Epigenetic programming of obesity and diabetes by in utero exposure to gestational diabetes mellitus

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It is now well accepted that offspring exposed to maternal undernutrition, obesity, or gestational diabetes mellitus have an increased risk for chronic diseases later in life, supporting the theory of the early origins of chronic diseases. However, the molecular mechanisms through which the exposure to an altered in utero environment translates into the development of chronic diseases are not yet well understood. Recently reported promising results help to resolve this issue. They suggest that epigenetic modifications are a potential mechanism for fetal metabolic programming. This review provides an overview of the relationship between the exposure to an altered intrauterine environment and fetal metabolic programming, focusing on gestational diabetes mellitus and epigenetic variations at adipokine candidate genes.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is a carbohydrate intolerance first diagnosed during pregnancy and characterized by an imbalance between insulin resistance and beta-cell dysfunction, leading to maternal hyperglycemia.\(^1\) Its prevalence ranges from 1% to 14%, depending on the population studied and the diagnostic criteria used.\(^2\)

In the 1970s, Dörner and Plagemann\(^3\) were among the first to provide evidence that exposure to a hyperglycemic intrauterine environment increases the risk of obesity and diabetes in offspring.\(^3\) These findings were then supported by a large number of epidemiological studies showing that children exposed to GDM have an increased risk of developing obesity, type 2 diabetes mellitus (T2DM), and the metabolic syndrome, among other chronic diseases, later in life.\(^4\)–\(^10\) The risk of developing chronic diseases after exposure to altered in utero conditions led to the proposition of the fetal metabolic programming hypothesis (Barker's hypothesis).\(^11\) Several hypotheses were proposed to explain the association between GDM and the increased risk of obesity and diabetes in offspring. Catalano and Hauguel-De Mouzon\(^12\) suggested that maternal hyperglycemia gives rise to increased glucose transfer to the fetus, thereby increasing fetal insulin secretion. This, in turn, leads to the stimulation of fetal growth and macrosomia, which has been associated with later obesity.\(^13,14\) Dörner and Plagemann\(^3\) proposed that fetal and/or neonatal hyperinsulinism occurring during a critical period of brain development leads to permanent malorganization of the hypothalamic regulation centers for metabolism and, hence, to malprogramming of the energy regulation systems. Despite these hypotheses, however, the molecular mechanisms through which the intrauterine exposure to hyperglycemia would translate into the development of obesity and diabetes are still not well understood. Nevertheless, recently published studies provide supporting evidence that epigenetic modifications comprise one of the potential mechanisms involved. Indeed, accumulating evidence suggests that both fetal hyperinsulinism and/or maternal hyperglycemia during early developmental stages epigenetically program a predisposition for the development of obesity and T2DM later in life.\(^15\)

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This review outlines the relationships among exposure to GDM (hyperglycemic in utero environment), epigenetic modifications, and fetal metabolic programming. Although data in humans are still limited, studies carried out at the molecular level to support the involvement of epigenetics in newborn metabolic programming are discussed. An overview of research showing the potential importance of epigenetic adaptations of adipokine genes as a result of GDM exposure is also presented.

**EPIGENETIC MODIFICATIONS AND THEIR CONTRIBUTION TO THE ETIOLOGY OF OBESITY AND T2DM**

Although the genetic code is shared by most cells of an organism, each individual cell type possesses its own gene-expression pattern that defines its biological fate. The cellular epigenetic profile is deeply involved in establishing the tissue-specific gene-expression profile, which is a normal and essential process in cell development and differentiation. Epigenetics refers to the heritable regulation of gene transcription (and, ultimately, protein synthesis) independent of the DNA sequence. Epigenetic marks are mitotically stable but can also be subject to reprogramming in response to both stochastic and environmental stimuli such as changes in diet, physical activity, in utero environment, and pharmacological treatment.

Nevertheless, preliminary results suggest that only a fraction of the epigome shows plasticity and that a large number of epigenetic marks are relatively stable over time.

The main types of epigenetic processes are DNA methylation, histone modification, and microRNA regulation. DNA methylation is the most stable and best understood epigenetic system and involves the addition of a methyl group on the fifth carbon of the cytosines within cytosine guanine dinucleotides (CpGs). CpGs are usually found in DNA elements called CpG islands located within the regulatory regions of about 50–60% of the transcribed genes. In most instances, highly methylated DNA regions (especially promoter regions) act to reduce gene expression. Since epigenetic marks have the potential to modulate gene expression, which is a major determinant in many diseases, these marks have been suggested as a possible mechanism through which the exposure to a detrimental in utero environment translates into the development of diseases, such as obesity and T2DM, among offspring.

Although data from humans are still limited, a number of studies have provided insights into the potential epigenetic mechanisms involved in the developmental programming of obesity and T2DM. Three candidate-gene-approach studies, conducted by the same group, have reported very significant results. The group of Heijmans et al. showed that individuals who were prenatally exposed to famine during the Dutch Hunger Winter in 1944–1945 exhibited, six decades later, a lower DNA methylation of the imprinted IGF2 gene locus compared with their unexposed, same-sex siblings. The association was specific, with only periconceptional exposure having an effect on DNA methylation of the gene. These results reinforce the hypothesis that environmental conditions in early life can cause epigenetic changes in humans that will last throughout their lives. The authors further characterized DNA methylation at 15 genomic loci harboring genes related to growth, development, and energy metabolism. Of these genes, six demonstrated DNA methylation differences in individuals at adulthood according to prenatal exposure to famine. DNA methylation of the INSIGF gene was lower among individuals who were periconceptionally exposed to famine compared with their unexposed same-sex siblings, whereas methylation of the IL10, LEP, ABCA1, GNASAS, and MEG3 genes was higher. The same research group investigated whether infants born small for gestational age (reflecting prenatal growth restriction) and those born appropriate for gestational age exhibited different patterns of DNA methylation at the IGF2, GNASAS, INSIGF, and LEP gene loci. However, DNA methylation levels of these genes were not significantly different between individuals who were small versus appropriate for gestational age. Another recent candidate-gene study reported an association between high birth weight and an increased placental methylation of the glucocorticoid receptor gene, which is a well-known candidate gene for obesity.

To identify genes and metabolic pathways showing epigenetic dysregulation that may be associated with short-term (i.e., at birth) or long-term (i.e., childhood or adulthood) outcomes, recent studies have surveyed the human genome using epigenome-wide approaches. Yuen et al. examined the DNA methylation at 1,505 CpG sites within 807 genes in 13 normal placentas (fetal side). While most loci showed a continuous pattern of DNA methylation levels, the WNT2, TUSC3, and EPHB4 genes were shown to have a polymorphic “on-or-off” pattern at their promoter region. The lead findings were further investigated in a larger population sample, where the TUSC3 DNA methylation was found to be correlated with late-onset preeclampsia, a condition that can have lasting consequences on the offspring’s health. Others found loci where cord blood DNA methylation showed nominally significant (P < 0.05) associations with birth weight and phenotypes of body composition in 9-year-old children.

In summary, the findings from the few studies conducted in humans suggest that DNA methylation is associated with phenotypic variations. These results likely...
reflect a response to the fetal environment, which may have lasting effects on an individual’s health throughout life. Further, it seems that genes associated with growth and energy metabolism will be preferentially affected by fetal programming. However, more research is needed to better define the contribution of epigenetics to the metabolic programming hypothesis.

ASSOCIATION OF GDM EXPOSURE WITH EPIGENOMIC PROFILE ADAPTATIONS IN NEWBORNS

A few years ago, a research program was undertaken by Dr. L. Bouchard and colleagues, which was aimed at examining whether the in utero exposure to GDM alters the DNA methylation profile in newborns. Pregnant women were monitored from the first trimester of pregnancy to delivery. GDM was diagnosed following a 75-g oral glucose tolerance test (OGTT) between weeks 24 and 28 of gestation (2 h post-OGTT glucose ≥7.8 mmol/L). The program’s longitudinal follow-up of women allowed metabolic changes occurring throughout pregnancy (potential confounding factors) to be taken into account and permitted the correct identification of women who developed gestational hyperglycemia, thus avoiding misclassification of those who may have had undiagnosed diabetes.

To investigate the impact of GDM on the epigenetic profile of newborns, the leptin and adiponectin genes (LEP and ADIPOQ, respectively) were selected first. These adipokines (literally, cytokines secreted by the adipocytes) are well-known obesity and T2DM candidate genes, as they are involved in energy metabolism and regulation of insulin sensitivity.34 Bouchard et al.35,36 examined LEP and ADIPOQ DNA methylation levels in placenta and cord blood samples exposed or not exposed to GDM; the results showed that the placental DNA methylation profiles of these two adipokines were dysregulated by exposure to maternal hyperglycemia. On the fetal side of the placental tissue, DNA methylation levels of LEP and ADIPOQ were found to decrease with increased blood glucose levels (2 h post-OGTT glucose), suggesting that maternal hyperglycemia (and likely the associated fetal hyperinsulinemia) had similar effects on both genes.35,36 However, in samples taken on the maternal side of the placental tissue, DNA methylation of LEP increased with post-OGTT hyperglycemia, whereas the DNA methylation of ADIPOQ decreased with increased insulin resistance (as assessed by the Homeostasis Model of Assessment – Insulin Resistance). The observations were apparently counterintuitive, since insulin is known to upregulate leptin transcription and downregulate adiponectin gene expression.37,38 However, it was hypothesized that epigenetic modifications at LEP and ADIPOQ may have been induced to partially counterbalance the effects of maternal hyperinsulinemia on the regulation of these genes’ expression.

Leptin is suspected to affect GDM pathophysiology and has been reported to play an important role in the fetal programming of obesity.39 Increased levels of maternal circulating leptin in early pregnancy are a predictor for GDM, independent of maternal prepregnancy adiposity.40 This increase has been attributed, in part, to an enhanced synthetic capacity of the placenta41 and visceral adipose tissue,42 suggesting that the risk of GDM is associated with similar phenomena (perhaps an epigenetic phenomenon) in these two tissues. At term, the placental expression of LEP and the leptin receptor gene (LEPR) has been reported to be higher in diabetic than in non-diabetic pregnancies.41 Interestingly, Bouchard et al.36 found that placental LEP expression was correlated with the gene’s DNA methylation levels as well as with circulating leptin levels in hyperglycemic women, suggesting that placental epigenetic marks at LEP are functional. Since DNA methylation is mitotically fairly stable,19 the observed LEP DNA methylation adaptations to GDM, as well as the parallel transcriptomic responses, may have profound long-term phenotypic effects and could help explain why newborns exposed to a detrimental fetal environment such as GDM have an increased risk of developing obesity and T2DM later in life. This long-term effect has been suggested for LEP in a previous study,24 arguing that epigenetic marks at birth could have long-term functional impacts on energy metabolism and insulin sensitivity, thus triggering long-term susceptibility to obesity and T2DM in offspring. Of note, it has been shown that the postnatal leptin treatment of the offspring of rats programmed to develop obesity conferred protection against obesity.43 The above data,36 along with those of previous studies, support the important role of leptin in the fetal programming of obesity,38 which may be partially explained by LEP epigenetic adaptations to an altered in utero environment.

Although the potential role of adiponectin in fetal programming is less clear than that of leptin, recent results suggest that epigenetic variations around ADIPOQ could also be one of the mechanisms involved in the epigenetic programming of metabolic perturbations in the offspring women with GDM.35 Adiponectin is produced exclusively and abundantly by adipose tissue and has putative insulin-sensitizing, anti-inflammatory and antiatherosclerotic properties.44 Unlike circulating concentrations of leptin, those of adiponectin are inversely associated with measures of overall adiposity and with insulin resistance, and adiponectin levels are low in patients with obesity and T2DM.44 In normal pregnancies, concentrations of circulating adiponectin increase in the first half of the pregnancy and then decrease in
proportion to weight gain and pregnancy-induced insulin resistance. Adiponectin levels are lower in pregnant women affected by GDM. Interestingly, a correlation was found between adiponectin levels throughout pregnancy and DNA methylation in both the fetal and maternal sides of the placenta. Given that the placenta itself does not express adiponectin (when investigated at or near term), the results of Bouchard et al. suggest that changes in placental DNA methylation on the maternal side are surrogate measures of the changes taking place in the maternal adipose tissue. However, it cannot be excluded that the adiponectin gene may have been expressed at some point in the placenta during the course of pregnancy. Studies in adipose tissue would clearly be helpful to better understand this phenomenon.

Recently, 14 candidate genes for metabolic programming were analyzed in 251 cord blood and placenta samples exposed and not exposed to GDM. The maternally imprinted MEST gene and the nonimprinted glucocorticoid receptor NR3C1 gene showed significantly decreased methylation levels in GDM samples compared with controls in both types of tissues analyzed. Furthermore, additional analyses showed that adults with morbid obesity had decreased blood MEST methylation compared with normal-weight controls. These findings suggest that epigenetic malprogramming of MEST, which is essential for regulation of fetal and placental growth, somatic differentiation, and neurobiological and behavioral functions, may contribute to the development of obesity.

**EPIGENETIC CHANGES ASSOCIATED WITH MARKERS OF FETAL GROWTH AND DEVELOPMENT**

To further investigate the impacts of epigenetic programming in obesity, St. Pierre et al. selected the insulin-like growth factor 2 (IGF2) and H19 genes, located within a cluster of imprinted genes. Genomic imprinting is an epigenetic phenomenon by which certain genes are expressed in a parent-of-origin manner and is known to impact genes important in placental and fetal growth and development. The IGF2 is paternally expressed in humans and is known to play a key role in fetal growth and development. IGF2 overexpression results in overgrowth symptoms and is associated with the Beckwith-Wiedemann syndrome, a condition involving prenatal overgrowth and enlarged placenta. H19 is maternally expressed and has growth-inhibitory effects, thus providing an example of direct antagonism between paternally expressed and maternally expressed genes. Importantly, the regulation of gene expression of each of the two copies according to their parental origin is under the control of DNA methylation at IGF2/H19 differentially methylated regions. Epigenetic mechanisms implicated in fetal programming likely include both DNA methylation and genomic imprinting phenomena.

St. Pierre et al. tested whether placental DNA methylation levels at the IGF2/H19 locus were associated with markers of fetal growth and development in the newborn and whether the exposure to GDM affected this association in a normal pediatric population. Although DNA methylation levels at IGF2/H19 were not affected by GDM, they were associated with fetal development indices and birth weight, as well as with neonatal circulating levels of IGF2. The findings suggest that epigenetic marks at the IGF2/H19 locus are functional and may act as a modulator of the IGF2-driven fetal growth of newborns in a normal pediatric population.

Taken together, the data summarized here provide supportive evidence for epigenetic adaptations in genes involved in fetal growth, energy, and glucose metabolism and support the concept of fetal metabolic programming of obesity and its metabolic complications. In addition, they clearly suggest that other genes may be responsive to an altered in utero environment (e.g., GDM) and associated with birth weight.

**FUTURE AVENUES AND CHALLENGES**

Future investigations exploring the impact of the fetal environment on the epigenetic programming of obesity and its metabolic perturbations will face a number of challenges but, most importantly, many exciting opportunities as well. In addition to candidate-gene studies, epigenome-wide studies are awaited, which might identify highly relevant new loci involved in the development of obesity-related perturbations among the offspring of mothers with GDM. Such genes and pathways would, in turn, be targeted for the identification of new biomarkers for the development of preventive and therapeutic strategies applicable before and during pregnancy and in the postpartum period, as well as for the long-term follow-up of children.

Moreover, although accumulating evidence now strongly supports an association between a detrimental in utero environment and epigenetic modifications in newborns, further research is needed to increase confidence in those results. One strategy is to expand epigenetic analyses to other “-omic” sciences. Epigenetic, transcriptomic, and proteomic results, when converging, provide strong arguments to support the findings and to increase confidence that the observed associations reflect true biological mechanisms. Translational approaches using animal models are also important to support findings observed in humans.

Further research is also needed to examine whether epigenetic marks observed at birth have an impact on
the long-term health of the offspring. Large longitudinal cohorts, with repeated (from birth to childhood/adolescence) measurements of phenotypes, genotypes, and epigenotypes, would be fundamental to validate the concept that epigenetics is involved in fetal metabolic programming. If such studies were to confirm the relationships between detrimental in utero environments, epigenetic modifications, and long-term development of metabolic perturbations, additional research would be needed to explore whether lifestyle interventions during pregnancy, as well as earlier screening and treatment for pregnancy complications, may restore or induce a more favorable DNA methylation profile in at-risk newborns. Animal models have provided strong evidence that the maternal diet alters the offspring’s body composition and epigenetic profile in genes involved in metabolic control. In humans, prenatal lifestyle intervention studies that include recommendations about nutrition and/or physical activity appear to be effective in preventing unfavorable pregnancy outcomes, such as excessive gestational weight gain or GDM. However, none of them examined whether these positive effects were accompanied by changes in DNA methylation. Only one known retrospective study explored the effects of maternal lifestyle on methylation status and offspring outcomes. Godfrey et al. measured the DNA methylation status of 68 CpGs from five candidate genes in umbilical cord tissue from healthy neonates and related methylation status to maternal pregnancy diet and child’s adiposity at 9 years of age. The authors reported that the DNA methylation of the RXRA and eNOS genes had independent associations with sex-adjusted childhood fat mass. Additionally, a higher DNA methylation of the RXRA gene was associated with higher neonatal adiposity and lower maternal carbohydrate intake in early pregnancy. This study is the first to suggest that maternal nutritional habits may influence epigenetic factors and infant outcomes; it thus opens the path to future lifestyle intervention studies.

Finally, as shown in the Hunger Winter Families Study, the effects of an altered in utero environment on epigenetic modifications are likely time-specific. In this respect, a time-specific effect of prenatal exercise has been observed on fetal growth. It has been reported that beginning an exercise program in early pregnancy (i.e., first trimester) has a stimulatory effect on placental growth, which may increase infant birth weight. Although this may be a beneficial adaptation in lean and physically active women, it may have a less desirable effect in promoting excess fetal growth in overweight/obese women. It has never been assessed, but it may be speculated that such time-specific effects on fetal growth are supported by molecular adaptations occurring in different cell types, such as cord blood, placenta, or other fetal tissues (adipose tissue, skeletal muscle).

One of the most challenging issues in human epidemiological epigenetic studies is obtaining access to informative tissues. Currently, most studies rely on placental biopsies and cord blood samples, two cell types that are somewhat readily accessible and that also provide important and complementary information. On the one hand, the placenta plays a critical role in regulating fetal growth and development since it is a key regulator of fetomaternal nutrient exchange. Adverse in utero conditions, such as GDM, have been associated with alterations in placental anatomy and physiology, inducing perturbations in placental nutrient supply and, consequently, fetal growth and development. Despite its key role in fetal development and its fetal origin, the placenta remains an extraembryonic tissue. On the other hand, cord blood cells are of fetal origin, and, similar to adult white blood cells, they are involved in the regulation of inflammation (a central feature in obesity and T2DM) and are directly in contact with circulating stimuli such as toxins, macronutrients, and hormones. Interestingly, some of these biomolecules induce DNA methylation modifications. Nevertheless, neither the placenta nor cord blood captures all the tissue-specific epigenetic adaptations related to a detrimental fetal environment. This is especially evident for perturbations occurring during late gestation, when epigenetic adaptations are more likely to be tissue-specific. However, it is reasonable to postulate that perturbations during the periconceptional period (and early in cell differentiation processes) would be more likely to affect most cell types and tissues. Therefore, some epigenetic marks observed in the placenta and cord blood could be expected to reflect biological phenomena occurring in other tissues involved in growth, energy, and glucose metabolism, such as adipose tissue, the pancreas, skeletal muscle, the hypothalamus, etc. However, how the DNA methylation profile correlates between cell types and tissues remains mostly unknown, although it can be hypothesized that the correlation level will be loci-specific. Nevertheless, for obvious reasons, the only accessible tissues for large epigenetic studies in newborns and children are placenta, (cord) blood, and epithelium (buccal swabs). Although these tissues are not completely satisfactory, they are and will remain the only ones accessible in large enough numbers to conduct epidemiological epigenetic studies in the very important field of research on human fetal metabolic programming. Nevertheless, it should be feasible to investigate less accessible tissues, such as the adipose tissue, to verify, in at least a subsample, specific hypothesis derived from epidemiological epigenetics studies.
CONCLUSION

Strong evidence demonstrates that offspring exposed to a detrimental fetal environment related to the maternal nutritional status are programmed to develop a number of chronic diseases such as obesity and diabetes, perpetuating the vicious circle of obesity and diabetes across generations. Currently, only a few studies have been carried out in humans at the molecular level to support the contribution of epigenetics in fetal metabolic programming. Placentas exposed to maternal GDM have been shown to exhibit modifications in DNA methylation at the leptin and adiponectin genes. These epigenetic marks are likely functional and are expected to have long-lasting effects on the regulation of energy metabolism among offspring if they indeed reflect DNA methylation in other tissues and are thus able to trigger the development of chronic metabolic diseases such as obesity. The results presented here emphasize the need to develop preconceptional and early pregnancy healthcare programs aimed at preventing GDM and, eventually, the associated epigenetic modifications. However, to support such developments, interventional and longitudinal studies are needed to confirm the role of epigenetics in fetal metabolic programming induced by GDM.

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Declaration of interest. The authors have no relevant interests to declare.

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