylation and acetylation, c) cellular and metabolic function such as neurotransmitter expression and adipose leptin secretion. All of these changes have lasting effects on the child's lifelong organ function, metabolism and later susceptibility to disease.

Today, the drastic rise in childhood disorders such as autism, AD(H)D, asthma, obesity, autoimmunity, diabetes and more, may be the biggest challenge we face as a society. The untold economic and emotional burden that health-challenged offspring present to immediate family as well as to society are just now starting to be fully understood. Understanding the origins of such disorders may prove invaluable in the prevention and mitigation of these devastating trends. Acting now to change public awareness and increase individuals' motivation to become healthy *before pregnancy* may prove to be one of the most economic and effective solutions in turning the tide of disease and dysfunction in childhood and even into old age. While most diseases require additional post-natal inputs (poor diet, continued toxic exposure, micronutrient deficiency, etc.), **finding and modifying the initial disturbances (in utero) is proving capable of reducing the risk of future illness**.[14]

This article offers a review of the current scientific research pertaining to certain aspects in the DOHD so that awareness may be increased among health care providers and the public. Through an informed public and practitioner base, this may motivate parental preconception changes that, as far as can be understood now, may have preventative and beneficial outcomes for current and future offspring. It is also important to understand the pathophysiology of disease, especially at its very origins, in order to develop more effective treatments for those already afflicted with illness. **Clarifying epigenetic regulators and how to modify them will improve pregnancy outcomes and the ability to treat and prevent disorders that emerge much later in life, even as their origins begin very early in life.**

Epigenetics

The literal meaning of epigenetics is the factors "above and beyond" the DNA code sequence which influence how a gene functions. Epigenetics can be defined as the study of heritable changes in gene expression that do not involve alterations in the DNA sequence.[15] Epigenetics is the science of understanding how environmental (exogenous and endogenous) inputs impact gene functioning. The

main epigenetic influence on gene functioning occurs through processes that affect methylation and acetylation pathways. Other factors involving micro and macro nutrient availability and xenobiotic inputs also influence how a gene functions, and these changes can be either temporary or permanent. This is important because for most childhood and adult diseases including autism spectrum disorders (ASD), the environment has as much, if not more, influence on the development of these neurological and physiological pathologies than the genotype.[16]

Gene Function – A Brief Review

It is important to understand factors which affect gene function. This will help give the reader an understanding of *how* numerous inputs and stimuli during fetal development cause lasting changes in gene function. The following should provide a simple understanding of a very complex process.

A gene ultimately codes for an end product which is usually a protein or enzyme which has an integral part in the structure or function of the cell. There are by current estimates 35,000 genes in a human genome. Most of the gene sequences are constantly wrapped in histone complexes and methyl groups and are therefore "turned off" or unreadable. A few genes are constantly turned "on," usually those vital to cell functioning. Most genes are only turned on if environmental stimuli are received, such as when a hormone signals the DNA transcription factors to start "reading" the gene to make the end product. Many stimuli, such as hormones, will affect hundreds of genes at the same time, turning some on and some off. Estrogen, for example, may affect over 400 genes in a single cell, producing a multitude of actions within that cell. In order for a protein/enzyme to be produced, the gene must undergo a process whereby:

1. The histones unravel and the methyl groups surrounding that particular part of the gene move away.
2. The gene can now be transcribed by DNA polymerase.
3. mRNA can now come and "read" or transcribe the gene code.
4. The RNA is transferred out of the nucleus to the endoplasmic reticulum to be translated by ribosomes into a set of instructions for how to build a particular end product.[17]

Epigenetics describes the changes to *any part* of this mechanism (usually methylation) which alters how a gene is read, how often it is read (if at all), and how the endoplasmic reticulum puts the protein together. This occurs often and ultimately allows our genes some flexibility in response to our environment. As stated previously, many of the epigenetic changes are beneficial, while other are not so helpful and may lead to later disease states. **Factors which alter this process in a negative way include xenobiotics, reactive oxygen species, hormonal imbalances, psychological stress, nutrient deficiencies, UV radiation and more (see Figure 2).** Again, understanding these epigenetic influences provide therapeutic intervention loci.

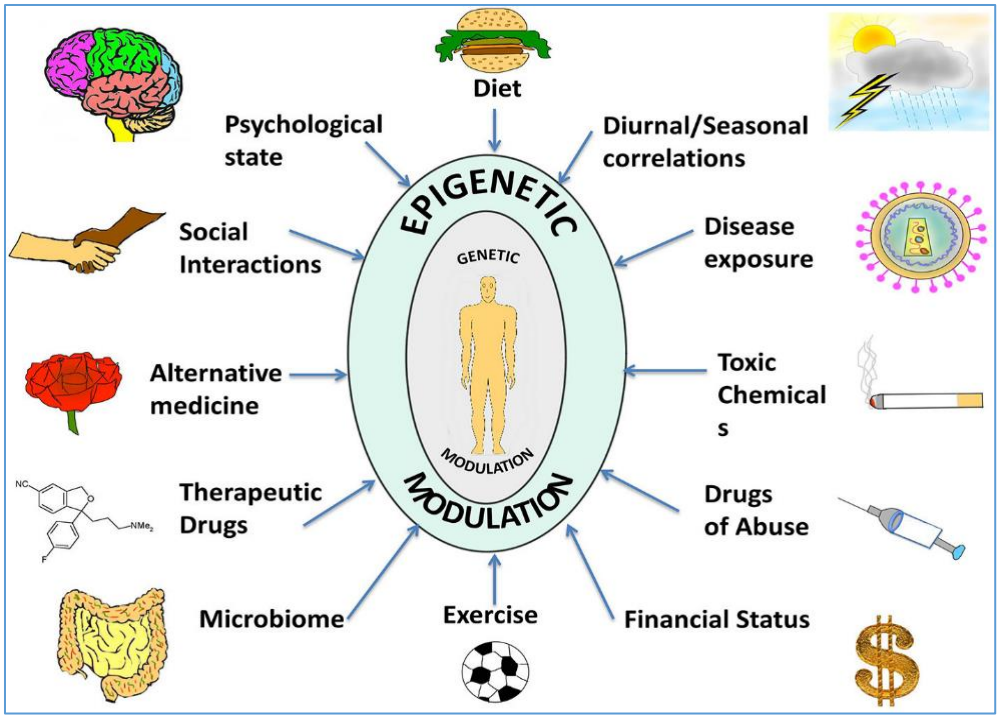

Figure 2. **Epigenetic influences across the lifespan.** Various environmental inputs have lasting or permanent effects on gene function. This is different than development of single nucleotide polymorphisms (SNPs) which are permanent alterations in gene sequence, although many of the same factors causing epigenetic changes can also cause *de novo* SNPs. Epigenetic modulation occurs at the level of gene methylation, histone acetylation and/or endoplasmic reticulum function which alters the amount and structure of the end product for which the gene is coding. *Used with permission from Kanherkar RR , Bhatia-Dey N, and Csoka AB. Epigenetics across the human lifespan. Front. Cell Develop Bio. 2014 Sep; 2(49): 1-19.*

It was once believed that genes mainly or solely determined the function of an organism. However, observations seemed to contradict this theory - such as why identical twins would often have very different health outcomes even when growing up in the same household. In fact, even in cases of genetically-linked diseases such as Type I diabetes, there is only a 30-50% correlation between same-sex genetically identical twins and lifetime prevalence of Type I Diabetes.[18] This apparent puzzle is solved by understanding that while many diseases do show genetic predispositions and have genetic pre-requisites, it is environmental stimuli (or epigenetic control) that completes all necessary requirements for disease initiation and progression. Many of these environmental inputs induce permanent changes in our gene expression, while others may cause only temporary changes.[19] It is beyond the scope of this article to expound upon each of these inputs and their effects. The references given can direct the reader to further information on this topic. Here, the focus is on critical periods of fetal development and the most detrimental known factors which affect the imprinting and permanent memory of fetal genetics.

Difference between SNPs and Epigenetics and How They Interact to Produce Genetic Plasticity

It is important to make a distinction between a single nucleotide polymorphism (SNP) and *epigenetic* alterations. While the two influence each other, they are inherently separate mechanisms which affect gene function. SNPs are not in and of themselves an epigenetic factor, but they may ultimately influence epigenetic factors. A SNP is a change to the genetic sequence of nucleobases and occurs in one of two ways: 1) inherited by one or both of the parents (*somatic*), or 2) acquired (*de novo*) at some point in the lifespan. Mutations range in size from a single DNA building block (DNA base – SNP) to a large segment of a chromosome (chromosomal mutation). *De novo* mutations which happen in utero or very early in life help explain genetic disorders in which an affected child has developed a gene mutation in every cell, but has no family history of the disorder. In other words, the damage to the gene or chromosome happened after fertilization by epigenetic influences. It is estimated that an adult acquires de novo mutations at a rate of two SNPs per year, doubling the amount of SNPs every 16 years.[20] **This is important for fetal development because as an individual ages, he/she acquires more *de novo* mutations which may be passed down to the offspring and have detrimental effects.** This is one reason that older parents tend to have children with more developmental disorders and adult diseases. Statistically, the age of optimal offspring outcomes appear to be between the ages of 22-32 for the female and 21-40 for the male.[45] However, the optimal age for reproduction varies according to lifestyle, nutrition, socio-economic and other factors.

Some individuals are more susceptible to epigenetic modifications than others. Certain SNPs make it more likely that an individual will develop de novo mutations (SNPs) throughout their lifetime. This also makes the genes more "plastic" and susceptible to epigenetic modification. For example, SNPs which code for methylation, such as MTHFR, MTRR, COMT, BHMT, etc. will significantly change the methylation patterns in the DNA. This will, in turn, make it more likely that certain genes will either be read when they are not needed or not read when they are needed.[21] If an offspring inherits or acquires significant mutations in this pathway, it may leave the genes abnormally methylated, meaning either hyper- or hypo-methylated. Interestingly, global hypo-methylation, as indicated by lab tests such as Homocysteine or SAM: SAH ratio, can actually produce regionally hyper-methylated areas on the chromosomes, thus blocking RNA transcription.[22] This may produce disease by not allowing the gene to be regulated properly and respond to environmental inputs. In addition, there may be **acquired** methylation deficits that are induced through nutrient deficiencies such as folate, B12, methionine, betaine, B6, B2, zinc, molybdenum and choline (methyldietary constituents).[23] **It is imperative to point out that one may have few to no SNPs which affect methylation, but if the individual is deficient in cofactors and methyl donors (B12, folate, etc.) he/she will have nutrient-deficient abnormal methylation with the same outcome as if many SNPs existed.** Furthermore, many people, including mothers, have the compounded problem of both genetic (SNP) and acquired (suboptimal methyldietary nutrients) methylation deficiencies. Inadequate enzyme activities and imbalances of the methyldietary constituents may cause homocysteine and S-adenosylhomocysteine accumulation. Therefore, the addition or removal of methyl groups on DNA and histones are the primary mechanisms of changing the epigenetic landscape.[24] Much focus has been put on the early life implications of altered methylation status, and this is discussed in more detail below. The essential point here is that SNPs and epigenetics are different mechanisms but they interact with one another. Certain SNPs, such as MTHFR, if positive, may not only have immediate consequences for gene function, but also leave that individual *more* susceptible to future SNP formation and altered gene function.

Critical Windows in Fetal Development

The ability to modify early life risk factors for later disease requires an understanding of timing of organ development. **The first 1000 days of life, which includes gestation and the first two years of postnatal life, seems to be the period of the entire lifespan where most epigenetic changes occur.** Organs undergo differing rates of rapid growth during fetal development which leave those organs highly susceptible to negative stimuli, such as deficient nutrient supply and oxygen delivery, which causes changes that are *irreversible*.[25,26] If conditions are not optimal, there will be *permanent* changes in the structure and function of the organ. Understanding these time-sensitive periods can have important implications as to when and how to optimize that particular organ's development (as well as when to protect it from harmful inputs) in order to promote greater offspring success. Again, this may be independent of gene function (i.e. iron deficiency), or a consequence of gene function (e.g. methylation SNP).

Perhaps the earliest and most well-studied living example of human fetal epigenetics and long-term consequences for health pertains to the Dutch Hunger Winter of 1944-1945 (Figure 3).[27,28,29]

The Dutch Hunger Winter has illuminated many associations with adult diseases and is especially valuable because it is a "natural" experiment which, for obvious ethical and moral reasons, is impossible to do on humans in a controlled environment. As can be seen in Figure 3, the extent and type of adult disease depended upon when the fetus was exposed to the famine. During that time, most women were restricted to less than 1000kcal/day for a period of about 8 -10 months. Depending on ***when*** the child was conceived during that time, different organs and biological systems were affected. This aligns with current embryological evidence as to when certain organs begin formation and are therefore more susceptible to developmental changes. Figure 4 further highlights the critical windows of organ development and, in this case, when exposure to environmental pollution caused the most detrimental changes to particular organs.

Epigenetic Mismatch and Catch-up Growth

What appears to be especially damaging is when the child is exposed to intrauterine nutrient and/or oxygen restriction, increased exposure to pollutants, and altered hormonal signals such as leptin – all of which are related to low birth weight. When in postnatal life the environment switches to a macronutrient excess, this shift overwhelms the child's epigenetic changes that were caused by the inadequate uterine environment. This is known as the thrifty gene hypothesis,[31,32,33] and leaves the offspring engaged in a lifelong struggle with metabolic processes that are not fit for current conditions. This often manifests as accelerated "catch-up growth" seen in low birth weight (LBW) or small for gestational age (SGA) babies. Catch-up growth which happens in the first few months of post-natal life is related to increased risk for diabetes, asthma, ASD, cardiovascular disease, hypertension, behavioral problems and even cancer.[34-43]

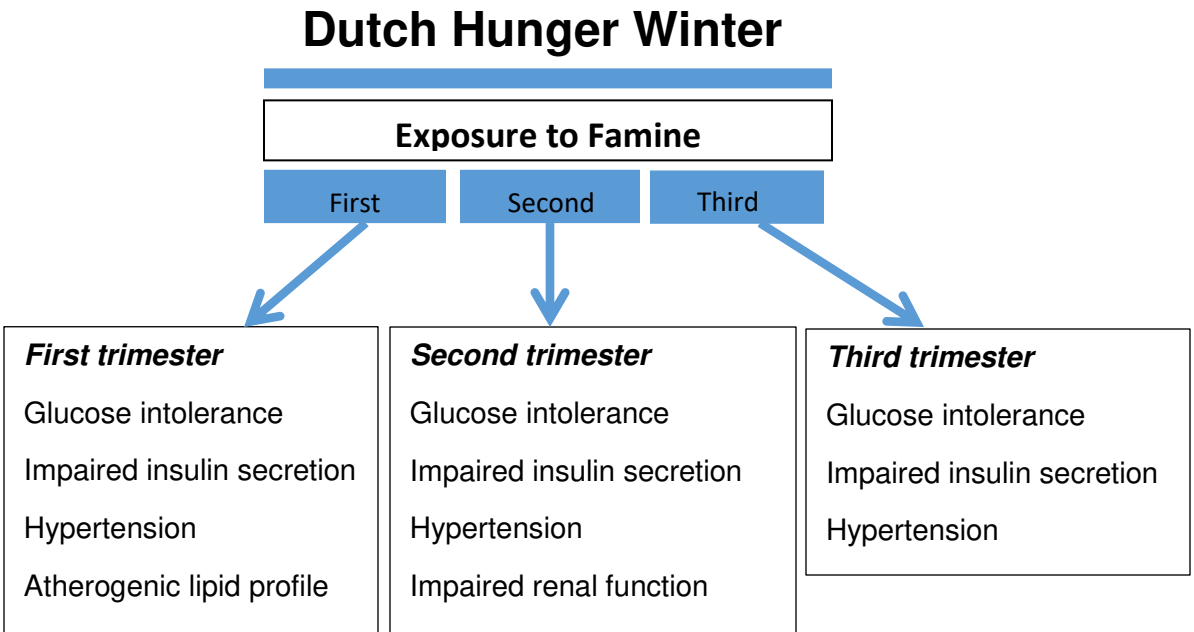

Figure 3 Postnatal consequences of the Dutch Hunger Winter. This diagram details the postnatal consequences of gestational specific exposure to the starvation rations. It is clear that exposure during the first trimester had the most severe effects.

Early Pregnancy Influences on Fetal Programming and Later Disease Risk

As seen in Figure 4, every major organ in the human body begins to develop in the first three weeks of life. All organ development is dependent upon placental nutrient availability. Therefore, the implantation of the embryo could prove to be the most important event related to future development and disease risk. Approximately half of all pregnancies are unplanned,[44] which makes the embryonic period subject to unknowing mothers who may be nutrient deficient, and who may continue to drink alcohol or engage in other risky behaviors, thereby altering their metabolism and increasing environmental exposure to smoke and pollutants. The materno-fetal supply line begins to develop very early in gestation and rapid growth occurs soon after. Note that because the early embryonic and placental developmental period is so important, interventions need to be initiated *before pregnancy* or immediately after if possible.

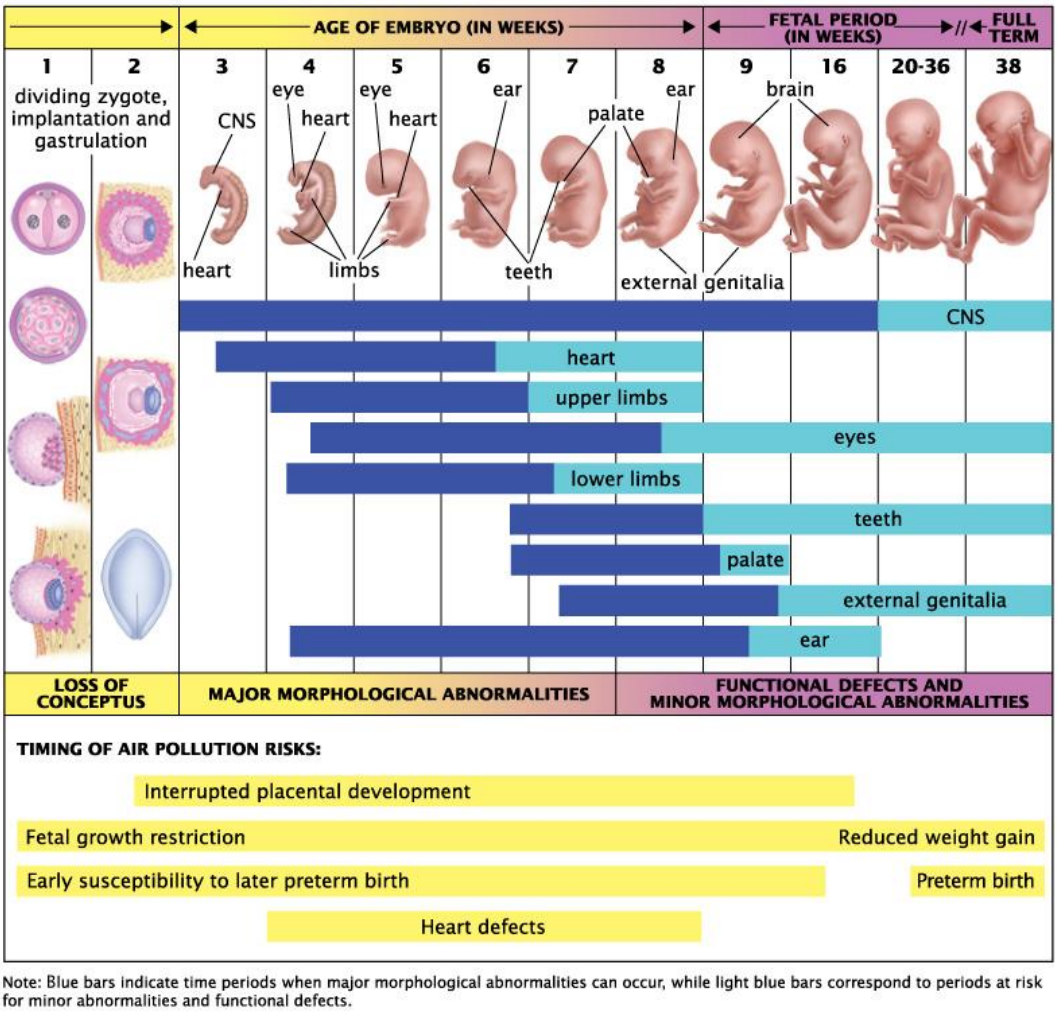

Figure 4. **Critical windows and developmental milestones of organogenesis in the embryo and fetus.** Most organs begin development in the first 8 weeks of life, which represents perhaps the most critical of all the windows in organogenesis. This graph also illustrates the complications that arise from air pollution exposure and timing of that exposure. Ritz B, Willhelm M. Air Pollution Impacts on Infants and Children. UCLA Institute of the Environment. 2008. Accessed November 2014 from http://www.environment.ucla.edu/media/files/air-pollution-impacts.pdf).[30]

During the first seven days after fertilization, the embryo is entirely dependent upon passive diffusion from surrounding extracellular fluid. The placenta has not developed yet. Therefore, if there are xenobiotics, low nutrient status, insufficient oxygen and increased inflammatory signals, then the embryo may never survive, and if it does, is already set up for altered fetal growth trajectories due to epigenetic changes. According to the British Nutrition Foundation's report on Nutrition and Development, "the nutritional status of a woman before pregnancy is critical to both her baby's and her own health. It determines her well-being and that of the fetus and child, and in turn the health and reproductive capacity of the next generation."[45] One of the most detrimental early-life environment is being overweight *before* conception. Since more and more women are overweight before birth, this has serious consequences for future generations and is also an important therapeutic intervention point.

Nutrient Deficiencies/Excesses Contributing to Altered Fetal Growth

Teenage Pregnancy

One of the most prevalent contributing factors to nutrient deficiencies in the developed world is teenage pregnancy, which continues to comprise a significant portion of births. Approximately 10% of births in the U.S. are from very young teenage mothers.[46] This population is at an increased risk of birth complications such as pre-eclampsia and are more likely to have low birth weight babies.[47] Adolescents are at a higher risk of developing nutritional insufficiencies due to their higher macro and micro nutrient needs resulting from their own growth requirements. Teenage mothers are also at a higher risk of developing later osteoporosis, since the fetal calcium requirements will be met by taking calcium from the mother's bones, even as she herself is still continuing to grow.[48] Also, the diets of teenage girls differ significantly from those of older women, and adolescents tend to east fewer fruits and vegetables and more inflammatory foods such as grains, sugar and packaged foods.[49,50] Younger girls are also at increased risk for anemia and deficiencies in iron, folate, calcium, vitamin E, vitamin D and other nutrients.[51,52] **This leaves their children at greater risk for low birth weight, which is the number one perinatal risk factor for increased incidence of childhood and adult dysfunction, especially if accompanied by catch-up growth in which the child crosses centiles**.[53]

Iron in Pregnancy

It is estimated that 50-75% of all pregnant women are iron deficient in the US and other developed countries. Iron status further decreases with parity as well.[54,55] Women are at a much higher risk of iron deficiency than men. Supplementing with iron in women who show lower iron status during pregnancy has long been known to improve health outcomes of the child.[56] Iron deficiency in pregnancy can lead to adult hypertension.[57,58] This is mediated through altered vascular system and kidney development and it appears that the critical period is the first few weeks of embryonic development. The primary role of iron is delivering crucial oxygen to meet the high energy requirements of fetal cells. Iron deficiency impairs organogenesis, and in the case of adult hypertension risk, the lower number of nephron formation during critical periods of kidney formation appears to be a major contributor. This puts the child at a lifetime higher risk of hypertension and kidney dysfunction.[59] Many miscarriages are also a result of insufficient iron because trophoblastic implantation and placental development are severely impaired.[60]

All organs depend on adequate oxygen delivery, especially the brain which undergoes the most rapid and complete development during pregnancy. Iron deficiency is the leading cause of hypoxia to the fetus.[60] Development of the hippocampus and frontal cortex are particularly vulnerable to fetal deficiencies, which result in later neurological impairments such as lower IQ and developmental disorders such as ASD.[61,62] In fact, Schmidt shows that lower maternal intake of iron is associated with a double risk for autism, and when combined with other risk factors such as maternal/paternal age and obesity, the risk jumps to a ***five-fold*** increase compared to mothers who have higher intakes of iron both pre- and post-pregnancy. Other neurological consequences of maternal low iron includes altered myelination, altered dopamine and serotonin metabolism, and even changes in gene expression in the hippocampal region affecting synaptic function and plasticity. Alterations in the blood-brain barrier are also seen in fetuses of mothers who are iron deficient, which increases the exposure of the developing brain to neuro-active hormones and organic acid metabolites.[63] Furthermore, there are additive effects of inflammation and iron deficiency. In one study, maternal iron deficiency anemia (IDA) together with an infection, increased cytokines and immune activation in the mother, which triggered production of

fetal brain antibodies and greater neurological deficits compared to either infection or IDA alone, and much greater than controls without either IDA or infection.[64] Iron deficiency in pregnancy is also related to preeclampsia, preterm delivery, LBW babies, as well as asthma and atopy in children. [65,66]

Currently there is no consensus on optimal iron intake. There are also subpopulations that need either more or less iron depending on genetics and circumstances.[67] For example, women with the HFE gene C282Y or H63D may need less iron, and taking too much iron could create an epigenetic imprint on the fetus for further development of hemochromatosis in male offspring. There has not been an investigation to date of perinatal iron status and risk of later development of hemochromatosis, but given the increased prevalence of iron overload it may very well prove to be developmental in origin. Low iron status at birth may also be a risk factor for development of hemochromatosis later in life. As always, too much or too little seem to have similar effects. Other populations where iron should be given with caution are those at risk of, or who have developed, preeclampsia. In this case, red blood cell lysis releases excess iron into the blood stream and increases fatty acid oxidation and free radical formation, both of which can be detrimental to fetal development.[68] This highlights the importance of individualization in supplemental regimens.

It appears most risks of altered health outcomes decline when ferritin is at least 15 ng/ml and continues to improve up to 65 ng/ml. There do not seem to be any adverse effects of iron supplementation with ferritin levels up to 100 ng/ml, unless the rise in ferritin is associated with inflammation (see the section on maternal inflammation, below). IDA may never fully manifest, even though iron status is insufficient for optimal fetal growth. Measuring ferritin, serum iron, and percent saturation is preferable over just hemoglobin and hematocrit as IDA is a late manifestation of iron deficiency and may not be revealed until critical windows have already passed.[69]

Methyldietary Nutrients in Pregnancy (Folate, B12, B6)

Methylation, as discussed above, is the most important epigenetic factor in fetal development. Despite the fortification of many foods with folic acid, dietary intakes are very often below recommended minimums for pregnancy, especially in the first few weeks of life.[45] The RDI of folate is largely based on the prevention of neural tube defects. However, it is now becoming more evident that the neurological role of folate has to do with its role in methylation and that it has global neurological influences. Many pregnant women are found to be deficient in one or more of the methyldietary nutrients, even if supplementing.[70] Furthermore, traditional methods of testing folic acid serum or RBC folates may be inadequate and do not give a functional status of folate levels, especially if MTHFR genotypes are present.[71]

Some of the early and most revealing studies on the epigenetic effects of methyl nutrients happened in agouti mice.[72-74] These studies were the first to highlight the lifelong effects of insufficient methyldietary nutrients. Agouti dams who were not given methyldietary nutrients during pregnancy gave birth to more unhealthy offspring than the control group who were given methyl nutrients. The mice who were given methyldietary nutrients were healthier than controls. They exhibited a healthier phenotype in which they did not gain weight, show signs of cardiovascular disease or develop diabetes throughout their entire lifespan, even though the postnatal environment and diet was exactly the same for both groups. Since then several studies have highlighted the association of methyl donor availability and later disease. **Through the methylation pathway, suboptimal levels of maternal folate appear to drive epigenetic patterns which relate to offspring development of ASD, neurocognitive decline, heart disease, behavioral problems, altered neurotransmitter signaling, cancer, and many other disorders** as

will be discussed below. In fact, it is speculated that most epigenetic changes which impair mental development, health and later psychology in the offspring are nutritional in origin.[75]

Elevated Homocysteine in Pregnancy

The functional status of methyldietary availability may be best tested by homocysteine (Hcys). While altered methylation subtypes such as MTR or BHMT polymorphisms may not always display as elevated Hcys, this test will pick up the majority of methylation defects and results above 12 nmol/ml represent nearly all of the methylation defects, no matter whether they are genetic or acquired (nutrient intake). Additionally, those women with an MTHFR genotype are at greater risk for decreased folate status and elevated homocysteine. A causal relationship has been found between elevated homocysteine (an inverse marker of B12 and/or folate status) and low fetal birth weight.[76] Again, LBW is associated with later life risk of many disease states such as diabetes, CVD, neurocognitive impairment and cancer. Even if mothers with MTHFR or other methylation gene SNPs have adequate intake of folic acid, it does not ensure that the folic acid is able to be methylated and therefore available for use in genetic replication, repair and other enzyme systems. It may be more beneficial to provide these women with *methyl*-folate and to check functional markers of folate status such as Hcys or fomiminoglutamate.[71] Supplementation with folic acid during pregnancy has been shown to lower Hcys in population-based studies.[77] Doses up to 5 mg of folic acid have been shown to reduce the risk of fetal growth restriction by 66%.[78]

In addition to LBW, elevated Hcys during pregnancy is associated with adverse offspring outcomes such as down syndrome,[79] spontaneous abortions, preterm delivery, hypertensive disorders of pregnancy such as eclampsia,[80,81] various neurological disorders,[82] autism,[83] insulin resistance, obesity, diabetes,[84] neonatal hyperbilirubinemia,[85] hypertension and chronic kidney disease,[86] and intrauterine growth restriction,[87] to name just a few. If methylation is altered, there are a multitude of downstream effects. For example, leptin has protective effects on the offspring against later obesity through changes in promoter methylation of the hypothalamic POMC gene.[88] Diet-induced methylation deficits of POMC and SOCS-3 genes have been shown to promote an altered leptin-insulin feedback loop, predisposing the child to lifelong leptin resistance.[89-91]

Folate

Optimal levels of folate must again be individualized. There are many subpopulations of women who do not absorb or utilize folic acid very well and therefore must take more than the RDI. For example, women with celiac disease, diabetes, and those who are overweight or taking certain medications may need much more folic acid than the RDI.[92] Those with a history of NTDs are advised in the UK to take 5 mg, whereas the RDI during pregnancy for most women is only 800 mcg. Because of the concern for folate masking of B12 deficiency, it may be prudent to provide B12 during folate supplementation. Most women do not take folic acid before conception and this may lead to altered outcomes due to deficiencies during the critical windows of neurological development, even if folate supplementation starts sometime later in the first trimester.[70] This is one reason why increased public and health provider awareness is so important. **Recommendations for certain at-risk women to take increased methyldietary nutrients above current RDI's, even when not planning to get pregnant, may prove to be the single greatest intervention for improving health outcomes for current and future generations.** Very few pregnant women receive Hcys testing prior to or during pregnancy. Instituting Homocysteine testing for pre-pregnant and pregnant women as a public policy change and adding it to obstetric panels may be the most beneficial test added in decades.

Could folic acid supplementation be <u>contributing</u> to neurological disorders?

There is an interesting hypothesis about the relationship of folic acid supplementation and the autism epidemic which seems to warrant investigation. Folic acid supplementation (FAS) was instituted in the 1990s to reduce NTDs and in this regard has been quite successful. However, this approach may have created an unforeseen consequence. Prior to FAS (here, we reference FAS as synthetic fortification of foods and not to methylfolate, which may prove beneficial) it is hypothesized that many children with the MTHFR-positive phenotype were spontaneously aborted due to insufficient folate metabolism. Mothers who take folic acid prevent miscarriage and NTDs but consequently more babies are now born with this phenotype. This results in more babies with a greater need for folate and methyldietary nutrients. However, most women stop taking folic acid supplements after birth, even if they are breastfeeding, and the **postnatal folic acid requirements for MTHFR-positive children has largely been ignored**. MTHFR-positive children have a much higher incidence of ASD (as well as many other disorders). Because there are more children with MTHFR-positive status, it is apparent that the need for methyldietary fortification extends throughout the lifetime of these children, who in turn grow up to have more children with MTHFR-positive status, thus perpetuating the trans-generational effect.[93]

<u>B12</u>

Vitamin B12 deficiencies in pregnancy are less studied than folate deficiencies, but nonetheless represent important potential pathways for altered epigenetic programming. Intrauterine B12 deficiency significantly impacts bone growth and other organ development.[94] Furthermore, low maternal vitamin B12 status is associated with increased risk of low lean mass and excess adiposity, increased insulin resistance, impaired neurodevelopment and altered risk of cancer in the offspring.[95] Many pregnant women are B12 deficient, especially vulnerable groups including women who are vegetarians, are obese or overweight, have hypothyroid, have various autoimmune disorders including pernicious anemia, have MTHFR SNPs, experience intestinal malabsorption syndromes, smokers, and teenagers.[50,96,97] Another often-overlooked subpopulation at risk for B12 deficiency during pregnancy involves bariatric surgery recipients. While the benefits of losing weight before pregnancy may make the intervention beneficial for offspring, micronutrient deficiencies are more often found in those women who received the surgery.[98]

Rather than testing for B12 deficiency per se, much of the research centers on homocysteine (Hcys) which may be related to each of the methyldietary nutrients (B12, B6, folate, methionine, etc.) in differing amounts. Clinically, this is why it may be more important to test for functional methylation markers and supplement with a variety of methyl nutrients until status improves. Rarely does taking one single methyl nutrient resolve elevated Hcys. There may not be enough time to tease out which nutrients are the most deficient during pregnancy which may result in delay of supplementation, thus missing critical windows in fetal development. Therefore, it may be advisable to take a combination of methyldietary nutrients at the same time. Another reason this may be a more beneficial strategy is based on the observation that providing an increase in a single nutrient may increase the body's need for other nutrients. Thus, providing only B12 may create a de facto deficiency in other nutrients, especially the methyldietary ones.

There is substantial evidence that supplementing with B12 during pregnancy, especially in vulnerable subgroups of women such as those described above has significant beneficial outcomes for offspring. In one study, Torres-Sanchez and colleagues showed that supplementing with B12 changed the length-to-weight ratio (which is a risk factor for lifelong obesity, neurological and cardiovascular abnormalities) of children born from mothers with MTHFR SNPs, especially the C667T allele.[99] Reinforcing the importance of poly-supplementation, supplementing women with riboflavin who have this allele and then develop hypertension during pregnancy appears to be quite effective at lowering hypertension and related

complications.[70] Improving diet with clean, organic, animal-based foods and by following a Mediterranean-style diet (MSD) also improves B12 status in pregnant women.[100]

The importance of diet as a significant epigenetic factor in fetal programming cannot be overstated. In fact, Chatzi et al. demonstrated that if a mother adheres to a strict Mediterranean-style diet with plenty of plant based foods, nuts, seeds and a moderate amount of animal protein in the form of fish and organic meats, this significantly lowers the childhood risk of wheeze and atopy with a follow-up period of 6.5 years. [101-104] *Postnatal* diet was not associated with later risk of atopy and wheeze, highlighting the fact that the fetal environment preprogrammed offspring for not only later dietary habits, but immunological responses well into childhood. Those women who ate the most junk food had babies who were more likely to grow up with food sensitivities and asthma, even after adjusting for a multitude of variables such as socioeconomic class, age and adiposity at birth. Of course, this does not mean that all women should follow a strict MSD, as a more personalized approach based on food sensitivity testing, cultural awareness and genetic preferences may prove more beneficial. However, these studies highlight the important effects that a diverse, natural foods-based peri-conceptual diet has on the long-term health of offspring.[105]

B6

Vitamin B6 is another important methyldietary nutrient. Studies show that while deficiency in this vitamin may be less common than other B vitamins and minerals needed for proper methylation, the effects to fetal development are not inconsequential.[106] Although rare, status epilepticus is a result of vitamin B6 deficiency in those with genetic SNPs altering B6 metabolism. Studies have shown that pregnancy increases demand for B6 in both mother and child, and treatment may require very high doses of B6.[107]

Zinc

Zinc (Zn) is essential for brain and organ development in prenatal and postnatal life.[45] Zinc deficiency is one of the most common worldwide, including in developed countries. Several reports of zinc deficiency in pregnant women show that 50-75% of women have inadequate Zn levels.[108] Zn is needed for DNA and RNA replication and deficiencies have been shown to reduce fetal brain growth and synaptogenesis.[109] Zinc is needed throughout the body as a cofactor for many enzymes and thus affects development of connective tissue, heart and lungs. Zinc supplementation reduces the risk for premature birth and may be especially important to supplement in those children born preterm or LBW.[45] Zinc may help prevent recurrent spontaneous abortions as well, due to its immunological properties.[110] Optimal levels of zinc intake for most women may be 7 – 25 mg/day depending on other dietary and genetic factors. Levels of 75 mg/day during pregnancy have been reported as safe. Individualized requirements may be more accurately determined through individual nutrient evaluation and testing. These tests are becoming more available to the general public at relatively low cost.

Other Nutrients to Consider

Iodine, vitamin D, EFAs, vitamin A, calcium, selenium, molybdenum and other trace minerals are extremely important for both fetal development and maternal health. Up to 75% of women have a deficiency in one or more of these important nutrients.[50,111] It appears that selenium and vitamin D are the most common deficiencies during pregnancy.[45] Ethnicity and time of year affect vitamin D levels as darker skin pigment and fall/winter/early spring are associated with lower levels. In one study, 80% of women were deficient in vitamin D.[112]

It is beyond the scope of this article to give a complete nutritional synopsis of every individual nutrient and the potential adverse outcomes associated with excess or deficiency. However, the important role of many micronutrients in epigenetics and fetal development cannot be overstated. It is also apparent that many women (in both developed and undeveloped countries) are extremely deficient in many of these essential nutrients, which makes timely supplementation with these nutrients crucial. It is the potential of modern "individualized medicine" that more and more women (and men) can be tested and given the exact nutrients in which they are found to be deficient. In the meantime, it may beneficial for many individuals to receive these nutrients in a multi-vitamin, multi-mineral and Omega 3-6-9 supplement form as the benefits of supplementation generally outweigh the risks,[113] with the exception of vitamin A which is teratogenic at high doses.

Maternal Inflammation Affects Neuro-Endocrine Development

Obesity

Obesity is now considered to be epidemic in industrialized countries. It is estimated that in the U.S., two-thirds of pregnant women are or will be overweight or obese even before getting pregnant.[114] It is well know that obesity creates a wide range of metabolic and hormonal changes including activation of the human inflammasome.[115] These include altered leptin signaling, disruption in neurotransmitter metabolism, increase in cytokine production, alterations in immune activation, mitochondrial and endoplasmic stress, to name a few.[116] In adults, evidence is clear that these changes lead to diabetes, CVD, Alzheimer's, Parkinson's, cancer, hypertension and psychiatric disorders.[117-121] What is recently being discovered, however, is that these same changes in the *maternal inflammatory milieu* have extensive life-long consequences on the developing fetus.[122] The purpose of this article is not to give a comprehensive review of the inflammatory exposome, but rather to highlight a few of the most alarming epigenetic changes to offspring known to produce detrimental life outcomes - especially related to brain and metabolic sequelae.

Adipose tissue is an endocrine and immune influencing "gland." Increased maternal adiposity is associated with elevated cytokines such as interleukin (IL)-6, tumor necrosis factor-alpha (TNF-a), IL-1B, monocyte chemotactant protein (MCP)-1, C-reactive protein (CRP), leptin, insulin and estrogens, all of which play a role in the development of insulin resistance, Type II diabetes and hypertension.[123] Obesity leads to over nutrition in the fetus with concomitant micronutrient deficiency. Maternal obesity leads to offspring being born either overweight or underweight. Interestingly, the average birth weight plot graph used to look like a bell shaped curve. However, as obesity has increased, the distribution now resembles a U-shaped curve with fewer babies being born with average weight, and more being born on both ends of the scale. Similarly, both LBW and high birth weight (HBW) show remarkably similar epigenetic programming and later disease profiles.[124,125] Perhaps the most telling of these epigenetic changes is shown in Table 1, which depicts the cardiovascular and metabolic changes which happen to offspring born in inflammatory environments.

Obese mothers also experience different birth outcomes and are more likely to deliver babies via C-section, which is also independently related to childhood obesity, presumably due to the change in microbiome formation. The gut bacteria of children born vaginally differ significantly from those born by C-section and it appears that these changes are long-lasting.[126]

Parameters	Effect	Maternal obesity	Offspring age (years)
Blood pressure	Increased	eGWG	9, 21
		ePPW	Neonates, 6, 17
Body fat	Increased	eGWG	9
		ePPW	Neonates, 6
BMI	Increased	eGWG	9, 21
		ePPW	6, 17
IL-6	Increased	eGWG	9
		ePPW	Neonates
CRP	Increased	eGWG	9
Abdominal fat	Increased	eGWG	9
		ePPW	6
Leptin	Increased	eGWG	9
		ePPW	Neonates
HDL	Decreased	eGWG	9
		ePPW	6
ApoA1	Decreased	eGWG	9
Insulin	Increased	ePPW	6
HOMA-IR	Increased	ePPW	Neonates

Table 1. **Offspring outcomes associated with maternal obesity, timing of obesity and effects on offspring metabolic parameters from neonate to age 21.** This illustrates how maternal obesity preprograms the child for lifelong metabolic dysfunction with a higher risk of obesity, insulin resistance, diabetes and heart disease. The offspring grow up with these parameters which in turn get passed on to future generations, compounding the trans-generational perpetuation of disease.

ePPw, excessive pregnancy weight; eGWG, excessive gestational weight gain; BMI, body mass index; IL-6, interleukin 6; CRP, c-reactive protein; HDL, high-density lipoprotein; ApoA1, apolipoproteinA-1, HOMA-IR, homeostasis model assessment for insulin resistance.

Maternal Microbiota and Fetal Development

The influence of the maternal microbiota on fetal development is only now beginning to be understood but it appears that gastrointestinal health may be the main mechanism by which inflammatory and other immune and neurological signals exert epigenetic effects on the developing fetus.[127] It used to be thought that the GI tracts of newborns were sterile. New evidence suggests that fertilization and colonization of neonatal bacteria begin in utero.[128] It has been demonstrated that in preterm babies and in mothers who experience premature rupture of membranes (PROM), the fetus is colonized before birth. However, even in full term babies metagenomic sequencing has discovered a rich *placental* microbiome. This placental microbiome likely provides important metabolic and immune contributions to the growing fetus. In some cases, the placenta selects for certain bacteria to cross and allows for early

colonization resembling the microbiota of the mother. If the mother has dysbiosis or altered systemic bacterial balance, then the child will experience much of the same consequences of this altered microbiome, even before birth.[129] This is one reason why perinatal antibiotics can be so devastating to the lifelong microbiota of the mother and child.[130] In addition to possibly initiating the human microbiome colonization in utero of the child's skin, eyes, respiratory, genito-urinary and GI systems, the xenobioitic influence of bacterial organic acids also provide a direct pathway into neuro-endo-immune patterning.

The literature is replete with the lifelong consequences that altered microbiome, insulin signaling and obesity have on such diseases as CVD, diabetes, etc. However, the literature on the epigenetic consequences to the *developing fetus* is relatively young in its evolution. The brain appears to be most susceptible to this influence through brain-immune crosstalk. Bolton et al. gives an excellent review of the neurological consequences of inflammation due to not only altered microbiome but also how factors such as maternal obesity, infection, pollutant exposure, and mitochondrial dysfunction contribute to altered neurological growth.[37] Cytokines, in addition to their immune duties, also serve as growth factors for the developing brain and other organs. Disruption in the levels of these inflammatory and metabolic signals are proving to be very disorganizing for neurocognitive trajectories. **The inflammatory environment in the mother, through brain-immune interactions, may eventually be seen as the primary *environmental* risk factor for developing ASD, behavioral disorders, lower IQ and other neurological disturbances**. It is evident that this is primarily a non-genetic phenomenon, as opposed to mostly being driven by genetic code. In the absence of major genetic anomalies such as trisomy 21, it is the in utero environment which produces changes in the offspring brain and can lead to expression of a phenotype more susceptible to neuropsychiatric illness. In his research at Cornell University, Toth explored this non-genetic determinant of offspring anxiety traits.[131] In his experiment, he took genetically identical embryos harvested from a mouse mother who did not have genetic abnormalities in serotonin function. This embryo with a normal phenotype was then transferred to different mothers for the entirety of gestation. One mother had a knock-out (KO) gene for serotonin which made her display signs of confusion and anxiety. The other embryo was transferred to a normal wild-type (WT) mother. The WT mother gave birth to normally behaved offspring as expected. However, the offspring that was gestated in the KO mother displayed the same traits of anxiety and confusion as the KO mother herself, even though the offspring did not have the KO gene. This was repeated multiple times and was found to be independent of post-natal factors. This research illustrates how the fetal exposome, including the maternal microbiome, influences the characteristics of neurobiological behavior in a non-genetic way, which may explain part of the reason we are seeing such a rise in neurological, learning and behavioral disabilities, even when parents or close relatives did not display such abnormalities.

Inflammatory Mechanisms of Altered Fetal Growth

Even if the offspring appears to develop normally in childhood and early adulthood, many studies are now confirming that certain fetal events predispose the progeny to neurological consequences *even into much later years in life*. Parkinson's and Alzheimer's diseases are now being connected to fetal events such as obesity, inflammation, stress, and micronutrient deficiencies.[132] This is called the "two-hit" or "multiple-hit" theory which attempts to explain the pathophysiology of these late neurological diseases. It appears that those altered fetal events represent the first hit, and postnatal events such as continued inflammation and glycation are the subsequent multiple hits necessary for the development of these diseases. This is extremely relevant given the dissemination of neurological problems among all age groups. Again, the most important target of intervention and prevention for later age disease appears to be the first 1000 days of life.

Much of the damaging inflammatory mechanisms are brought about through NFkB activation.[133-135] There are many dietary and nutrient interventions that have proven to dampen this NFkB response and slow down or even reverse the effects of inflammation in the adult. Fewer studies have been done on pregnant women and their offspring, but a few reports are encouraging. After lifestyle and dietary management of the risk factors associated with inflammation, nutraceutical intervention appears to be very promising.[136] Employing probiotics, curcumin, omega 3 -6-9, quercetin, resveratrol, methyldietary nutrients, lipoic acid, NAC, sulforaphane, and green tea extracts and other polyphenols have all been shown to lower inflammation and NFkB activation. Ly et al provide an excellent review of the use of the above nutraceuticals in pregnancy in mediating the inflammatory, oxidative and advanced glycation endproduct formation.[137] In addition, sulforaphane has recently been shown to improve ASD symptoms,[138] implying that inflammatory mechanisms are at play in the autistic brain. These inflammatory pathways affect the developing brain through metabolic endpoints such as altered detoxification, enzyme activation, glutathione metabolism and neurotransmitter function.[37,139]

Infection, Inflammation and Fetal Programming

A large body of evidence exists pointing to the role infections play not only in inducing inflammatory mechanisms and contributing to disrupted fetal epigenetics, but also direct effects on fetal immune activation and preference throughout life.[140-143] Epidemiological studies have confirmed that maternal infection and immune activation (mIA) is positively associated with ASD, schizophrenia, cerebral palsy and other neurological disorders in offspring.[142,144,145] Animal models have further confirmed that mIA is a profound risk factor for neurochemical and behavioral abnormalities in affected progeny.[146] As such, a complete preconception workup must include looking for symptomatic as well as asymptomatic clinical infections which may contribute to maternal immune activation with subsequent altered fetal growth outcomes. In addition to the typical obstetrical workup which should include cytomegalovirus, rubella and toxoplasmosma, the clinician may consider testing for more clinically “silent” infections such as candida, herpes, H. pylori, Borrelia burgdorferi (Lyme), mycoplasma, Epstein Barr, cytomegalovirus and mycoplasma, as these have been shown to have profound influences on the central nervous system and inflammatory activation.[147] Other conditions which contribute to mIA must also be identified and resolved. These factors may include leaky gut, stress, periodontitis, dysbiosis and parasite infections. A comprehensive stool analysis which looks at both commensal and potentially pathogenic bacteria can give the practitioner a deeper view of the microbiota and maternal immune system. Please see the section on recommendations, below, for specifics. For further instruction and training on identifying adult infections that contribute to autoimmune, Alzheimer’s disease, Parkinson’s disease and more, I refer the reader to www.infectionconnection.com.

Pollutants and Xenobiotics

Another important epigenetic factor in fetal development includes pollutants and heavy metals. It is beyond the scope of this article to delve into the plethora of evidence linking mercury, arsenic, lead, phthalates and PDBEs to altered growth, brain and metabolic trajectories of offspring. However, in dealing with the inflammatory milieu of the mother, this topic must be investigated by the practitioner. Studies in both North America and Europe have confirmed that a higher exposure to pollutants, mainly from car exhaust and other polycyclic aromatic hydrocarbons, is associated with impaired neurocognitive development and even autism.[148] Proximity of mothers to a freeway and exposure to pollutants is positively associated with ASD after adjusting for numerous other variables.[149] Pesticides and herbicides are now being recognized as a contributor to neurological deficits in offspring such as autism, schizophrenia and learning and behavioral disorders.[150] In the latest study, living within a relatively close proximity to pesticide applications (such as organophosphates, chloropyriphos, and pyethroids) increased the odds ratio of having a child with ASD by an unmistakable 60%. The exposure

windows which seemed most associated with ASD or developmental delay were preconception (within three months of pregnancy) and the second and third trimester, depending on the pesticide involved. Understanding the mechanisms of pesticide action makes it easier to understand how pesticides damage fetal brain development. Most pesticides are neurotoxic and readily cross the placental barrier. They work on the CNS in many different ways: from inhibition of acetylchline esterase, to increasing gamma amino butyic acid (GABA) which is both an inhibitory neurotransmitter and a growth factor in the developing brain.[150] Agricultural use of pesticides is not the only cause of exposure. Many urban and densely populated cities and towns across the US use pyethroids for control of mosquitos and other insects. Residential exposure is a concern and steps may need to be taken to eliminate this type of widespread use.

Research has started to elucidate vulnerable subsets of individuals who show even greater statistical significance between exposure and neurotoxic effects.[151] In fact many studies are now linking medication exposure, either maternally or in the infant, to greater risk of ASD. Acetaminophen use by mothers or administered to a child postnatally may increase production of toxic metabolites in children with impaired detoxification SNPs.[152] Given the widespread use of acetaminophen by mothers during pregnancy and also the practice of administering it prophylactically to children during vaccination, this association seems to make sense when viewed in light of genetic susceptibility. It is conceivable that one day soon most children will be tested for genetic vulnerabilities. Until then, it may prove beneficial to limit its use.

The most vulnerable subsets of children are those with impaired detoxification and methylation SNPs. Those mother-children pairs will likely benefit the most from increased methyldietary nutrients and avoidance of pollution, as well as nutrient supplementation with glutathione precursors N-acetyl cysteine and lipoic acid. Detoxification must happen *preconcepetually* as releasing pollutants and heavy metals during pregnancy is detrimental, highlighting the need for early pre-pregnancy assessment and intervention.

Endocrine Disruptors

BPA, phthalates and many other chemicals found ubiquitously are known endocrine disruptors (EDs) and are found in human amniotic fluid, breast milk, cord blood, placenta and semen.[155] These EDs have a myriad of effects on the hormonal status of both adults and children and have been linked to many adult diseases such as thyroid disruption, prostate and breast cancer, diabetes, obesity, CVD, asthma, infertility, ADHD, autism and dementia.[156-160] However, their effects on fetal development and infertility have only recently been the focus of scientific research. There have been several articles and books written in the last 30 years,[161,162] on this topic, but not until a recent boom in research has it become clear just how damaging these EDs can be for the lifelong neuroendocrine health of offspring if exposed in utero. The research on BPA and other chemicals is complex and controversial, and a deeper look into the nuances of toxicology and development would exceed the scope of this article. However, it is clear that the risk to fetal development is real and the scientific community is building consensus that these chemicals are indeed extremely harmful to fetal development and the long-term health of future generations, *especially* at low doses that reflect common everyday exposure.

Bisphenol-A (BPA) acts as a xenoestrogen and therefore can influence the function and structure of fetal endocrine and brain tissue. It is known that BPA readily crosses the placental barrier and is inversely associated with birth weight.[163] Furthermore, because BPA is an estrogenic compound, it may have different effects on males and females. In females, fetal exposure appears to increase life-long risk of breast cancer.[164,165] In a recent study, Watkins et al. found that in utero exposure to BPA and phthalates

is associated with earlier age at menarche which is itself a risk factor for later breast cancer.[166] The age of menarche has been decreasing for decades and it is believed that EDs are the main culprit. The authors summarize their findings by saying that efforts to control exposure of phthalates in utero should be a high priority. In addition to age at menarche, higher levels of testosterone, estrogen and certain estrogen receptors were also found which explains why many studies have also found a link between in utero and postnatal exposure to ED chemicals such as PDBEs, PFOAs and dioxins (now termed obesogens for their ability to disrupt leptin and other adipose tissue signaling), and low birth weight, neurological developmental delays and later development of depression, adiposity and diabetes.[167-170]

In males with in utero exposure to EDs, testes development is inhibited, contributing to later reproductive dysfunction, and exposure is also linked to the rise of hypospadias and other reproductive disorders.[171] Since testosterone production and function in utero is altered with exposure to estrogenic compounds, the fetal male brain is also uniquely affected and may explain some of the male predominance of autism, learning disabilities and ADHD.[172] Since both testosterone and estrogen are needed for proper neurological development and both act as trophic factors for synaptogenesis and neurotransmitter metabolism, it is no wonder that exposure to BPA, phthalates, PCBs and other chemicals have all been found to affect both male and female brain development and subsequent cognitive and behavioral disorders including ASD, ADHD, lower IQ, anxiety and depression.[173-175] **These changes in brain morphology appear to not only last throughout the lifetime of the offspring, but may be passed down to subsequent generations – even without further exposure to BPA or other chemicals**.[176] Reducing exposure to these potentially harmful chemicals is of paramount importance for fetal and reproductive health.

Maternal Antidepressant Use and Altered Fetal Growth

Another class of medications getting some attention for its role in epigenetic influences on fetal development is antidepressants. Given their widespread use during the perinatal period, evidence linking this class of medications to altered fetal growth will be important in shaping future recommendations. Indeed, much evidence is surfacing that these medications may have unwanted consequences to the fetus.[153] The majority of effects to the fetus appear to be short- and long-term motor and language development. In utero exposure to either selective serotonin reuptake inhibitors (SSRI) or monoamine oxidase (MAOI) inhibitors increases the risk for ASD three-fold.[154] It is true that untreated depression also poses a risk to the fetus, but it appears that taking antidepressant has far worse effects on the fetus. Given the multitude of natural and safe treatments for depression (such as EFAs, SAMe, methyl folate, St. Johns Wort, etc.), it may be prudent to advise women on the alternatives and risks of all available treatment options.

Thyroid

Perhaps the greatest *single* hormonal influence on fetal development is thyroid hormone, which drives nearly every component of cell division and metabolism of every organ and cell in utero. The implications of even minor perturbations in thyroid hormone on fetal development and long term health outcomes are only now being elucidated. This is a very large topic and will only be briefly mentioned here due to limitations in space. However, the reader is highly encouraged to look at the growing body of evidence showing the thyroid as the primary hormone in neurological and organ development, and if altered even slightly, will have serious implications for fetal development.[177-182] It is imperative that a full functional medicine evaluation of the thyroid be done on all persons of reproductive age *before*

pregnancy by checking TSH, free and total T3, free and total T4, reverse T3 and thyroid antibodies (see recommendations below).
The greatest need for thyroid hormone is in the first eight weeks of development and most women of reproductive age have some level of thyroid dysfunction[180]. This may be mediated through iodine deficiency, infection, autoimmune factors, stress, inflammation and oxidative damage. It is considered by this author and many functional medicine practitioners to be malpractice if a **full** thyroid workup is not done on all patients, especially the pre-pregnant population.

Recommendations for Improving Fetal Epigenetics and Offspring Outcomes

Most women do not take supplements before learning they are pregnant which leaves the fetus in the first four to six weeks of life without adequate nutrition in many cases.[45] As discussed previously, this is perhaps the most critical time window for ensuring healthy fetal outcomes and subsequent adult health. Furthermore, it is frequently the case that lifestyle changes are not made during this critical window, further adding to the negative impact of the fetal exposome. Given what is known so far about the fetal origins of childhood and adult diseases, it seems that there is no longer time to waste in raising awareness, changing lifestyle habits and administering proper testing and individualized supplementation when needed. **The costs of doing preconception planning and supplementation are infinitesimally small compared with the cost of complicated pregnancies and altered fetal development, and the lifetime cost of diseases such as obesity, autism, behavioral and learning disorders, asthma, cardiovascular disease and more.** The consequences of waiting until scientific, public and medical consensus is reached seems foolish and may have devastating consequences, since this may not happen for quite some time, if at all. The risk – benefit analysis of increasing supplementation, changing dietary and lifestyle habits and improving preconception planning and care, are much in the favor of *aggressively* targeting these risk factors –now – which are relatively easy to detect and modify.

Beneficial therapeutic intervention and education of parents-to-be is not much different than testing and treating any person that walks into a functional or alternative medicine office: it simply involves a full functional medicine workup and evaluation, treating the root causes of what is found and getting that person as healthy as their circumstances will allow. What is different is that they themselves are not the only patients being treated, but rather all the future generations are receiving treatment as well. The health of parents-to-be has the potential to either create or devastate life, depending on their choices and the clinician's expertise.

Much of the rationale for the following recommendations has been given in the preceding pages. This is meant to serve as a guide for the practitioner and to aid the implementation of practical clinical services which could very well turn the tide of the epidemic childhood and adult disorders which have their origin in the very beginning of life. It is important to note that the above information and following recommendations are also **extremely useful in addressing fertility and egg and sperm quality** as many of the same factors affecting fertility also affect fetal development.

Raising Awareness

Simply opening up dialogue with patients of reproductive age is important. It is essential to help people understand that what they are doing before they get pregnant may have the greatest impact on the lifelong health of their yet to be conceived child. Increasing understanding that some of the most devastating childhood and adult diseases may indeed be prevented with simple dietary, lifestyle and supplementation modifications may go a long way towards motivating individuals to take charge of their health and the health of their future offspring. This may be compelling even for adolescents, who often

do not think about the consequences that their actions may have on themselves, let alone their unborn children.

Lab Testing

Simple lab testing can highlight nutrient deficiencies, inflammatory states and even clinically silent autoimmune tendencies, which may have significant impacts on the fetus and which need to be addressed before conception. The following is suggested as a preconception panel and has been used in the author's clinic for many years. If the reader is not proficient at reading blood chemistry from a functional medicine standpoint, it is highly advisable to consult with one who is. Reading blood chemistry and seeing potential problems takes more than a superficial understanding of each analyte being tested, and merely depending on computer-based programs to interpret results may lead to many under-, over- or mis-diagnoses. As always, lab results must be interpreted together with history and physical findings.

<u>Comprehensive Blood Chemistry</u>

A comprehensive blood chemistry test should include:

1. CBC w/diff - to check for overt/covert infections; micro or macro-cytosis of RBC morphology heralding methylation abnormalities, iron or B12 deficient anemias, platelet production which could cause abnormal bleeding and predict complicated pregnancies, parasite infection, and more)

2. CMP 24 - to check multiple organ function such as kidney, liver, gallbladder, pancreatic and heart function. Blood sugar handling abnormalities should be suspected with fasting blood glucose over 90. Also, HA1c should be checked and brought to within an optimal range of 4.9-5.4 for pregnancy. This can be tested instead of an oral glucose tolerance test (OGTT) which may actually harm the fetus due to advanced glycation endproduct formation and insulin spike.

3. Lipid panel to include total cholesterol, LDL, HDL, VLDL and apoA1.

4. Inflammatory and Methylation markers such as hsCRP, Ferritin, ESR, IL-6 and Homocysteine. *Hcys may represent the simplest, most economical and most modifiable risk factor in all of preconception evaluations.* Due to its importance, it is this author's belief that every pregnant woman should have their Hcys tested routinely before and during pregnancy.

5. Iron status (serum iron, percent saturation, ferritin and total iron binding capacity).

6. Thyroid panel to include TSH, free and total T3 and T4, reverse T3 and T 3 uptake – which may be used to asses PCOS, insulin resistance or other issues affecting sex hormone binding globulin such as estrogen or liver dysfunction.

7. Urinalysis *with microscopic examination* – may be able to detect asymptomatic urinary tract infections which may need to be addressed before pregnancy. May also detect early kidney stone formation more accurately than just a dipstick test.

<u>Comprehensive Stool Analysis</u>

A comprehensive stool analysis should be performed to check for parasites, dysbiosis, inflammatory states, malabsorption and leaky gut, all of which can have significant impact on nutrient, immune and inflammatory status. Correcting commensal microbiota imbalances before or during pregnancy is proving to be one of the most important ways to influence fetal development and lifelong health of offspring. Please see above section on microbiota and fetal development.

<u>Heavy Metals and Porphyrins</u>

As discussed above, heavy metals pose a significant threat to fetal health. Addressing heavy metal and toxicant burden represents an important therapeutic endpoint for ensuring fertility, egg, sperm and

offspring quality. This should be addressed at least three to six months prior to conception as it is not advisable to mobilize and remove heavy metals during pregnancy or lactation.

SNPs

Due to the low cost of genome wide sequencing testing such as 23andme, this may be an option for more and more people. A special emphasis should be put on interpretation of methylation pathways so that at-risk populations may be given proper dosages and forms of folate and other cofactors needed for epigenetic regulation of fetal development. Other companies also offer specific methylation SNP testing with interpretation and clinical suggestions, making this a good option as well.

Infection

If an infection is suspected on blood chemistry, a history and physical may further guide infectious disease investigation. The most commonly tested for and found in many functional medicine offices are Lyme disease, Herpes varieties, EBV, CMV, candidiasis, parasites such as giardia and more.

EFA Ratios

Testing EFA ratios may be very useful in dosing fish and plant oil supplementation as there appears to be a wide range of need, and dosing everyone with just fish oils can be just as dangerous as low EFA status.

Hormones

One of the most widely used and beneficial tests for fertility and fetal development are hormone tests which analyze circadian adrenal function, DHEA, pregnenalone, progesterone and testosterone. Bringing abnormal findings back into balance with adrenal, hypothalamic and pituitary support is standard in fertility clinics that stay on top of current research and practices. Again, what is positive for fertility also seems to be supportive of fetal health as well. The use of bioidentical hormones for fertility is widespread but must be done with an experienced practitioner if continued in smaller amounts throughout pregnancy. In most cases it is advisable to stop hormonal replacement, with the exception of very low dose DHEA or pregnenolone when needed. 50-75mg DHEA supplementation has been shown to reduce miscarriage and chromosomal abnormalities, especially in those with low ovarian reserve or poor responders to IVF.[183,184] Pregnenolone is showing promise as an active growth-promoting neurosteroid supporting brain development.[185]

Nutrient Supplementation Basics

The following information is for informational purposes only. Each practitioner must weigh the risks/benefits of supplementation with the patient and their specific needs. Many supplements have not been studied sufficiently to establish their safety during pregnancy. However, the following suggestions have been used safely in many clinics around the country. It remains the responsibility of the practitioner to use the information wisely. There are some basic supplements that all women (and men) may benefit from, including a multivitamin with activated forms of vitamins such as methylcobalamin, methylfolate, pyridoxyl-5-phosphate (B6) and vitamin K2; a multimineral to include calcium, iron, zinc, potassium, selenium, molybdenum, etc.; Vitamin D (5-10K IU/day)**;** and EFAs to include Omega 3-6-9 balanced ratios, instead of just fish oils.

In addition to these basics, it may be important to supplement with the following nutrients as these have been shown to have a good safety profile and beneficial effects on pregnancy and fetal outcomes:[186]

1. Probiotics with a balance of acidophilus and bifidobacter species. Changing maternal microbiota is proving to be a beneficial epigenetic modifier.[187]

2. Coq10: 250 – 1000 mg. Low CoQ10 levels are associated with low birth weight and obstetric complications.[188] Higher Coq10 levels are associated with improved fetal outcomes.[186]
3. Lipoic Acid (LA): 500 – 1000 mg. LA or alpha lipic acid (ALA) has been used safely during pregnancy for neuropathic pain (i.e. sciatica).[189] It has also been shown to prevent neural tube defects, prevent beta cell damage from AGE formation, and support brain development of the fetus.[190-195] Populations which may particularly benefit from ALA supplementation are older parents, diabetics, dysglycemic or PCOS, and those with autoimmune tendencies.
4. N-acetyl cysteine (NAC): 500 – 2000 mg. Especially important for dysglycemic mothers. Good for increasing egg and sperm quality as well as protection of fetus from xenobiotics.[196-200]
5. Methylation Support: Additional methyl nutrients (mehtylfolate 1-5mg, methyl cobalamin 500-2,000mcg), especially if MTHFR positive, intrinsic factor antibody positive, obese or H. pylori positive as all of these are associated with diminished absorption/utilization as well as greater need for methyl nutrients.[45,70-75] Supporting proper methylation prevents unwanted genes from being turned on and expressed during fetal development.
6. Bioitin – (100-1000mcg) May be needed in greater amounts than most women are getting.[201]
7. L-carnitine – (500-200mg) Carnitine can help improve fertility in PCOS patients as well as improve lipid status.[202] Carnitine can improve mitochondrial function and support fetal development, especially in women who are overweight or obese either before or during pregnancy.[203] As a PPAR-a agonist, it may also improve fetal cardiac development and function, thus decreasing future susceptibility to cardiovascular disease.[204,205]
8. **Adaptogens** – HPA modulators such as panax ginseng may help limit fetal HPA and endocrine disruption, especially if the mother is exhibiting heightened stress responses.[206]
9. Anti-inflammatories – Resveratrol, ginger and curcumin are potentially of great benefit to mothers, especially those who exhibit elevated levels of CRP, LPS, ESR or other inflammatory markers .[207]

Relaxation Response for Modulating Stress hormones
Practicing a relaxation technique is one of the most powerful epigenetic modifiers in fetal development.[208] Elevated cortisol levels during fetal development represent one of the most studied inducers of altered brain growth and later cognitive and behavioral developmental disorders.[209-213] Practicing yoga or some other relaxation technique during pregnancy changes stress responses and improves autonomic function of mother and fetus.[214]

Lifestyle
Avoidance of toxic beauty products and household chemicals is paramount to protecting fetal growth. Exercise every day is essential, involving a balance of some cardio, weights, stretching and core work, which can assist with a smooth delivery. Social support is also positively associated with lowered cortisol levels and improved fetal outcomes.[215]

Maternal Diet and Weight Gain
As mentioned above, a high-fat maternal diet independently raises inflammation and alters fetal outcomes. Chatzi et al. provided evidence that a strict adherence to a Mediterranean diet provided offspring with lower risk of atopy, wheeze and adiposity later in life.[101-104] There is no need for calorie increase until the third trimester as maternal weight gain is associated with offspring obesity, altered leptin signaling, CVD risk and altered brain development.[37,45,216] A gluten-free diet also lessens the risk for offspring leaky gut, LPS formation, hypersensitivity and altered fetal growth trajectories.[217] There is emerging evidence that increased intestinal permeability begins in utero due to maternal stress, diet and infections.[218] Therefore, treating the mother's gut is of paramount importance.

Postnatal Influences

Careful for Catch-up Growth

As mentioned above, it may be more beneficial to curb catch-up growth rather than encourage unrestricted weight gain in LBW or SGA babies, which is what is currently promoted in obstetric offices around the world. For extremely LBW or SGA babies, gaining weight is important. However, rapidly crossing centiles is associated with later risk for obesity, diabetes, CVD etc.[31-43]

SNP Testing

Child SNP testing post natal for possible additional need of methyldietary nutrients. Testing an infant for MTHFR SNP's and glutathione status may elucidate subpopulations at risk for toxicant-related neurological damage.

Breastfeeding

While breast feeding, some children may benefit from additional supplementation of methyldietary nutrients as breast milk, even if the mother is supplementing, may not be enough to provide optimal levels of some nutrients.[45]

Conclusion

Childhood and adult disease begins in utero. The fetal exposome imparts permanent changes to fetal DNA and organ function with lifelong consequences. It must be understood that the rise in childhood and adult diseases such as autism, diabetes, obesity, CVD and cancer are not just a product of later-life influences or simply genetic in cause. Rather, pathogenesis begins even before conception, in the maternal and paternal environment. If we want to improve the health of our society, it must begin with improving egg, sperm and fetal quality. Simple changes can be made in diet, lifestyle and nutritional status that will have unprecedented positive effects on current and future generations. It is clear that if we want healthy babies to grow up to be fully functioning and productive adults, then implementation of simple yet powerful dietary and lifestyle modifications should be offered to those of reproductive age. It begins with opening a dialogue and increasing awareness and understanding about the developmental origins of health and disease. The future can indeed be much brighter if the promise of individualized, personal medicine can be applied to our current and future parents-to-be. This will allow identification of at risk populations which would benefit from early intervention. Public policy and practitioner standards should be adjusted to address the most detrimental fetal programming influences such as nutrient deficiencies, obesity, inflammation, infection, hormonal imbalances and toxicant/endocrine disrupting chemical exposures.

References

1. Bateson P, et al. Developmental plasticity and human health. *Nature*. 2004 Jul 22; 430(6998):419-21.

2. Gluckman PD et al. Environmental influences during development and their later consequences for health and disease: implications for the interpretation of empirical studies. *Proc Biol Sci.* 2005 Apr 7; 272(1564):671-7.

3. Vrijheid, M et al. The human early-life exposome (HELIX): project rationale and design. *Environ Health Perspect.* 2014 Jun; 122(6):535-44.

4. Jedrychowski, W et al. Pre-pregnancy dietary vitamin A intake may alleviate the adverse birth outcomes associated with prenatal pollutant exposure: epidemiologic cohort study in Poland. *Int J Occup Environ Health.* 2007 Apr-Jun; 13(2):175-80.

5. Ellis-Hutchings, RG et al. The effects of marginal maternal vitamin A status on penta-brominated diphenyl ether mixture-induced alterations in maternal and conceptual vitamin A and fetal development in the Sprague-Dawley rat. *Birth Defects Research Part B-Developmental and Reproductive Toxicology*. 2009; 86:48-57.

6. Bateson P, et al. Developmental plasticity and evolutionary biology. *Journal of Nutrition.* 2007 April 137(4): 1060-1062.

7. Bateson P, et al. The biology of developmental plasticity and the Predictive Adaptive Response hypothesis. *J Physiol.* 2014 Jun 1; 592(Pt 11):2357-68.

8. Langley-Evans, SC. Nutritional programming of disease: unravelling the mechanism. *J. Anatomy.* 2009, 215(1):36-51.

9. Vehaskari ,VM, Aviles DH, Manning J. Prenatal programming of adult hypertension in the rat. *Kidney Int.* 2001; 59: 238-245.

10. Hoppe CC, Evans RG, Bertram JF et al. Effects of dietary protein restriction on nephron number in the mouse. *Am J Physiol Regul Integr Comp Physiol*. 2007 May;292(5):R1768-74.

11. Watkins AJ, Lucas ES, Torrens C, Cleal JK, Green L, Osmond C, Eckert JJ, Gray WP, Hanson MA, Fleming TP. 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 Jun; 103(12):1762-70.

12. Yeung, EH, et al. Developmental origins of cardiovascular disease. *Curr Epidemiol Rep*. 2014 Mar 1; 1(1):9-16.

13. Barker, DJ. Developmental origins of adult health and disease. *J Epidemiol Community Health.* 2004 Feb; 58(2):114-5.

14. Shenderov, BA, Midtvedt T. Epigenomic programing: a future way to health? *Microb Ecol Health Dis*. 2014 May 8;25.
15. Riggs AD, Martienssen RA, Russo VE. *Epigenetic Mechanisms of Gene Regulation.* Plainview, NY; Cold Springs Harbor Laboratory Press, 1996:1-4.

16. Rangasamy S, D'Mello SR, and Narayanan V. Epigenetics, autism spectrum, and neurodevelopmental disorders. *Neurotherapeutics.* 2013 Oct; 10(4):742-56.

17. University of Illinois. Online Lecture: Control of Genetic Systems in Prokaryotes and Eukaryotes. Accessed November 2014 from http://www.uic.edu/classes/bios/bios100/lectures/genetic_control.htm

18. U.S. Department of Health and Human Services. *Genetic and Fetal Antecedents of Disease Susceptibility.* National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development, 2010.

19. Kanherkar RR , Bhatia-Dey N, and Csoka AB. Epigenetics across the human lifespan. *Front. Cell Develop Bio.* 2014 Sep; 2(49): 1-19.

20. Kong, A, Frigge ML, Masson G, Besenbacher S, Sulem P, Magnusson G et al. Rate of de novo mutations and the importance of father's age to disease risk. *Nature*. 2012 Aug 23; 488(7412):471-5.

21. Singh, SK, PalBhadra, M, Girschick, HJ. Micro-RNA-micro in size but macro in function. *FEBS J.* 2008; 275, 16.

22. Ziller,MJ. Charting a dynamic DNA methylation landscape of the human genome. *Nature* 2013 Aug 22; 500(7463):477–481.

23. Muskiet, FA. The importance of (early) folate status to primary and secondary coronary artery disease prevention. *Reprod Toxicol*. 2005 Sep-Oct; 20(3):403-10.

24. Cedar H, and Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. 2009; *Nat.Rev.Genet.* 10, 295–304.

25. Vickers, MH. Early life nutrition, epigenetics and programming of later life disease. *Nutrients.* 2014 Jun 2; 6(6):2165-78.

26. Mahajan, S, Aalinkeel R, Shah P, Singh S, Gupta N, Kochupillai N. Nutritional anaemia dysregulates endocrine control of fetal growth. *Br J Nutr*. 2008 Aug;100(2):408-17.

27. Roseboom ,TJ, van der Meulen JH, Ravelli AC, Osmond C, Barker DJ, Bleker OP. Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. *Twin Res.* 2001 Oct;4(5):293-8.

28. Roseboom T, de Rooij S, Painter R. The Dutch famine and its long-term consequences for adult health. *Early Hum Dev.* 2006 Aug; 82(8):485-91.

29. El Hajj N, Schneider E, Lehnen H, Haaf T. Epigenetics and consequences of an adverse nutritional and diabetic intrauterine environment. *Reproduction*. 2014 Sep.

30. Ritz, B and Willhelm, M. Air Pollution Impacts on Infants and Children. UCLA Institute of the Environment. 2008. Accessed November 2014 from http://www.environment.ucla.edu/media/files/air-pollution-impacts.pdf.

31. Neel, J. Diabetes mellitus: a “thrifty” genotype rendered detrimental by “progress”? *Am J Hum Genet*. 1962 Dec; 14(4): 353–362.

32. Cooper, R. Diabetes and the thrifty gene. *Lancet.* 1994 Dec 10; 344(8937):1648.

33. Ravussin, E, and Bogardus, C. Energy expenditure in the obese: is there a thrifty gene? *Infusionstherapie.* 1990 Apr; 17(2):108-12.

34. Corpeleijn, WE, Kouwenhoven, SM, and van Goudoever, JB. Optimal growth of preterm infants. *World Rev Nutr Diet.* 2013; 106:149-55.

35. Kerkhof, G et al. Health profile of young adults born preterm: negative effects of rapid weight gain in early life. *J Clin Endocrinol Metab.* 2012 Dec; 97(12):4498-506.

36. Sonnenschein-van der Voort, A et al. Preterm birth, infant weight gain, and childhood asthma risk: a meta-analysis of 147,000 European children*. J Allergy Clin Immunol*. 2014 May; 133(5):1317-29.

37. Bolton, JL and Bilbo, SD. Developmental programming of brain and behavior by perinatal diet: focus on inflammatory mechanisms. *Dialogues Clin Neurosci.* 2014 Sep; 16(3):307-20.

38. Walker, CL and Ho, SM. Developmental reprogramming of cancer susceptibility. *Nat Rev Cancer.* 2012 Jun 14; 12(7):479-86.

39. Abel, KM et al. Deviance in fetal growth and risk of autism spectrum disorder. *Am J Psychiatry.* 2013 Apr; 170(4):391-8.

40. Campbell, DJ, Chang, J and Chawarska, K. Early generalized overgrowth in autism spectrum disorder: prevalence rates, gender effects, and clinical outcomes. *J Am Acad Child Adolesc Psychiatry.* 2014 Oct; 53(10):1063-73.e5.

41. Petterssen, E et al. Birth weight as an independent predictor of ADHD symptoms: a within-twin pair analysis. *J Child Psychol Psychiatry.* 2014 Jul 15.

42. Bilder, DA et al. Maternal prenatal weight gain and autism spectrum disorders. *Pediatrics.* 2013 Nov; 132(5):e1276-83.

43. Mahoney, AD et al. Autism spectrum disorders and prematurity: a review across gestational age subgroups. *Adv Neonatal Care.* 2013 Aug; 13(4):247-51.

44. Finer, LB and Zolna, MR. Shifts in intended and unintended pregnancies in the United States, 2001-2008. *Am J Public Health.* 2014 Feb; 104 Suppl 1:S43-8.

45. British Nutrition Foundation, Ed. *Nutrition and development: short- and long-term consequences for health.* West Sussex, UK: John Wiley & Sons; 2013.

46. U.S. National Center for Health Statistics. Births: Preliminary Data for 2009. *National Vital Statistics Reports (NVSR).* 2010 Dec; 59(3).

47. Bloom, L and Escuro, A. Adolescent pregnancy: where do we start? *Handbook of Nutrition and Pregnancy*. Ed. Lammi-Keefe, CJ, Couch, SC, and Philipson, EH. Towtowa, New Jersey: Humana Press; 2008.

48. Cho, GJ et al. Adolescent pregnancy is associated with osteoporosis in postmenopausal women. *Menopause*. 2012 Apr; 19(4):456-60.

49. Gregory, J et al. *National diet and nutrition survey: young people aged 4-18 years. Vol 1. Report of the diet and nutrition survey.* London: HMSO; 2000.

50. Bates, B et al. *National diet and nutrition survey headline results from years 1, 2 and 3 (combined) of the Rolling Programme (2008/2009-2010/2011).* Department of Health; 2012.

51. Moran, VH. A systematic review of dietary assessments of pregnant adolescents in industrialised countries. *British J Nutr*. 2007; 97:411-425.

52. Moran, VH. Nutritional status in pregnant adolescents: a systematic review of biochemical markers. *Maternal & Child Nutrition*. 2007; 3:74-93.

53. Baker, PN et al. A prospective study of micronutrient status in adolescent pregnancy. *Am J Clin Nutr*. 2009; 89:1114-1129.

54. Mahajan, S, Aalinkeel, R, Shah, P, Singh, S, Gupta, N and Kochupillai, N. Nutritional anaemia dysregulates endocrine control of fetal growth. *Br J Nutr.* 2008 August; 100(2): 408-417.

55. Miller,EM. Iron status and reproduction in US women: national health and nutrition examination survey, 1999-2006. *PLoS One*. 2014 Nov 6; 9(11):e112216.

56. Taylor, D, Mallen, C, MCDougall, N and Lind, T. Effect of iron supplementation on serum ferritin levels during and after pregnancy. Br J Obstet Gynaecol. 1982 Dec; 89(12): 1011-7.

57. Crowe, C et al. The effects of anemia on heart, placenta and body weight, and blood pressure in fetal and neonatal rats. *J Physiol.* 1995; 488:515-519.

58. Gambling, L et al. Iron deficiency during pregnancy affects postnatal blood pressure in the rat. *J Physiol.* 2003; 552:603-610.

59. Langley-Evans, S and McMullen, S. Developmental origins of adult disease. *Med Princ Pract.* 2010 February; 19(2): 87-98.

60. Burke, RM, Leon, JS, and Suchdev, PS. Identification, prevention and treatment of iron deficiency during the first 1000 days. *Nutrients*. 2014 Oct; 6(10): 4093-4114.

61. Schmidt, RJ et al. Maternal intake of supplemental iron and risk of autism spectrum disorder [published online ahead of print 2014 Sept]. *Am. J. Epidemiol.* 2014.

62. Greminger, AR et al. Gestational iron deficiency differentially alters the structure and function of white and gray matter brain regions of developing rats. *J Nutr.* 2014 Jul; 144(7):1058-66.

63. McCann, JC and Ames, BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *Am J Clin Nutr*. 2007; 85:931-945.

64. Harvey, L and Boksa, P. Additive effects of maternal iron deficiency and prenatal immune activation on adult behaviors in rat offspring. *Brain Behav Immun*. 2014 Aug; 40: 27-37.

65. Clowse, ME et al. Predictors of preterm birth in patients with mild systemic lupus erythematosus. *Ann Rheum Dis*. Sept 2013; 72(9): 1536-1539.

66. Nwaru, B et al. An exploratory study of the associations between maternal iron status in pregnancy and childhood wheeze and atopy. *Br J Nutr*. 2014 Oct; 24:1-10.

67. Arija, V et al. Adapting iron dose supplementation in pregnancy for greater effectiveness on mother and child health: protocol of the ECLIPSES randomized clinical trial. *BMC Pregnancy Childbirth*. 2014 Jan; 14:33.

68. Rayman, MP et al. Abnormal iron parameters in the pregnancy syndrome preeclampsia. *Am J Obstet Gynecol*. 2002 Aug; 187(2): 412-418.

69. Herbert, V. Iron disorders can mimic anything, so always test for them. *Blood Rev*. 1992 Sept; 6(3): 125-132.

70. McNulty, H et al. Nutrition throughout life: folate. *Int J Vitam Nutr Res*. 2012 Oct; 82(5): 348-354.

71. Shane, B. Folate status assessment history: implications for measurement of biomarkers in NHANES. *Am J CLin Nutr*. 2011 Jul; 94(1): 337S-342S.

72. Wolff, GL et al. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *FASEB J.* 1998 Aug; 12(11):949-57.

73. Rakyan, VK et al. The marks, mechanisms and memory of epigenetic states in mammals. *Biochem J.* 2001 May 15; 356(Pt 1):1-10.

74. Cooney, CA, Dave, AA and Wolff, GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. *J Nutr.* 2002 Aug; 132(8).

75. McGowan, PO, Meaney, MJ and Szyf, M. Diet and the epigenetic (re)programming of phenotypic differences in behavior. *Brain Res*. 2008; 1237:12-24.

76. Yajnik, CS et al. Maternal homocysteine in pregnancy and offspring birthweight: epidemiological associations and Mendelian randomization analysis. *Int J Epidemiol*. 2014 Oct;43(5):1487-97.

77. Bjørke-Monsen, AL et al. Maternal B vitamin status in pregnancy week 18 according to reported use of folic acid supplements. *Mol Nutr Food Res.* 2013 Apr; 57(4):645-52.

78. Papadopoulou, E et al. The effect of high doses of folic acid and iron supplementation in early-to-mid pregnancy on prematurity and fetal growth retardation: the mother-child cohort study in Crete, Greece (Rhea study). *Eur J Nutr.* 2012; 52:327–336.

79. Rai, V et al. Maternal methylenetetrahydrofolate reductase C677T polymorphism and down syndrome risk: a meta-analysis from 34 studies. *PLoS One.* 2014 Sep 29; 9(9):e108552.

80. Mascarenhas, M, Habeebullah, S and Sridhar, M. Revisiting the role of first trimester homocysteine as an index of maternal and fetal outcome. *J Pregnancy*. 2014; 2014:123024.

81. Reilly, R et al. MTHFR 677TT genotype and disease risk: is there a modulating role for B-vitamins? *Proc Nutr Soc*. 2014 Feb;73(1):47-56.

82. Ansari, R et al. Hyperhomocysteinemia and neurologic disorders: a review. *J Clin Neurol*. 2014 Oct; 10(4):281-8.

83. Kirsch, S, Hermann, W and Obeid, R. Genetic defects in folate and cobalamin pathways affecting the brain. *Clin Chem Lab Med.* 2013 Jan; 51(1):139-55.

84. Ngo, S et al. Elevated S-adenosylhomocysteine alters adipocyte functionality with corresponding changes in gene expression and associated epigenetic marks. *Diabetes.* 2014 Jul; 63(7):2273-83.

85. Sukla, K et al. Low birthweight (LBW) and neonatal hyperbilirubinemia (NNH) in an Indian cohort: association of homocysteine, its metabolic pathway genes and micronutrients as risk factors. *PLoS One*. 2013 Aug 6; 8(8):e71587.

86. Laskowska, M et al. A comparison of maternal serum levels of endothelial nitric oxide synthase, asymmetric dimethylarginine, and homocysteine in normal and preeclamptic pregnancies. *Med Sci Monit.* 2013 Jun 5; 19:430-7.

87. Murphy, MM and Fernandez-Ballart, JD. Homocysteine in pregnancy. *Adv Clin Chem.* 2011; 53:105-37.

88. Palou, M et al. Protective effects of leptin during the suckling period against later obesity may be associated with changes in promoter methylation of the hypothalamic pro-opiomelanocortin gene. *Br. J. Nutr.* 2011 Sept; 106(5):769–778.

89. Vickers, MH et al. Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. *AM J Physiol-Endocrinology and Metabolism*. 2000; 279:E83-87.

90. Vickers, MH et al. The effect of neonatal leptin treatment on postnatal weight gain in male rats is dependent on maternal nutritional status during pregnancy. *Endocrinology*. 2008; 149:1906-1913.

91. Vicker, MH, Ikenasio, BA, and Breier, BH. IGF-I treatment reduces hyperphagia, obesity, and hypertension in metabolic disorders induced by fetal programming. *Endocrinology*. 2001; 142:3964-3973.

92. Rasmussen, SA et al. Maternal obesity and risk of neural tube defects: a metaanalysis. *Am J Obstet Gynecol*. 2008; 198:611-619.

93. Rogers, E. Has enhanced folate status during pregnancy altered natural selection and possibly Autism prevalence? A closer look at a possible link. *Med Hypotheses*. 2008 Sep; 71(3):406-10.

94. Roman-Garcia, P et al. Vitamin B_{12}-dependent taurine synthesis regulates growth and bone mass. *J Clin Invest.* 2014 Jul 1; 124(7):2988-3002.

95. Rush EC, Katre P, and Yajnik CS. Vitamin B12: one carbon metabolism, fetal growth and programming for chronic disease. *Eur J Clin Nutr.* 2014 Jan; 68(1):2-7.

96. Frisancho, AR et al. Developmental and nutritional determinants of pregnancy outcome among teenagers. *Am J Phys Anth*. 1985; 66:247-261.

97. Alberg, A. The influence of cigarette smoking on circulating concentrations of antioxidant micronutrients. *Toxicology.* 2002; 180:121-137.

98. Gadgil, MD et al. Laboratory testing for and diagnosis of nutritional deficiencies in pregnancy before and after bariatric surgery. *J Womens Health*. 2014 Feb; 23(2):129-37.

99. Torres-Sanchez, L et al. Maternal dietary intake of folate, vitamin B12 and MTHFR 677C>T genotype: their impact on newborn's anthropometric parameters. *Genes Nutr.* 2014 Sep; 9(5):429.

100. Balci, YI et al. Serum vitamin B12 and folate concentrations and the effect of the Mediterranean diet on vulnerable populations. *Pediatr Hematol Oncol.* 2014 Feb; 31(1):62-7.

101. Chatzi, L and Kogevinas, M. Prenatal and childhood Mediterranean diet and the development of asthma and allergies in children. *Public Health Nutr.* 2009 Sep; 12(9A):1629-34.

102. Chatzi, L et al. Mediterranean diet in pregnancy is protective for wheeze and atopy in childhood. *Thorax.* 2008 Jun; 63(6):507-13.

103. Chatzi, L et al. Diet, wheeze, and atopy in school children in Menorca, Spain. *Pediatr Allergy Immunol.* 2007 Sep; 18(6):480-5.

104. Chatzi, L et al. Protective effect of fruits, vegetables and the Mediterranean diet on asthma and allergies among children in Crete. *Thorax.* 2007 Aug; 62(8):677-83.

105. Pedersen, M et al. Bulky dna adducts in cord blood, maternal fruit-and-vegetable consumption, and birth weight in a European mother-child study (NewGeneris). *Environ Health Perspect.* 2013 Oct; 121(10):1200-6.

106. Dror, DK, and Allen, LH. Interventions with vitamins B6, B12 and C in pregnancy. *Paediatr Perinat Epidemiol.* 2012 Jul; 26 Suppl 1:55-74.

107. Schulze-Bonage, A et al. Pharmacorefractory status epilepticus due to low vitamin B6 levels during pregnancy. *Epilepsia.* 2004 Jan; 45(1):81-4.

108. Naem, NE et al. Zinc status and dietary intake of pregnant women, Alexandria, Egypt. *J Egypt Public Health Assoc.* 2014 Apr; 89(1):35-41.

109. Benton, D. Micronutrient status, cognition and behavioral problems in childhood. *Eur J Nutr*. 2008; 47 Suppl 3:38-50.

110. Zare, A et al. Correlation between serum zinc levels and successful immunotherapy in recurrent spontaneous abortion patients. *J Hum Reprod Sci.* 2013 Apr; 6(2):147-51.

111. Datta, S et al. Vitamin D deficiency in pregnant women from a non-European ethnic minority population: an interventional study. *Brit J Obstet Gynecol*. 2002; 109:905-908.

112. Darling, AL et al. Vitamin D deficiency in UK South Asian women of childbearing age: a comparative longitudinal investigation with UK Caucasian women. *Osteoporis Int.* 2013; 24:477-488.

113. Christian, P et al. Antenatal micronutrient supplementation and third trimester cortisol and erythropoietin concentrations. *Matern Child Nutr.* 2014 Jul 24.

114. Centers for Disease Control and Prevention. Obesity and overweight. Accessed November 2014 from http://www.cdc.gov/nchs/fastats/obesity-overweight.htm.

115. Lee, C et al. Obesity, adipokines and cancer: An update. *Clin Endocrinol (Oxf).* 2014 Nov 13.

116. Xu, J et al. Maternal circulating concentrations of tumor necrosis factor-alpha, leptin, and adiponectin in gestational diabetes mellitus: a systematic review and meta-analysis. *ScientificWorldJournal.* 2014; 2014:926932.

117. De Kort, S, Keszthelyi, D and Masclee, AAM. Leaky gut and diabetes mellitus: what is the link? *Obesity Reviews.* 2011; 12:449–458.

118. Hotamisligil GS, and Erbay, E. Nutrient sensing and inflammation in metabolic diseases. *Nat Rev Immunol.* 2008; 8:923–934.

119. McNaull, B et al. Inflammation and anti-inflammatory strategies for Alzheimer's disease - a mini-review. *Gerontology.* 2010; 56:3-14.

120. Toni R et al. New paradigms in neuroendocrinology: relationships between obesity, systemic inflammation and the neuroendocrine system. *J Endocrinol Invest*. 2004 Feb; 27(2):182-6.

121. Harris RE, Casto, BC, and Harris, ZM. Cyclooxygenase-2 and the inflammogenesis of breast cancer. *World J Clin Oncol.* 2014 Oct; 5(4):677-92.

122. Westermeier, F et al. Programming of fetal insulin resistance in pregnancies with maternal obesity by ER stress and inflammation. *Biomed Res Int.* 2014; 2014:917672.

123. Das, UN. Is obesity an inflammatory condition? *Nutrition.* 2001; 17:953-966.

124. Levin, BE. Metabolic imprinting: Critical impact of the perinatal environment on the regulation of energy homeostasis. *Philos Trans R Soc Lond B Biol Sci.* 2006; 361:1107-1121.

125. Dunn, GA and Bale, TL. Maternal high-fat diet promotes body length increases and insulin insensitivity in second-generation mice. *Endocrinology*. 2009; 150:4999-5009.

126. Solt, I. The human microbiome and the great obstetrical syndromes: A new frontier in maternal-fetal medicine. *Best Pract Res Clin Obstet Gynaecol*. 2014 Aug; pii: S1521-6934(14)00172-2.

127. Gohir, W, Ratcliffe, EM, and Sloboda, DM. Of the bugs that shape us: maternal obesity, the gut microbiome, and long-term disease risk [published online ahead of print 2014 Oct]. *Pediatr Res.* 2014.

128. Wassenaar, TM and Panigrahi, P. Is a foetus developing in a sterile environment? [published online ahead of print 2014 Oct 1]. *Lett Appl Microbiol*. 2014.

129. Romano-Keeler, J, and Weitkamp, JH. Maternal influences on fetal microbial colonization and immune development [published online ahead of print 2014 Oct]. *Pediatr Res.* 2014.

130. Munyaka, PM, Khafipour, E, and Ghia, JE. External influence of early childhood establishment of gut microbiota and subsequent health implications. *Front Pediatr.* 2014 Oct 9; 2:109.

131. Toth, M. Mechanisms of non-genetic inheritance and psychiatric disorders [published online ahead of print 2014 Jun 3]. *Neuropsychopharmacology.* 2014.

132. Faa, G et al. Fetal Programming of the human brain: is there a link with insurgence of neurodegenerative disorders in adulthood? *Curr Med Chem.* 2014; 21(33):3854-76.

133. Zhang, Y et al. Roles of PPARγ/NF-κB signaling pathway in the pathogenesis of intrahepatic cholestasis of pregnancy. *PLoS One.* 2014 Jan 29;9(1):e87343.

134. Geisert, RD et al. Cytokines from the pig conceptus: roles in conceptus development in pigs. *Journal of Animal Science and Biotechnology*. 2014, 5:51.

135. Sykes, L et al. Changes in the Th1:Th2 cytokine bias in pregnancy and the effects of the anti-inflammatory cyclopentenone prostaglandin 15-deoxy-Δ(12,14)-prostaglandin J2. *Mediators Inflamm.* 2012; 2012:416739.

136. Zhao, M et al. Folic acid protects against lipopolysaccharide-induced preterm delivery and intrauterine growth restriction through its anti-inflammatory effect in mice. *PLoS One.* 2013 Dec 6; 8(12):e82713.

137. Ly, C et al. The effects of dietary polyphenols on reproductive health and early development [published online ahead of print 2014 Nov 5]. *Hum Reprod Update.* 2014; pii: dmu058.

138. Singh, K et al. Sulforaphane treatment of autism spectrum disorder (ASD). *Proc Natl Acad Sci U S A.* 2014 Oct 28; 111(43):15550-5.

139. Mikaelsson MA, et al. Placental programming of anxiety in adulthood revealed by Igf2-null models. *Nat Comm.* 2013; 4:2311.

140. Manuelpillai U et al. Identification of kynurenine pathway enzyme mRNAs and metabolites in human placenta: Up-regulation by inflammatory stimuli and with clinical infection. *Obstet Gynecol*. 2005; 192:280-288.

141. Noto Llana, M et al. Salmonella enterica serovar enteritidis enterocolitis during late stages of gestation induces an adverse pregnancy outcome in the murine model. *PLoS One*. 2014 Nov 3; 9(11):e111282.

142. Samsam M, Ahangari, R, and Naser, SA. Pathophysiology of autism spectrum disorders: revisiting gastrointestinal involvement and immune imbalance. *World J Gastroenterol*. 2014 Aug 7; 20(29):9942-51.

143. Armstrong-Wells, J et al. Inflammatory predictors of neurologic disability after preterm premature rupture of membranes [published online ahead of print 2014 Sept 16]. *Am J Obstet Gynecol.* 2014: pii: S0002-9378(14)00958-2.

144. Knuesel, I et al. Maternal immune activation and abnormal brain development across CNS disorders. *Nat Rev Neurol.* 2014 Nov; 10(11):643-660.

145. Poletaev, AB et al. Adaptive maternal immune deviations as a ground for autism spectrum disorders development in children. *Folia Med (Plovdiv).* 2014 Apr-Jun; 56(2):73-80.

146. Straley, ME et al. LPS alters placental inflammatory and endocrine mediators and inhibits fetal neurite growth in affected offspring during late gestation. *Placenta.* 2014 Aug; 35(8):533-8.

147. Hornig, M. The role of microbes and autoimmunity in the pathogenesis of neuropsychiatric illness. *Curr Opin Rheumatol.* 2013; 25(4):488-495.

148. Guxen, M et al. Air pollution during pregnancy and childhood cognitive and psychomotor development: six European birth cohorts. *Epidemiology.* 2014 Sep; 25(5):636-47.

149. Volk, H et al. Residential proximity to freeways and autism in the CHARGE study. *Environ Health Perspect.* 2011 Jun; 119(6):873-7.

150. Landrigan, P et al. What causes autism? Exploring the environmental contribution. *Curr Opin Pediatr.* 2010 Apr; 22(2):219-25.

151. Ghanizadeh, A. Acetaminophen may mediate oxidative stress and neurotoxicity in autism. *Medical Hypotheses*. 2012 Feb; 78(2):351.

152. Shaw, W. Evidence that increased acetaminophen use in genetically vulnerable children appears to be a major cause of the epidemics of autism, attention deficit with hyperactivity, and asthma. *Journal of Restorative Medicine*. 2013; 2:1-16.

153. Suri, R et al. Acute and long-term behavioral outcome of infants and children exposed in utero to either maternal depression or antidepressants: a review of the literature. *J Clin Psychiatry*. 2014 Oct; 75(10):e1142-52.

154. Rai, D et al. Parental depression, maternal antidepressant use during pregnancy, and risk of autism spectrum disorders: population based case-control study. *BMJ.* 2013; 346: f2059.

155. Martinez-Arguelles, D et al. Fetal origin of endocrine dysfunction in the adult: the phthalate model. *J Steroid Biochem Mol Biol*. 2013 Sep; 137:5-17.

156. Park, S et al. Association between phthalates and externalizing behaviors and cortical thickness in children with attention deficit hyperactivity disorder [published online ahead of print 2014 Nov 12]. *Psychol Med.* 2014.

157. Rossignol, DA, Genuis, SJ, and Frye, RE. Environmental toxicants and autism spectrum disorders: a systematic review. *Transl Psychiatry.* 2014 Feb 11; 4:e360.

158. Goodman, M, Lakind, JS, and Mattison, DR. Do phthalates act as obesogens in humans? A systematic review of the epidemiological literature. *Crit Rev Toxicol.* 2014 Feb; 44(2):151-75.

159. Zeliger, HI. Lipophilic chemical exposure as a cause of type 2 diabetes (T2D). *Rev Environ Health.* 2013; 28(1):9-20.

160. Crinnion, WJ. Toxic effects of the easily avoidable phthalates and parabens. *Altern Med Rev.* 2010 Sep; 15(3):190-6.

161. Colborn, T, Dumanoski, D, and Meyers, JP. Our stolen future: are we threatening our fertility, intelligence and survival? A scientific detective story. New York: Dutton, 1996.

162. Fett, R. It starts with the egg: how the science of egg quality can help you get pregnant naturally, prevent miscarriage, and improve your odds in IVF. New York: Franklin Fox Publishing, 2014.

163. Troisi, J et al. Placental concentrations of bisphenol A and birth weight from births in the Southeastern U.S. Placenta. 2014 Nov; 35(11):947-52.

164. Paulose, T et al. Estrogens in the wrong place at the wrong time: fetal BPA exposure and mammary cancer [published online ahead of print 2014 Sept 29]. *Reprod Toxicol*. 2014; pii: S0890-6238(14)00249-4.

165. Dhimolea, E et al. Prenatal exposure to BPA alters the epigenome of the rat mammary gland and increases the propensity to neoplastic development. *PLoS One*. 2014 Jul; 9(7):e99800.

166. Watkins, DJ et al. In utero and peripubertal exposure to phthalates and BPA in relation to female sexual maturation. *Environ Res.* 2014 Aug; 134C:233-241.

167. La Merrill, M and Birnbaum, LS. Childhood obesity and environmental chemicals. *Mount Sinai Journal of Medicine.* 2011; 78:22–48.

168. Mendez, MA et al. Prenatal organochlorine compound exposure, rapid weight gain and overweight in infancy. *Environ Health Perspect.* 2011 Feb; 119(2):272-8.

169. Lilienthal, H et al. Effects of developmental exposure to 2,2,4,4 ,5-pentabromodiphenyl ether (PBDE-99) on sex steroids, sexual development, and sexually dimorphic behavior in rats. *Environ Health Perspect.* 2006 Feb; 114(2):194–201.

170. Apelberg, BJ et al. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. *Environ Health Perspect.* 2007 Nov; 115(11):1670–1676.

171. Rouiller-Fabre, V, Habert, R, and Livera, G. Effects of endocrine disruptors on the human fetal testis. *Ann Endocrinol*. 2014 May; 75(2):54-7.

172. de Cock, M, Maas, YG, and van de Bor, M. Does perinatal exposure to endocrine disruptors induce autism spectrum and attention deficit hyperactivity disorders? *Acta Paediatr*. 2012 Aug; 101(8):811-8.

173. Tando, S et al. Bisphenol A exposure disrupts the development of the locus coeruleus-noradrenergic system in mice [published online ahead of print 2014 Jul 2]. *Neuropathology.* 2014.

174. Xu, X et al. Bisphenol A promotes dendritic morphogenesis of hippocampal neurons through estrogen receptor-mediated ERK1/2 signal pathway. *Chemosphere.* 2014 Feb; 96:129-37.

175. Kundakovic, M et al. Sex-specific epigenetic disruption and behavioral changes following low-dose in utero bisphenol A exposure. *Proc Natl Acad Sci USA*. 2013 Jun 11; 110(24):9956-61.

176. Jang, Y et al. High dose bisphenol A impairs hippocampal neurogenesis in female mice across generations.*Toxicology.* 2012 Jun 14; 296(1-3):73-82.

177. Carreón-Rodríguez, A et al. Clinical implications of thyroid hormones effects on nervous system development. *Pediatr Endocrinol Rev*. 2012 Mar; 9(3):644-9.

178. Medici, M et al. Maternal early pregnancy and newborn thyroid hormone parameters: the Generation R study*. J Clin Endocrinol Metab*. 2012 Feb; 97(2):646-52.

179. Su, P et al. Maternal thyroid function in the first twenty weeks of pregnancy and subsequent fetal and infant development: a prospective population-based cohort study in China. *J Clin Endocrinol Metab*. 2011 Oct; 96(10):3234-41.

180. Gärtner, R et al. Thyroid disorders during pregnancy. *Dtsch Med Wochenschr*. 2009 Jan; 134(3):83-6.

181. LaFranchi, S et al. Is thyroid inadequacy during gestation a risk factor for adverse pregnancy and developmental outcomes? *Thyroid.* 2005 Jan; 15(1):60-71.

182. Chen, L et al. Effects of Subclinical Hypothyroidism on Maternal and Perinatal Outcomes during Pregnancy: A Single-Center Cohort Study of a Chinese Population. *PLoS One.* 2014 Oct 29; 9(10):e109364.

183. Mamas, L, and Mamas, E. Dehydroepiandrosterone supplementation in assisted reproduction: rationale and results. *Curr Opin Obstet Gynecol.* 2009 Aug; 21(4):306-8.

184. Fusi, F et al. DHEA supplementation positively affects spontaneous pregnancies in women with diminished ovarian function. *Gynecol Endocrinol.* 2013 Oct; 29(10):940-3.

185. Zamudio-Bulcock, PA, and Valenzuela, CF. Pregnenolone sulfate increases glutamate release at neonatal climbing fiber-to-Purkinje cell synapses. *Neuroscience.* 2011 Feb 23; 175:24-36.

186. Bruin, JE, et al. Maternal antioxidants prevent β-cell apoptosis and promote formation of dual hormone-expressing endocrine cells in male offspring following fetal and neonatal nicotine exposure. *J Diabetes.* 2012 Sep; 4(3):297-306.

187. Thum, C et al. Can nutritional modulation of maternal intestinal microbiota influence the development of the infant gastrointestinal tract? *J Nutr.* 2012 Nov; 142(11):1921-8.

188. Giannubilo, S et al. Amniotic coenzyme Q10: is it related to pregnancy outcomes? *Antioxid Redox Signal*. 2014 Oct 10; 21(11):1582-6.

189. Costantino, M et al. Peripheral neuropathy in obstetrics: efficacy and safety of α-lipoic acid supplementation. *Eur Rev Med Pharmacol Sci.* 2014 Sep; 18(18):2766-71.

190. Al Ghafli, M et al. Effects of alpha-lipoic acid supplementation on maternal diabetes-induced growth retardation and congenital anomalies in rat fetuses. *Mol Cell Biochem.* 2004 Jun; 261(1-2):123-35.

191. Moore, R et al. Alpha-lipoic acid inhibits tumor necrosis factor-induced remodeling and weakening of human fetal membranes. *Biol Reprod.* 2009 Apr; 80(4):781-7.

192. Bentov, Y et al. The use of mitochondrial nutrients to improve the outcome of infertility treatment in older patients. *Fertil Steril.* 2010 Jan; 93(1):272-5.

193. Moore, R et al. Alpha-lipoic acid inhibits thrombin-induced fetal membrane weakening in vitro. *Placenta.* 2010 Oct; 31(10):886-92.

194. Miller, SL, Wallace, EM, and Walker, DW. Antioxidant therapies: a potential role in perinatal medicine. *Neuroendocrinology.* 2012; 96(1):13-23.

195. Yin, Q et al. AGEs induce cell death via oxidative and endoplasmic reticulum stresses in both human SH-SY5Y neuroblastoma cells and rat cortical neurons. *Cell Mol Neurobiol.* 2012 Nov; 32(8):1299-309.

196. Chang, E et al. N-acetylcysteine prevents preterm birth by attenuating the LPS-induced expression of contractile associated proteins in an animal model. *J Matern Fetal Neonatal Med.* 2012 Nov; 25(11):2395-400.

197. Chang, E et al. N-acetylcysteine attenuates the maternal and fetal proinflammatory response to intrauterine LPS injection in an animal model for preterm birth and brain injury. *J Matern Fetal Neonatal Med*. 2011 May; 24(5):732-40.

198. Falluel-Morel, A et al. N-acetyl cysteine treatment reduces mercury-induced neurotoxicity in the developing rat hippocampus. *J Neurosci Res.* 2012 Apr; 90(4):743-50.

199. Buhimschi, I, Buhimschi, CS, and Weiner, CP. Protective effect of N-acetylcysteine against fetal death and preterm labor induced by maternal inflammation. *Am J Obstet Gynecol.* 2003 Jan; 188(1):203-8.

200. Xu, D et al. Effect of N-acetylcysteine on lipopolysaccharide-induced intra-uterine fetal death and intra-uterine growth retardation in mice. *Toxicol Sci.* 2005 Dec; 88(2):525-33.

201. Perry, C et al. Pregnancy and lactation alter biomarkers of biotin metabolism in women consuming a controlled diet. *J Nutr.* 2014 Dec; 144(12):1977-84.

202. Ismail, A et al. Adding L-carnitine to clomiphene resistant PCOS women improves the quality of ovulation and the pregnancy rate. A randomized clinical trial. *Eur J Obstet Gynecol Reprod Biol.* 2014 Sep; 180:148-52.

203. Tipi-Akbas, P et al. Lowered serum total L-carnitine levels are associated with obesity at term pregnancy. *J Matern Fetal Neonatal Med.* 2013 Oct; 26(15):1479-83.

204. Lie, S et al. Exposure to rosiglitazone, a PPAR-γ agonist, in late gestation reduces the abundance of factors regulating cardiac metabolism and cardiomyocyte size in the sheep fetus. *Am J Physiol Regul Integr Comp Physiol.* 2014 Mar 15; 306(6):R429-37.

205. Magliano, D et al. Peroxisome proliferator-activated receptors-alpha and gamma are targets to treat offspring from maternal diet-induced obesity in mice. *PLoS One.* 2013 May 20; 8(5):e64258.

206. Wanderley, M et al. Foetal exposure to Panax ginseng extract reverts the effects of prenatal dexamethasone in the synthesis of testosterone by Leydig cells of the adult rat. *Int J Exp Pathol.* 2013 Jun; 94(3):230-40.

207. Segovia, S et al. Maternal obesity, inflammation, and developmental programming. *Biomed Res Int.* 2014; 2014:418975.

208. Rossi, EL Stress-induced alternative gene splicing in mind-body medicine. *Adv Mind Body Med*. 2004 Summer; 20(2):12-9.

209. Li, H, Gudmundsson, S, and Olofsson, P. Acute changes of cerebral venous blood flow in growth-restricted human fetuses in response to uterine contractions. *Ultrasound Obstet Gynecol.* 2004 Oct; 24(5):516-21.

210. Jensen Peña, C, Monk, C, and Champagne, FA. Epigenetic effects of prenatal stress on 11β-hydroxysteroid dehydrogenase-2 in the placenta and fetal brain. *PLoS One*. 2012; 7(6):e39791.

211. Crudo, A et al. Glucocorticoid programming of the fetal male hippocampal epigenome. *Endocrinology.* 2013 Mar; 154(3):1168-80.

212. Crudo, A et al. Effects of antenatal synthetic glucocorticoid on glucocorticoid receptor binding, DNA methylation, and genome-wide mRNA levels in the fetal male hippocampus. *Endocrinology.* 2013 Nov; 154(11):4170-81.

213. Duthie, L, and Reynolds, RM. Changes in the maternal hypothalamic-pituitary-adrenal axis in pregnancy and postpartum: influences on maternal and fetal outcomes. *Neuroendocrinology.* 2013; 98(2):106-15.

214. Satyapriya, M et al. Effect of integrated yoga on stress and heart rate variability in pregnant women. *Int J Gynaecol Obstet.* 2009 Mar; 104(3):218-22.

215. Giesbrecht, G et al. The buffering effect of social support on hypothalamic-pituitary-adrenal axis function during pregnancy. *Psychosom Med.* 2013 Nov-Dec; 75(9):856-62.

216. Ferezou-Viala, J et al. Long-term consequences of maternal high-fat feeding on hypothalamic leptin sensitivity and diet-induced obesity in the offspring. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology.* 2007; 293:R1056-1062.

217. Hansen, C et al. A maternal gluten-free diet reduces inflammation and diabetes incidence in the offspring of NOD mice. *Diabetes.* 2014 Aug; 63(8):2821-32.

218. Moloney, R et al. Early-life stress induces visceral hypersensitivity in mice. *Neuroscience Letters.* 2012 Mar; 512(2):99-102.