Epigenetic Mechanisms in Fetal Programming of Metabolic Syndrome.

Abstract

The fetal origin of disease hypothesis posits that adverse conditions during fetal development, such as maternal undernutrition or placental insufficiency, can lead to long-term health consequences in adulthood, including hypertension, insulin resistance, and cardiovascular diseases. These conditions are thought to arise from adaptive responses by the fetus to a suboptimal intrauterine environment, which results in permanent changes to organ structure and function. This concept is supported by epidemiological and experimental studies, demonstrating a correlation between low birth weight and increased risk of metabolic syndrome, type 2 diabetes, and coronary heart disease in later life. Research indicates that insulin resistance, a central factor in metabolic syndrome, may be programmed by these early-life adverse events. Furthermore, epigenetic mechanisms, such as DNA methylation and chromatin remodeling, play a crucial role in maintaining these adaptations, linking early environmental factors to long-term gene expression changes. Epigenetic marks serve as a memory of early exposure to adverse conditions, leading to the developmental origins of the health and disease (DOHaD) hypothesis. These findings underscore the importance of maternal nutrition and stress management during pregnancy in shaping future health outcomes. The need for further research to identify factors affecting fetal growth and to develop strategies for optimizing maternal and fetal health is paramount. Understanding these mechanisms can potentially reduce the prevalence of chronic diseases and address health inequalities.

Keywords: Fetal origin of disease hypothesis, Maternal undernutrition, Hypertension, Insulin resistance, Epigenetics

1.0 INTRODUCTION

Fetal programming or prenatal programming is a phenomenon that links environmental conditions during sensitive embryonic and fetal development with the risk of diseases later in life (Kwon and Kim, 2017; Lindsay *et al*., 2019; őztűrk and Tűrker, 2021). The central idea about fetal programming is that during the development of the embryo and fetus, important physiological parameters can be altered by environmental events and more importantly the resetting can endure into adulthood and even affect the following generation to produce a trans-generational non-genetic disorder (Marciniak *et al*., 2017; őztűrk and Tűrker, 2021).

Fetal development is a very complex process (Kwon and Kim, 2017). At different stages of development different aspects can be changed by specific environmental factors (Kwon and Kim, 2017; Lindsay *et al*., 2019). The external factors affect the development of the fetus and some of these changes last for a life time (Lindsay *et al*., 2019). The local fetal cellular environments can change gene expression during the developmental construction of tissues and organs, and these changes can result in long-range consequences for the function of those tissues and organs during childhood and adulthood (Lindsay *et al*., 2019).

Fetal programming can be changed by various factors like altered nutrition or maternal stress. This effect does not always become obvious at once but sometimes shows up later in life (Öztürk and Türker, 2021). Numerous studies indicate that early-life events play an important role in influencing later susceptibility to certain chronic diseases (Kwon and Kim, 2017; Marciniak *et al*., 2017; Stevenson *et al*., 2020). Such events might be over- or under-nutrition, exposure to environmental toxins, but also changes in hormones, in particular stress hormones (Marciniak *et al*., 2017; Stevenson *et al*., 2020). Those events are triggered by the environmental challenges of the mother (Öztürk and Türker, 2021).

Recent studies have shown that paternal environmental or nutritional factors affect the phenotype of the offspring as well (Öztürk and Türker, 2021). The maternal environmental factors act on the

phenotype of the offspring via epigenetic modification of its genome (Öztürk and Türker, 2021). Several Studies showed that impaired intrauterine growth and adult metabolic and cardiovascular disorders such as coronary heart disease, type 2 diabetes, and insulin (INS) resistance, are strongly associated (Marciniak *et al*., 2017; Elsakr *et al*., 2019; Armengaud *et al*., 2021).

Clinico-epidemiological evidence suggests a close interaction between fetal and maternal environment and modulation of gene expression that starts very early in life and passed across generations (Monteiro *et al*., 2016; Stevenson *et al*., 2020). Hence, the notion about epigenetic modifications such as DNA methylation and covalent posttranslational histone modifications, which mediate phenomena such as genomic imprinting and chromatin remodeling, emerged as the most suitable molecular explanation of fetal metabolic programming (Monteiro *et al*., 2016; Stevenson *et al*., 2020).

1.1 Aims and Objectives

1.1.1 Aim

The aim of this literature is to review fetal programming.

1.1.2 Objectives

The objectives of this literature are enumerated as follows:

i. To review the fetal programming hypothesis.
ii. To review fetal programming and epigenetics.
iii. To review fetal programming and adult health.

1.2 Scope of the Study

The scope of this literature is to review fetal programming in relation to adult health.

2.0 FETAL PROGRAMMING HYPOTHESIS

The fetal period is a time of enormous neurological changes and experiences during this period can dramatically influence development (Davis and Thompson, 2014). The fetal programming or developmental origins of disease models posit that during periods of rapid development or change the organism is susceptible to environmental influences and that these influences exert persisting consequences for health and disease risk (Barker, 1998; Kwon and Kim, 2017). The fetal programming hypothesis, dependent on the ability to study individuals across the entire lifespan, has an interesting origin. Empirical support for the fetal programming hypothesis exists, in part, because of an intervention established by the British government in the early 1900s (Davis and Thompson, 2014).

2.1 Barker's Hypothesis

At the start of the 20th century the British government was concerned about the declining health of the British population and the poor health of young men attempting to enlist in the army. An enlightened intervention was established. Ethel Margaret Burnside was named as the country's first "chief health visitor and lady inspector of midwives." She established a team of nurses to travel around the country to advice mothers on how to care for their infants. One extraordinary consequence of this intervention was that meticulous records were kept related to infant weight at birth and over the first postnatal year (Barker, 2003).

More than 60 years later Dr. David Barker was able to link these birth records to death records. Using these data he made an astounding observation. A disproportionate number of deaths from coronary heart disease occurred among individuals with low birth weight (Barker, 2003). These data have since been replicated in numerous epidemiological studies. Size at birth is predictive of variety of later health outcomes including heart disease, diabetes, and obesity as well as psychiatric dysfunction (Nathanielsz, 1999; Barker, 2003) illustrating the importance of considering fetal origins of later health and disease.

The `fetal origins' hypothesis proposes that alterations in fetal nutrition and endocrine status result in developmental adaptations that permanently change structure, physiology and metabolism, thereby predisposing to cardiovascular, metabolic and endocrine disease in adult life (Barker, 1998). For example, it is thought that coronary heart disease may be a consequence of fetal adaptations to under-nutrition that are beneficial for short-term survival, even though they are detrimental to health in post-reproductive life (Barker, 1998).

In fetal life the tissues and organs of the body go through what are called `critical' periods of development. These may coincide with periods of rapid cell division. In common with other living creatures, human beings are `plastic' in their early life, and are moulded by the environment (Godfrey and Barker, 2001). Although the growth of a fetus is influenced by its genes, studies in humans and animals suggest that it is usually limited by the environment, in particular the nutrients and oxygen received from the mother (Godfrey and Barker, 2001). There are many possible evolutionary advantages in the body remaining plastic during development, rather than having its development driven only by genetic instructions acquired at conception (Godfrey and Barker, 2001). ‘Programming' describes the process whereby a stimulus or insult during a critical period of development has lasting or lifelong effects (Lucas, 1994). Experimental studies in animals have documented many examples of fetal programming, with recent studies showing that alterations in maternal nutrition can have long-term effects on the offspring that are of relevance to human cardiovascular disease (Godfrey and Barker, 2001).

For example, feeding pregnant rats a low protein diet results in life-long elevation of blood pressure in the offspring (Langley-Evans and Jackson, 1994) Rats whose mothers had been fed a diet with a low ratio of protein to energy during pregnancy exhibited a permanently altered balance between hepatic glucose production and utilization; control rats fed the same diet during post-natal life had no alterations in hepatic glucose metabolism (Desai *et. al.,* 1995). Other notable long-term effects of alterations in maternal nutrition include changes in cholesterol metabolism, insulin secretion and renal development (Barker, 1998).

Though some effects of nutrition may be direct consequences of alterations in substrate availability, a number are thought to be mediated by hormonal effects (Barker, 1998). These may alter the development of specific fetal tissues during critical periods, or lead to long-lasting changes in hormone secretion or tissue hormone sensitivity. Experimental studies have implicated the fetal hypothalamus as a key site that can be programmed by transient changes in prenatal endocrine status (Godfrey and Barker, 2001).

2.2 Example of fetal programming

2.2.1 The Dutch Hunger Winter

Some of the most compelling evidence for fetal programming of adult disease comes from long-term follow-up studies of survivors of the Dutch "Hunger Winter." During the Nazi occupation of the Netherlands (winter 1944–1945) a severe famine was experienced in the western Netherlands resulting from a German embargo on rail transport and a severe winter. For approximately 9 months, a significant portion of the Dutch population was forced to subsist on less than 1,000 calories per day. During this time period many Dutch starved to death (Schreuder, 2007).

The famine ended abruptly with the Allied liberation of the Netherlands in the spring of 1945. This tragedy has provided unique information about the consequences of nutritional deprivation during pregnancy. Because the famine was circumscribed in time and place it was possible to determine the long-term consequences for fetuses that were exposed in specific gestational intervals (Godfrey and Barker, 2001). The national registries in the Netherlands provided the opportunity for long-term and intergenerational assessment of the consequences of fetal exposure to this famine. The findings from this large research literature indicate that although many of the immediate effects of maternal malnutrition on newborns (such as birth weight) were not strongly predictive of later outcomes, there were important latent effects (Lumey *et. al.,* 2011). Adults who were exposed as fetuses to the famine exhibited significantly heightened risk for a variety of physical and mental health problems, including adult obesity, diabetes, heart disease, and schizophrenic disorders, compared to pregnancies not affected by the famine or same-sex sibling controls (Lumey *et al.,* 2007; Lumey *et. al.,* 2011).

Many of these later outcomes are consistent with adult metabolic disorder, in which biological systems are functioning maladaptively to enhance cardiovascular risk. It appears that the period of early malnutrition caused biological adaptations to nutritional insufficiency in the fetus (such as decreased energy metabolism and growth rate) to prepare for a postnatal life of food scarcity (Lucas, 1994; Godfrey and Barker, 2001). When children instead grew up in conditions of insufficient food, their physiological systems were unprepared for this, thus contributing to later health problems (Lucas, 1994).

2.1.2 Ramadan Fasting

During Ramadan (of the lunar cycle), many Muslims around the world participate in a fast during the daylight hours of the lunar month (Glazier *et al*., 2018). This fasting usually entails abstaining from food or drink for the daylight hours of the month (Glazier *et al*., 2018). There are groups that are automatically exempted from having to participate like the young, sick and old but the list of exemption does not officially include pregnant women (though they are most often given exemption) (Baynouna *et al*., 2014). The majority of pregnant women however, choose to participate despite the stress accompanying the cultural and religious practice (Baynouna *et al*., 2014).

Despite the limited data regarding the effect of Ramadan fasting on the pregnancies that experienced the fasting, several reports have been conflicting and have shown contradicting outcomes (Kavehmanesh and Abolghasemi, 2004; Ozturk *et al*., 2011; Awwad *et al*., 2012; Petherick *et al*., 2014). The sample sizes of these studies are not considerable to evoke confidence about the outcomes (Kavehmanesh and Abolghasemi, 2004; Ozturk *et al*., 2011; Awwad *et al*., 2012; Petherick *et al*., 2014). Almond and Mazumder (2011) have indicated the negative health conditions that pregnancies exposed to Ramadan fasting have susceptibility to low birth weight. However, activated starvation which is the decrease in maternal glucose levels leading to biochemical changes in the fetal environment has been associated with Ramadan fasting (Almond and Mazumder, 2011). Also, high increase in the hormonal cortisol has been associated with Ramadan fasting in a study by Dikensoy *et al*. (2009). The combination of the effects of sharp decline in the maternal glucose levels

and heightened increase in the cortisol levels has been associated with Ramadan fasting. Timing has been reported to be important in the adverse effect of nutritional deficiencies of pregnant mothers that play a role in long-term illness associated with the adult lives of the children exposed to these nutritional deficiencies (Roseboom *et al*., 2001; Thornburg *et al*., 2010; Savitri *et al*., 2014). Fasting during the first trimester has been associated with low birth weight while the effect on the placental weight, which has been considered as a predictor of health outcomes in progeny (Thornburg *et al*., 2010; Savitri *et al*., 2014).

In uterus exposure to Ramadan fasting has a negative effect on male birth rate causing a skewed sex ratio for total births. When exposure to the Ramadan fast takes place a month after conception it is correlated with a 13% decline in total births. The effects on exposed males and females are drastically different. Where the male birth rate drops by 26%, the female birth rate drops only 2.5% leading to the assumption that "male vulnerability" may be to blame (Almond and Mazumder, 2011). In the same study by Almond and Mazumder (2011) conducted in Uganda and Iraq on the levels of disability among those exposed to the fast while in the uterus, it was concluded that disability rates were much higher for those exposed when controlling for outside factors. Though the measure for disability differs by country the effect is still noticeable (Almond and Mazumder, 2011). For those born 9 months after Ramadan the likelihood of disability is higher than the surrounding population (Almond and Mazumder, 2011). The mean rate of disability in Uganda is 3.8% for the country but for those exposed the number is drastically higher at 22% mean disability rate. A similar effect can be observed in Iraq where the mean rate of disability is 1.5% but the disability rate of those exposed is 23% as reported by Almond and Mazumder (2011). Those born 9 months after Ramadan were 33% more likely to be blind and 64% more likely to be deaf than those not exposed in uterus. The effects of exposure to the Ramadan fast can even be observed in mental disorders (Almond and Mazumder, 2011). The part of Almond and Mazumder (2011) study conducted in Uganda, it was concluded that exposure to the fast, early in a pregnancy effectively doubles the likelihood of a person having a cognitive disorder of some kind. In the same report, similar discovery was made in Iraq where 63% higher likelihood of a cognitive disorder relative to the mean was discovered for all those exposed (Almond and Mazumder, 2011).

Certain specific health effects have been observed for those exposed to uterus fasting. The damage of the kidney caused the anemia and the reported signs of anemia among the female adults were higher for those exposed during mid-gestation while all other points in the gestation period were found to be insignificant (*van Ewijk, 2009).*

2.2 Maternal Nutrition and Fetal Development

Nutrition is the major intrauterine environmental factor that alters expression of the fetal genome and may have lifelong consequences (Marshal, 2002). Maternal nutrition plays a critical role in fetal growth and development. Although considerable effort has been directed towards defining nutrient requirements of animals over the past 30 years, suboptimal nutrition during gestation remains a significant problem for many animal species (e.g., cattle, pigs, and sheep) worldwide (Bell and Ehrhardt, 2002). Despite advanced prenatal care for mothers and fetuses, ~5% of human infants born in the U.S. suffers from intrauterine growth retardation (IUGR) (Marshal, 2002). Over the past decade, compelling epidemiological studies have linked IUGR with the etiology of many chronic diseases in adult humans and animals (MacLennan *et. al.,* 2004; Ashworth *et. al.,* 2009).

2.2.1 The Intrauterine Environment as a Major Factor Contributing to IUGR.

Multiple genetic and environmental factors contribute to IUGR (Bell and Ehrhardt, 2002). Although the fetal genome plays an important role in growth potential in uterus, increasing evidence suggests that the intrauterine environment is a major determinant of fetal growth. For example, embryo-transfer studies show that it is the recipient mother rather than the donor mother that more strongly influences fetal growth (MacLennan *et. al.,* 2004). There is also evidence that the intrauterine environment of the individual fetus may be of greater importance in the etiology of chronic diseases in adults than the genetics of the fetus.

2.2.2 Under-nutrition and Interuterine Growth Restrictions (IUGR)

Maternal undernutrition during gestation reduces placental and fetal growth of both domestic animals and humans (Barker and Clark, 1997; Bell and Ehrhardt, 2002). Available evidence suggests that fetal growth is most vulnerable to maternal dietary deficiencies of nutrients (e.g., protein and

micronutrients) during the peri-implantation period and the period of rapid placental development. In animal agriculture, fetal under-nutrition frequently occurs worldwide. (Wu *et al.,* 1998; Sugden Holness, 2002; Waterland & Jirtle, 2004).

Unsupplemented grazing ewes lose a significant amount of body weight during pregnancy, and their health, fetal growth, and lactation performance are seriously compromised (Thomas and Kott, 1995). In pigs, a disproportionate supply of nutrients along the uterine horn results in 15–20% low-birth-weight piglets (<1.1 kg), whose postnatal survival and growth performance are severely reduced (Guoyao *et al*., 2004). Therefore, the poor performance of certain livestock during the postnatal growth and finishing phases may be a consequence of growth restriction in utero (Guoyao *et al*., 2004)

Undernutrition in pregnant women may result from low intake of dietary nutrients owing to either a limited supply of food or severe nausea and vomiting known as hyperemesis gravidarum (Snel *et al.,* 1998). This life-threatening disorder occurs in 1–2% of pregnancies and generally extends beyond the 16th week of gestation (Snel *et al.,* 1998). Pregnant women may also be at increased risk of undernutrition because of early or closely-spaced pregnancies (King, 2003). Since pregnant teenage mothers are themselves growing, they compete with their own fetuses for nutrients, whereas short inter-pregnancy intervals result in maternal nutritional depletion at the outset of pregnancy. Low birth weights and preterm deliveries in adolescent pregnancies are more than twice as common as in adult pregnancies, and neonatal mortality in adolescent pregnancies is almost three times higher than for adult pregnancies (King, 2003). Further, placental insufficiency results in reduced transfer of nutrients from mother to fetus, thereby leading to fetal undernutrition and IUGR (Bell and Ehrhardt, 2002).

Finally, due to competition for nutrients, multiple fetuses resulting from assisted reproductive technologies are often at risk of under-nutrition and therefore fetal growth restriction (Marshal, 2002). Thus, various nutritional and pathological conditions can result in IUGR (MacLennan *et. al.,* 2004).

2.2.3 Over-nutrition and IUGR.

Significant health problems for animals (particularly companion animals) and women of reproductive age also result from being overweight or obese due to overeating. Over-nutrition can result from increased intake of energy and/or protein. Wallace *et al.* (2003) reported that extensive studies shown that maternal over-nutrition retards placental and fetal growth, and increases fetal and neonatal mortality in rats, pigs, and sheep (Wallace *et al.,* 2003). Results of recent epidemiological studies indicated that almost 65% of the adult population in the U.S. is overweight [defined as a body mass index (BMI) > 25 kg/m^2], while 31% of the adult population is obese (defined as BMI > 30 kg/m^2) (Hill *et al.,* 2003). Many overweight and obese women unknowingly enter pregnancy and continue overeating during gestation (Castro and Avina, 2002; MacLennan *et. al.,* 2004). Castro and Avina (2002) in their study pointed out that these women usually gain more weight during the first pregnancy and accumulate more fat during subsequent pregnancies. The over-nutrition before or during pregnancy often leads to maternal obesity thus resulting in fetal growth restriction and increased risk of neonatal mortality and morbidity in humans (Castro and Avina, 2002).

2.2.4 Health Problems Associated with IUGR.

IUGR causes both perinatal and neonatal medical complications. For instance, Mashal (2002) had noted how IUGR is responsible for about 50% of non-malformed stillbirths in humans. Infants who weigh <2.5 kg at birth have perinatal mortality rates that are 5 to 30 times greater than those infants who have average birth weights, while those <1.5 kg have rates 70 to 100 times greater (Marshal, 2002). Surviving infants with IUGR are often at increased risk for neurological, respiratory, intestinal, and circulatory disorders during the neonatal period (Marshal, 2002; MacLennan *et. al.,* 2004).

Both epidemiological and experimental evidence suggest that IUGR contributes to a wide array of metabolic disorders and chronic diseases in adults. For example, individuals exposed to the Dutch

winter famine of 1944–1945 in utero had higher rates of insulin resistance, vascular disease, morbidity, and mortality in adulthood (Lumey, 1998).

Thus, the intrauterine environment of the fetus may alter expression of the fetal genome and have lifelong consequences. This phenomenon is termed “fetal programming,” which has led to the theory of “fetal origins of adult disease” (Barker and Clark, 1997). Namely, alterations in fetal nutrition and endocrine status may result in developmental adaptations that permanently change the structure, physiology, and metabolism of the offspring, thereby predisposing individuals to metabolic, endocrine, and cardiovascular diseases in adult life (Barker and Clark, 1997).

2.2.5 Biochemical Mechanisms of IUGR

The lack of knowledge about the mechanisms of IUGR has prevented the development of effective therapeutic options, such that the current management of growth-restricted infants is empirical and is primarily aimed at selecting a safe time for delivery (MacLennan *et. al.,* 2004). Because nutritional and developmental research often involves invasive tissue collections and surgical procedures, it is neither ethical nor practical to conduct these experiments with the human placenta and fetus. Thus, animal models (e.g., mice, rats, pigs, and sheep) are instrumental in defining the mechanisms of IUGR and developing therapeutic means. The available evidence, which is discussed in the following sections, suggests that arginine [a nutritionally essential amino acid for the fetus (Flynn *et al.,* 2002)] plays a key role in the development of the conceptus (embryo/fetus, associated placental membranes, and fetal fluids).

2.2.6 Molecular Mechanisms of Fetal Programming

Nutritional insult during a critical period of gestation may leave a permanent “memory” throughout life, and some of the effects (e.g., insulin secretion and action) may be gender specific (Sugden Holness, 2002). There is growing evidence that maternal nutritional status can alter the epigenetic state of the fetal genome and imprint gene expression. Epigenetic alterations (stable alterations of gene expression through covalent modifications of DNA and core histones) in early embryos may be carried forward to subsequent developmental stages (Waterland & Jirtle, 2004).

Two mechanisms mediating epigenetic effects are DNA methylation (occurring in 5'-positions of cytosine residues within CpG dinucleotides throughout the mammalian genome) and histone modification (acetylation and methylation) (Jaenisch and Bird, 2003). CpG methylation can regulate gene expression by modulating the binding of methyl-sensitive DNA-binding proteins, thereby affecting regional chromatin conformation. Histone acetylation or methylation can alter the positioning of histone-DNA interactions and the affinity of histone binding to DNA, thereby affecting gene expression (Jaenisch and Bird, 2003).

DNA methylation is catalyzed by DNA methyltransferases, with S-adenosylmethionine (SAM) as a methyl donor (Jaenisch and Bird, 2003). SAM is synthesized from methionine and ATP by methionine adenosyltransferase. One-carbon unit metabolism, which depends on serine, glycine, and B vitamins (including folate, vitamin B-12, and vitamin B-6), plays an important role in regulating the availability of SAM (Waterland and Jirtle, 2004).

Thus, DNA methylation and histone modifications may be altered by the overall availability of amino acids and micronutrients. This notion is supported by several lines of evidence. First, a deficiency of amino acids results in a marked reduction in genomic DNA methylation and aberrant expression of the normally silent paternal H19 allele (an imprinted gene) in cultured mouse embryos (Doherty *et al., 2000).*

Second, uteroplacental insufficiency causes hypomethylation of the p53 gene in postnatal rat kidney (Pharm *et al., 2003*), as well as global DNA hypomethylation and increased histone acetylation in postnatal rat liver (MacLennan *et al.,* 2004).

Third, maternal supplementation of methyl donors and cofactors (folic acid, vitamin B-12, choline, and betaine) increases CpG methylation at the A^{vy} locus of agouti mice, and the methylation patterns are retained into adulthood (MacLennan *et al.*, 2004).

It remains to be determined whether maternal nutrition affects CpG methylation of the genes for NOS, GTP cyclohydrolase I (the rate-limiting enzyme for BH_4 synthesis), and ODC, or alters histone modifications, in the uterus, placenta, as well as fetal and postnatal tissues (e.g., the vascular bed, adipose tissue, liver, kidney, skeletal muscle, or pancreas).

However, epigenetics may provide a molecular mechanism for the impact of maternal nutrition on fetal programming of postnatal disease susceptibility and on genomic imprinting (the parent-of-origin-dependent expression of a single allele of a gene (MacLennan *et al.*, 2004; Pharm *et al., 2003)*.

3.0 FETAL PROGRAMMING AND ADULT HEALTH

The 'fetal origin of disease' hypothesis proposes that adulthood hypertension, insulin resistance, and dyslipidemia, leading to markedly increased rates of cardiovascular disease and non-insulin-dependent diabetes in adult life, originate through adaptation that the fetus undergoes when the environment (for example: nutrition) in early life is poor, caused by either maternal under-nutrition or placental insufficiency. (Hocher, 2014).

These functional and structural changes of the newborn develop in likely different time windows, mainly during pregnancy, but also in very early childhood (Barker, 2004). It was proposed that an event occurring during a critical early period of life might permanently alter the organ structure and function in response to environmental factors. Such events may lead to cardiovascular/metabolic and renal diseases in later life (Hocher, 2014). Maternal under-nutrition or abnormal uteroplacental function reduces nutrient delivery to the fetus and may produce secondary adaptations in metabolism and gene expression that may be beneficial during intrauterine life, but that may contribute to disease risk in later life (Hocher, 2014). A key factor in the pathogenesis of the metabolic syndrome in adult life and its consequences (cardiovascular diseases, hypertension and type 2 diabetes) is insulin resistance (Nolan *et al.,* 2011).

Thus, it was proposed that adverse events in early life – typically maternal undernutrition – might program insulin resistance, leading to cardiovascular diseases and diabetes in later life. This hypothesis was proven in humans in two independent populations (Li *et al.,* 2011; Pfab *et al.,* 2006) with different genetic backgrounds (Caucasians versus Asians) and different eating behaviors (Asian food versus a Western diet). The study carried out in Southern China (Li *et al.,* 2011), furthermore revealed for the first time that the low-birth-weight phenotype is not just low birth weight, but also disproportional intrauterine growth due to brain sparing. In line with our data is a recent study in an animal model of intrauterine growth retardation (IUGR) showing organ-specific effects on the expression of glucose transporters, facilitating better glucose transport to the growing brain, compared with other tissues.

In other words, this animal model showed an organ-specific alteration of insulin resistance as a potential mechanism of brain sparing (Sadiq *et al.,* 1999). Subsequent studies have demonstrated a strong correlation among fetal liver volume, gestational age, and fetal biometric parameters (abdominal circumference and others) (Dos Santos Rizzi *et al.,* 2010; Vintzileos *et al.,* 1985). Therefore, reduced abdominal circumference mainly reflects reduced liver size. An animal model of IUGR by uterine artery ligation was likewise characterized by low birth weight, lower liver weight, and lower liver glycogen storage (Bueno *et al.,* 2010). Tissue resistance to the effects of insulin is viewed as a fetal response by which in a situation of malnutrition, blood glucose concentrations are maintained, e.g., for the benefit of the brain, but at the expense of glucose transportation to the muscles and insulin-mediated growth. This response may cause higher plasma glucose levels even as early as the end of pregnancy in children with low birth weight, and may thus explain the present findings (Li *et al.,* 2011; Pfab *et al.,* 2006).

The typical event-causal for fetal programming is maternal under-nutrition during pregnancy. This was first recognized in epidemiological studies and later confirmed in animal experiments (Barker *et al.,* 1989; Ravelli *et al.,* 1998; Gluckman *et al.,* 2008; Vehaskari *et al.,* 2001). Meanwhile, several other mechanisms caused by environmental conditions in early life leading to lifelong functional and structural alterations have been described, among them glucocorticoid exposure of the fetus due to 11 beta-hydroxysteroid dehydrogenase 2 deficiency of the placenta (Aufdenblatten *et al.,* 2009; Seckl *et al.,* 2000) or even a high protein diet during pregnancy (Thone-Reineke *et al.,* 2006). Maternal cortisol secretion was shown to be inversely linked to fetal brain growth (Li *et al.,* 2012). This study might therefore explain the finding that maternal stress during pregnancy, as analyzed by classical stress tests, is linked inversely to the academic performance of the offspring (Li *et al.*, 2012).

3.1 Fetal growth and coronary heart disease

At the start of this century, the incidence of coronary heart disease rose steeply in Western countries to become the most common cause of death. In many of these countries, the steep rise has been followed by a fall over recent decades that cannot be accounted for by changes in adult

lifestyle. The incidence of coronary heart disease is now rising in other parts of the world to which Western influences are extending, including China, India, and Eastern Europe (Godfrey and Barker, 2001). An important clue suggesting that coronary heart disease might originate during fetal development came from studies of death rates among babies in Britain during the early 1900's (Barker, 1998).

The usual certified cause of death in newborn babies at that time was low birth weight. Death rates in newborns differed considerably between one part of the country and another, being highest in some of the northern industrial towns and the poorer rural areas in the north and west. This geographical pattern in death rates was shown to closely resemble today's large variations in death rates from coronary heart disease (Barker, 1998), variations that form one aspect of the continuing north± south divide in health in Britain. One possible conclusion suggested by this observation was that low rates of growth before birth are in some way linked to the development of coronary heart disease in adult life. Although it had been suggested that events in childhood influence the pathogenesis of coronary heart disease, a focus on intrauterine life offered a new point of departure for research.

More direct evidence that an adverse intra-uterine environment might have long-term consequences came from follow-up studies of men and women in middle and late life whose body measurements at birth had been recorded. A study of people born in Hertfordshire, UK, showed for the first time that those who had had low birth weights had increased death rates from coronary heart disease in adult life (Barker, 1998; Osmond *et al.,* 1993). Thus, among 15 726 people born during 1911 to 1930, death rates from coronary heart disease fell progressively with increasing birth weight in both men and women (Barker, 1998; Osmond *et al.,* 1993). A small rise at the highest birth weights in men could relate to the macrosomic infants of women with gestational diabetes. Another study, of 1,586 men born in Sheffield during 1907 to 1925, showed that it was particularly people who were small at birth as a result of growth retardation, rather than those born prematurely, who were at increased risk of the disease (Barker *et al.,* 1993).

Replication of the UK findings has led to wide acceptance that low rates of fetal growth are associated with coronary heart disease in later life. For example, confirmation of a link between low birth weight and adult coronary heart disease has come from studies of 1,200 men in Caerphilly, South Wales, and of 70 297 nurses in the United States (Rich-Edwards *et al*., 1976). The latter study found a two-fold fall in the relative risk of non-fatal coronary heart disease across the range of birthweight9. Similarly, among 517 men and women in Mysore, South India the prevalence of coronary heart disease in men and women aged 45 years or older fell from 15% in those who weighed 2.5 kg or less at birth, to 4% in those who weighed 3.2 kg or more (Stein *et al.,* 1996).

3.2 Body proportions at birth and cardiovascular disease

The Hertfordshire records and the Nurses and Caerphilly studies did not include measurements of body size at birth other than weight. The weight of a newborn baby without a measure of its length is a crude summary of its physique. The addition of birth length allows the derivation of ponderal index (birthweight/length3) as a measure of thinness but cannot adequately distinguish variations in fat and lean mass. With the addition of head circumference, the baby whose body and trunk are small in relation to its head, as a result of `brain-sparing', can also be distinguished. Thinness, shortness, and a small trunk are thought to reflect differing fetal adaptations to under-nutrition, hypoxia, and other influences and they have different long-term consequences.

In Sheffield, death rates from coronary heart disease were higher in men who had had a short crown-heel length at birth (Martyn *et al*., 1996). The mortality ratio for coronary heart disease in men who were 18.5 inches [47 cm] or less in length was 138 compared with 98 in the remainder (Martyn *et al*., 1996). A low ponderal index and thinness at birth were also associated with coronary heart disease (Martyn *et al*., 1996). While low birth weight was associated with raised death rates from coronary heart disease, there was a stronger association with thinness at birth, especially in men born at term (Forsen *et al.,* 1997). Men who were thin at birth, measured by a low ponderal index, had death rates that were twice those of men who had a high ponderal index.

In Finland raised death rates from coronary heart disease were associated with low placental weight. In Sheffield, however, coronary heart disease showed a U-shaped relation with the ratio of placental weight to birth weight, the highest mortality ratios being at either end of the distribution. The pattern of body proportions at birth which predicts death from coronary heart disease may be therefore summarized as a small head circumference, shortness, or thinness, which reflects retarded fetal growth, and either low placental weight or an altered ratio of placental weight to birth weight. The pattern for stroke, which has only been reported in Sheffield, is different. Whereas stroke was similarly associated with low birth weight it was not associated with thinness or shortness.

Instead, there were increased rates among men who had a low ratio of birth weight to head circumference, or a low ratio of placental weight to head circumference (Martyn *et al*., 1996). One interpretation of these associations is that normal head growth has been sustained at the cost of interrupted growth of the body in late gestation, in association with inadequate growth of the placenta.

4.0 FETAL PROGRAMMING AND EPIGENETICS

All cells in the body have identical genomes. However, each cell has one of many epigenomes, unique sets of epigenetic instructions for establishing and maintaining lineage-specific expression profiles (Jenuwein , 2006). The genome is programmed to express appropriate sets of genes in particular tissues at specific time points during an individual's life. Epigenetic events create a memory of cell identity that serves to maintain genomic functions such as maintenance of cell identity after differentiation, propagation of essential features of chromosomal architecture, and dosage compensation (Probst *et al*., 2009).

Unlike genetic information, which is extremely stable, epigenetic events are reversible and respond to endogenous and exogenous (environmental) signals (Gabory *et al*., 2011). There is convincing experimental evidence to suggest that epigenetic marks serve as a memory of exposure in early life to inappropriate environments and that these marks induce long-term changes in gene expression, potentially leading to disease in later life. This is known as the developmental origin of health and disease (DOHaD) hypothesis (Gluckman *et al.,* 2009; McAllister, 2009).

Thanks to significant advances in analytic technologies, epigenome characterization is becoming a key element in increasing numbers of investigations (Meissner *et al.,* 2008; Mikkelsen *et al.,* 2008; Mikkelsen *et al.,* 2007; Fouse *et al.,* 2008; Shen *et al.,* 2008; Pauler *et al.,* 2009). Recently published data challenge prevailing views about the dynamics, relevant positions, and functions of many epigenetic marks and their complex patterns of crosstalk. The reversibility of the chromatin modification states that determine gene expression status is essential for interaction between the environment and the dynamic epigenome.

However, some epigenetic marks that are laid down early in development under the influence of environmental factors must remain stable, acting as a memory of the event long after exposure has ceased. The basis of this paradox—the need for both reversibility and stability—remains unclear (Gabory *et al*., 2011).

4.1 DNA Methylation Dogmas

Cytosine methylation is the only epigenetic modification that directly affects the DNA molecule. It is required for correct embryonic development in mammals (Gabory *et al*., 2011). The DNA of most vertebrates is depleted in CpG dinucleotides, the main target of DNA methylation. Furthermore, the role of DNA methylation in genome regulation, other than in genomic imprinting and X inactivation, remains unclear (Gabory *et al*., 2011). CpG islands (CGIs) and promoters have been studied in detail because they are easily accessible in terms of the techniques available and sequence specificity. However, other sequences should be taken into consideration (Gabory *et al*., 2011).

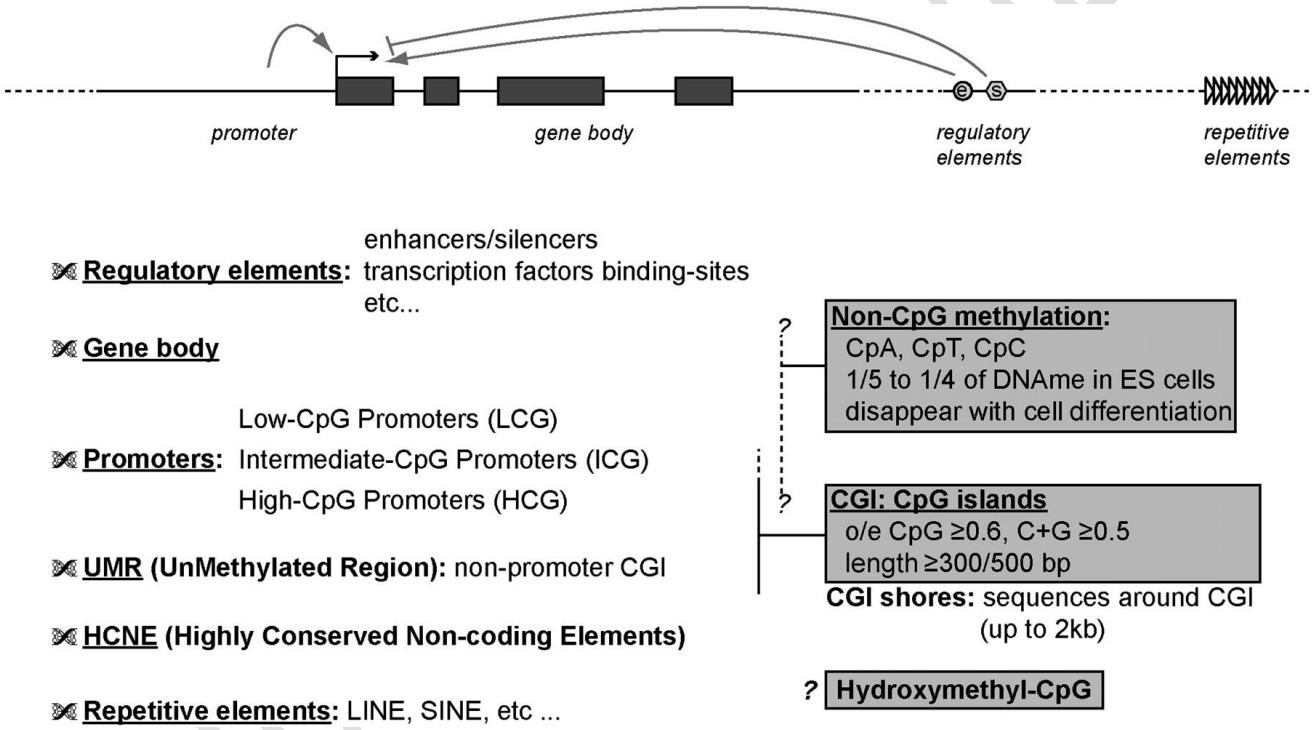

Figure 1: Target sequence for DNA methylation studies (Gabory *et al*., 2011)**.**

The majority of DNA methylation involves methylcytosine on CpG dinucleotides, but hydroxymethylcytosine and methylation on non-CpG sites were recently identified. Non-CpG methylation was reported in gene bodies, promoters, and repetitive elements; its expanse needs to be further investigated. CpG islands (CGIs) and gene promoters are preferred targets in many studies because they correspond to a tractable fraction of the genome with obvious regulatory

potential. CGIs are defined algorithmically as sequences with an observed-to-expected (o/e) ratio of CpG >0.6, content of guanine and cytosine >0.5, and, in most cases, length >500 base pairs (bp) (Gabory *et al*., 2011).

Three classes of promoters are defined according to their CpG content: low CpG, intermediate CpG, and high CpG. Low CpGs have the highest probability of being methylated, but methylation correlates poorly with transcription; high CpGs have a low probability of being methylated, but this correlates with gene expression. However, transcriptional regulation of genes also depends on distal regulatory elements such as enhancers, insulators, locus control regions, and silencing elements. In addition, recent studies have shown that gene bodies in active transcription sites are enriched in DNA methylation.

Moreover, nonpromoter CGI unmethylated regions (UMRs) were recently identified; initially unmethylated, they become methylated during development in a tissue-specific manner. CGI shore sequences were described around CGIs. (Gabory *et al*., 2011). Their methylation in normal tissues is highly conserved, tissue-specific, and strongly related to gene expression; and they were highly sensitive to DNA alterations in colon cancer as opposed to promoters or CGIs. Highly methylated repetitive elements and highly conserved noncoding elements can also be interesting targets for DNA methylation studies. DNAme, methylated DNA; ES, embryonic stem (Gabory *et al*., 2011).

Mammalian genomes are punctuated by CGIs, DNA sequences with an unusually high frequency of CpG sites (Illingworth and Bird, 2009). There is considerable evidence for a functional role of CGI-promoter methylation in transcription, but for many genes the correlation between CGI methylation and transcription status is poor. Recent studies defined CGI shores as sequences of ≤2 kb around CGI, with methylation that is highly conserved, tissue-specific, and strongly related to gene expression (Doi, 2009; Irizarry *et al.,* 2009).

Several large-scale methylation studies have called into question some of the prevailing views about the dynamics and function of DNA methylation (Weber *et al.,* 2007). Several large-scale methylation studies have called into question some of the prevailing views about the dynamics and function of DNA methylation. Weber *et al* investigated the function of DNA methylation in *cis*-regulatory

regions, and its impact on gene expression, by mapping DNA methylation throughout the genome with a methylated DNA immuno precipitation (MeDIP-chip) approach (Weber *et al.,* 2007). They defined 3 classes of promoters in terms of CpG frequency (low CpG, intermediate CpG, and high CpG) and showed that *1*) the methylation of CpG-poor promoters does not prevent gene expression; *2*) DNA methylation is not a general mechanism of gene repression, as most CGI promoters remain unmethylated even when inactive; and *3*) DNA methylation is principally involved in regulating key developmental genes.

Thus, promoter CpG density and gene function are the main predictors of the promoter methylation state. Shen and Waterlande (2007) reported that a subset of CGIs within the promoters of key developmental genes is subject to tissue-specific methylation during development. Such methylation had previously been reported only for imprinted and X-inactivated genes. This observation suggests the existence of a programmed mechanism of DNA methylation. Unmethylated regions (UMRs), recently identified as nonpromoter CGIs, become methylated during development in a tissue-specific manner, potentially modifying gene expression (Straussman *et al.,* 2009). Thus, the methylation of other regulatory elements may also be important for transcriptional regulation. Moreover, as first observed for the active X chromosome, gene-body methylation may be a hallmark of active genes in the whole genome (Hellman & Chase, 2007; Ball *et al.,* 2009; Illingworth and Bird, 2009).

4.2 How Early Nutrition sculpts our epigenomes

Throughout evolution, organisms have been faced with the challenge of sensing changes in their environment, such as food depletion and stress, and adapting to them to ensure their survival. These responses implicitly involve various mechanisms, such as chromatin targeting, for adapting the expression of fundamental genes and ensuring genome integrity. Environmental factors such as diet, nutrients, drugs, and the social environment can be linked to chromatin structure in several ways (Gabory *et al.,* 2011)**.**

4.2.1 Mechanistic pathways for environmental factors involved in epigenetic reprogramming

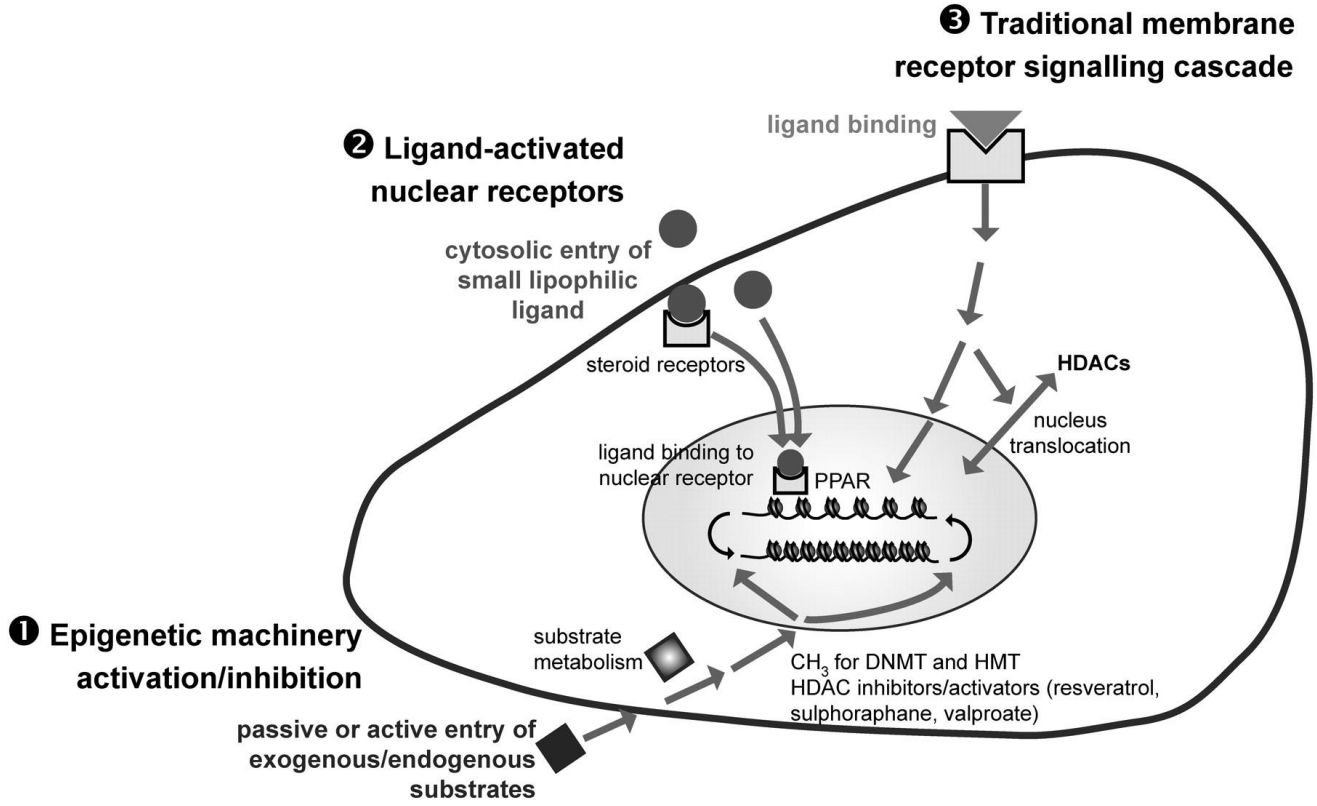

Figure 2: Mechanistic pathways for environmental factors involved in epigenetic reprogramming (Gabory *et al.,* 2011).

There are 3 ways to link environmental factors (e.g., nutrients and drugs) from the cell membrane to the chromatin structure:

1. Some environmental factors (e.g., aging and sex) may target chromatin, modifying enzymes or their substrate availability. After passive or active entry through the cell membrane is achieved, exogenous or endogenous substrates [e.g., methyl donors such as folates, and histone acetyl deacetylase (HDAC) inhibitors such as trichostatin A] undergo cell-specific metabolism. Thus, endogenous, or exogenous compounds may lead to the alteration of a critical balance of chromatin remodeling enzymes at the whole-genome level or to specific regions targeted by specific enzymes (e.g., HDACs).
2. Some other compounds (e.g., endocrine disruptors) that specifically bind to nuclear receptors (e.g., steroid receptors) may be present in the cytoplasm, bind to their ligand,

undergo several modifications, and subsequently be translocated to the nucleus where they bind to their responsive elements. The binding of other nuclear receptors [e.g., PPARs (peroxisome proliferator–activated receptors) and RXR (retinoid X receptor)] with their natural polyunsaturated fatty acid ligands or drugs (e.g., fibrates) leads to the recruitment of coactivators and chromatin remodeling factors. Appropriate modifications of the epigenetic marks at PPAR/RXR responsive elements in target gene promoters modulate the expression of a particular set of genes in a tissue-specific manner depending on the presence of appropriate cofactors (Gabory *et al.,* 2011).

3. Traditional membrane receptor-signaling cascades may be involved. It is possible, depending on the type of ligand, that different pathways can be used. Maintenance of epigenetic patterns depends on preserving the balance of factors such as DNA methyltransferases (DNMTs), histone acetyltransferases or HDACs, and histone methyltransferases (HMTs) or histone demethylases and on the translocation of these enzymes into the nucleus. Extra- or intracellular signaling pathways may trigger activation of one of these factors, resulting in loci-specific modifications (Gabory *et al.,* 2011).

4.3 Environmental conditions before conception and implantation

Preimplantation development in mammals has been shown to be sensitive to environmental conditions, both in vivo and in vitro, that can modify blastocyst potential and lead to long-term changes in fetal and postnatal health and physiology. Similarly, the environment inhabited by a breeding female before conception and early in pregnancy has striking effects on oocytes developing in the ovarian follicle and on embryos in the early stages of development in the reproductive tract. Environmental conditions at these stages may also alter behavior, cardiovascular function, and reproductive function throughout postnatal life (Thompson *et al.,* 1995; Sinclair *et al.,* 2007; Ecker *et al.,* 2004; Fernandez-Gonzalez, 2004).

Low maternal protein consumption or vitamin B and methionine status can lead to behavioral and cardiovascular abnormalities in offspring, sex-specific changes in hepatic gene expression in rat fetuses, and changes in imprinted gene expression in the rat embryo-fetal axis (Ashworth et al., 2009; Rook *et al.,* 2007; Watkins *et al.,* 2007).

It was recently shown that in vitro culture conditions, as used in assisted reproduction technology, may affect the global patterns of DNA methylation and gene expression (Katari *et al.,* 2009). Gametes or early embryos from couples undergoing treatment for infertility may therefore display epigenetic modifications. An association was observed between in vitro conception and changes in DNA methylation, potentially affecting the long-term pattern of expression of genes involved in chronic metabolic disorders such as obesity and T2D. Thus, identifying the specific features and functions of the epigenetic buildup at these stages and determining the mechanisms by which environmental factors may affect them in the long term will be a major milestone in the domain of DOHaD investigation (Corry *et al.,* 2009; Aranda *et al.,* 2009).

4.4 Sexual dimorphism of gene expression and epigenetics

The vast majority of common diseases, including atherosclerosis, diabetes, osteoporosis, asthma, and neuropsychological and autoimmune diseases, which often take root in early development, display a sex bias. Moreover, the risk of developing a complex disease in offspring often depends on the sex of the affected parent. The relevance of epigenetic mechanisms underlying the physiologic differences between sexes, particularly in drug metabolism, fits well into the epigenetic theory of complex disease (Gabory *et al.,* 2011).

This bias may be explained by the role of sex chromosomes, the different regulatory pathways that underlie the sexual development of most organs, and the lifelong fluctuating impact of sex hormones. Many tissues exhibit sexual dimorphism for a substantial proportion of the genes they express (Gabory *et al*., 2009; Yang *et al.,* 2006).

In fact, sex-specific expression appears to be under the control of sex-specific epigenetic marks. Environmental factors such as social behavior, nutrition, or chemical compounds can influence these flexible epigenetic marks in a sex-related manner during particular temporal windows of life. For example, modifications of histone H3 are sexually dimorphic in the developing mouse brain, and patterns of acetylation, but not methylation, are masculinized in females by testosterone in utero

(Tsai *et al.,* 2009). Studies have provided many examples of sex differences in the effects of prenatal and early postnatal life exposures on the risks of subsequent metabolic dysfunction (Gabory *et al*., 2009; Flanagan *et al.,* 2000; Sugden and Holness, 2002; Wilcoxon and Redei, 2004).

5.0 CONCLUSIONS AND RECOMMENDATIONS

Fetal programming postulates that important physiological parameters can be changed by environmental factors like maternal stress and maternal nutrition during embryonic and fetal life. These changes can prolong into adulthood and affect the following generations to produce a trans-generational genetic disorder. Investigators are still accumulating proofs of concept for fetal programming, which is based on the demonstration that a developmental insult (eg, diet, drugs, lifestyle, or social behavior) can lead to long-term consequences (eg, metabolic syndrome or psychiatric diseases).

Further studies need to identify the factors that set the trajectory of fetal growth and the influences that limit the maternoplacental delivery of nutrients and oxygen to the fetus. We also need to define how the fetus adapts to a limited nutrient supply, how these adaptations programme the structure and physiology of the body, and by what molecular mechanisms nutrients and hormones alter gene expression. A woman's own fetal growth, and her diet and body composition before and during pregnancy play a major role in programming the future health of her children, mothers will want to know what they can do to optimize the intra-uterine environment they provide for their babies. Research is also required to identify the barriers to healthy eating among young women, whose diets are important both for their own health and the health of the next generation. Such an approach may allow us to reduce the prevalence of major chronic diseases and diminish social inequalities in health.

Declarations:

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Institutional Review Board Statement: Not applicable. This manuscript didn't involve humans or animals studies.

Informed Consent Statement: Not applicable. This manuscript didn't involve involving humans.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

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