Maternal Nutrition and Epigenetics in Early Life

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Abstract There are increasing evidences that prenatal events influencing *in utero* milieu and fetal development are associated with risk of developing chronic adult conditions, such as obesity, diabetes, and cardiovascular diseases. Epigenetics is one of the potential molecular mechanisms explaining this phenomenon, often called “fetal metabolic programming.” Epigenetics is defined as heritable regulation of DNA transcription that is independent of the DNA sequence and include DNA methylation. In this article, we review recent human studies that investigated associations between epigenetic

marks in the offspring and maternal nutritional status, from extreme caloric restriction and nutrients deficiency during pregnancy to maternal obesity and gestational diabetes. Despite human studies being scarce, we are confident that this emerging field will increase our understanding of the biological mechanisms involved in developmental origins of health and adult diseases and inform interventions aimed at primordial prevention of chronic metabolic diseases in future generations.

Keywords Fetal programming · Metabolic diseases · Developmental origins of adult diseases · Epigenetics · DNA methylation · Maternal nutrition · Caloric restriction · Famine · Under-nutrition · Micronutrient deficiency · B12 · Folate · Choline · Macronutrient · obesity · Gestational weight gain · Gestational diabetes mellitus · Maternal hyperglycemia · Birth weight · In utero development

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Introduction

Many epidemiological observations have raised awareness about the importance of early life determinants of adult-onset chronic diseases. Prospective studies of famine survivors, for example, have shown that undernutrition preconception [1] and during critical periods of early growth and development [2–4] is associated with increased risk of metabolic and cardiovascular disease later in life, and in some instances these effects appear to be transgenerational and linked to the sex of the exposed grandparent [5, 6]. Conversely, offspring of diabetic mothers, who by virtue of the hyperglycemic intrauterine environment to which they are exposed are overnourished during early growth and development, are at elevated risk of obesity and type 2 diabetes later in life [7, 8]. Intrauterine life includes critical periods that are suspected to contribute to long-term programming of glycemic/lipids metabolism or energy balance regulation and are now believed to

play an important role in determining lifetime susceptibility to obesity, type 2 diabetes, and cardiovascular disorders [9, 10]. In the emerging field of developmental origins of adult health and disease, this phenomenon often is called “fetal metabolic programming”.

Epigenetics: Potential Mechanisms of Fetal Metabolic Programming

Many investigators have now suggested that epigenetics is part of the molecular mechanisms involved in fetal metabolic programming [11]. The term “epigenetics” refers to the potentially heritable regulation of DNA transcription that is independent of the DNA sequence. Common, measurable epigenetic modifications include DNA methylation, histone modifications, variation in small non-coding RNAs (microRNAs), and chromatin structure remodelling. The epigenetic regulation of cellular function is a normal and essential process in cell development and differentiation [12, 13]. DNA methylation is one of the most studied and better-understood epigenetic mechanisms. Methylation is more likely to occur at CpG dinucleotides, which are more common in promoter regions. In the majority of cases, high levels of methylation at promoter regions prevent the transcription factors binding and so is associated with lower genes expression (Fig. 1), but there are exceptions to this rule. Emerging data suggest that methylation is important for expression regulation of key developmental genes, especially for genes with promoter rich in CpG sites [14].

Most epigenetic marks are mitotically stable and enduring, conveying long-term, predictable effects on gene expression, whereas other epigenetic marks can be modulated by stochastic environmental stimuli [13]. For example, some epigenetic marks are sensitive to changes in the *in utero* environment [12], whilst overall, the epigenome is relatively stable, showing only marginal changes over decades in adulthood [15–17]. Specifically, it has been suggested that epigenetic programming during fetal development, with may induce somatic-wide effects, may have more enduring effects than age-related changes that are predominantly tissue-specific [15, 18, 19•, 20].

Results from animal models clearly show that epigenetics is part of the fetal programming induced by *in utero* milieu affected by maternal metabolism, diet, and other lifestyle factors [21], with a smaller body of literature describing human studies on this topic. In this review, we will discuss the most recent and relevant literature on fetal epigenetic adaptations related to maternal nutritional status in humans (Table 1). Maternal diet is an important determinant of fetal development, directly or indirectly (via maternal obesity status and/or metabolic impairment). This review will focus on both

under- and overnutrition *in utero* and its effects on fetal development and metabolic consequences over life.

Maternal Undernutrition

Severe Caloric Restriction: Dutch Winter Hunger Example

The classic examples of the impact of undernutrition during fetal development have come from “natural” experiments related to adverse events in human history, such as the Dutch Winter Hunger at the end of the Second World War, where average daily rations are estimated to have been 667 kcal (estimated macronutrients distribution: 12% proteins, 19% fat, and 69% carbohydrates). To investigate the effect of *in utero* exposure to caloric restriction on epigenetic variation, blood samples were collected in 58-year-old individuals who were exposed to food deprivation during gestation and DNA methylation was measured in circulating blood cells (from whole blood samples) and were compared to their same-sex sibling who was not exposed to famine during gestation [18]. They found that periconceptual (or early gestational) exposure to famine was associated with hypomethylation of the *IGF2/H19* loci [18]. In a subsequent report, it also was suggested that both famine exposure and *in cis* genetic variants (per allele) have similar magnitude additive effects on DNA methylation levels at the *IGF2/H19* loci [22]. *IGF2* is a key factor in human growth and development and is maternally imprinted (i.e., the paternal allele is expressed), whereas *H19* is transcribed into a noncoding RNA of still unclear function and is paternally imprinted. Genetic variants in the *IGF2* genomic region have been associated with risk of early-onset obesity in childhood [23] and with an adverse cardiometabolic profile in adult life [24].

In addition to *IFG2*, 15 candidate genes involved in weight regulation or metabolism were investigated in the same cohort: exposure to famine during the periconceptual period was associated with differences in methylation levels at *IL10*, *LEP*, *GNASAS*, *INSIGF*, and *ABCA1* [19•]. It appears that the regulation of methylation may be very sensitive to when in pregnancy nutritional deficit occurs, because exposure to famine late in gestation was not associated with differences in methylation levels except at *GNASAS* (and possibly at *LEP* in men only) [19•]. It is noteworthy that the magnitude of the difference in DNA methylation levels related to caloric restriction during pregnancy were still observable six decades later, despite being only 2–5%. It has been demonstrated that such a modest change could contribute gene expression regulation, although the strength of the correlation between DNA methylation and mRNA levels of the target gene varies depending of the loci and tissue analysed [25]. Whether the observed DNA methylation changes cause changes in gene expression in this specific population remains unknown. It

Fig. 1 Methyl groups are more likely to bind DNA at CpG sites. Approximately 85% of human promoter regions have CpG enriched sites. Highly methylated regions most often prevent binding of transcription

factors, which most commonly leads to reduction of transcription and expression, but there are exceptions to this rule

also is important to note that the effect of famine exposure seems to be locus specific, because markers of global genome-wide methylation (LINE1 and LUMA) did not vary according to prenatal famine exposure or not [26].

Seasonal Variation in Nutrient Availability: Examples from Rural Gambia

Recognizing the contrast between seasonal food access and physical demands on the farmers of rural Gambia, Waterland et al. compared DNA methylation levels in circulating blood cells of children conceived during the rainy season (low food availability and high energy expenditure due to agricultural workload) and those conceived out of the rainy season [27••]. In contrast to the studies of famine exposure described above, the authors did not observe differences in methylation levels at *IL10*, *GNASAS*, or *IGF2*; this discrepancy may be attributable to the greater extent of caloric deprivation in the Dutch Winter Hunger compared with seasonal variations in food availability in the Gambia. Nonetheless, Waterland et al. found that children conceived during the rainy season had higher methylation levels at *BOLA3*, *FLJ20433*, *PAX8*, *SLITRK1*, and *ZFYVE28* [27••]. These specific loci were selected and tested because of they appear to be human “metastable epialleles” (ME), which are characterized by stochastic and systemic interindividual epigenetic variation likely determined very early in fetal development. The higher levels of methylation at the ME in children conceived in the rainy season was unexpected (hypothesized to be lower because of methyl donor deficiency during that period) and led to further hypotheses related to the role of one-carbon (C_1) donor in epigenetic regulation.

Micronutrient Deficiency

C_1 metabolism is composed of two complementary but interdependent pathways (one folate dependent) and is essential to produce the methyl donors necessary for DNA methylation (see ref [28] for a recent comprehensive review). Diet is

the unique source of micronutrients resulting in methyl-group donor availability. Vitamin B12 is a coenzyme in the C_1 metabolism cycle; it is involved in DNA synthesis and regulation in all cells but has a specific role in blood cells formation and neurological functions. Folate is a key coenzyme in methyl transfer and is essential for DNA synthesis and repair. The amount of nutrients necessary for optimal C_1 metabolism and for matching methyl-donor demand is increased during pregnancy. Prepregnancy supplementation of folates is recommended and has been shown to prevent neural tube defects [29, 30]. Choline is considered as part of the B-complex vitamins and also is implicated in neural development in later part of the pregnancy [31].

Observational studies in India have reported that increased insulin resistance during childhood (measured by HOMA-IR at 6 years old) is related to *low* levels of vitamin B12 but *high* concentrations of erythrocyte folates in maternal circulation measured during midpregnancy [32]. It is possible that methylation processes and epigenetic regulation are part of the mechanisms underlying these associations, but this remains to be demonstrated in this specific population. The authors raised the importance of B12 deficiency in India and that folate supplementation might not always be as beneficial as previously thought, especially after the neural tube closure period [32].

Global methylation (measured by LINE-1) in circulating cord blood cells do not seem to be associated with maternal dietary intake of methyl-donor [33] or, specifically, of folic acid [34]. Moreover, prenatal intake of multivitamins does not appear to influence global methylation (estimated by LINE-1 or AluYb8) in placenta [35]. These findings are in line with the Dutch Winter Hunger reports mentioned above [26], where extreme maternal caloric restriction did not appear to affect the offspring’s global epigenetic profile, despite the positive findings at some of the specific loci of interest they tested [19••]. In contrast, McKay et al. reported that maternal circulating B12 levels were associated with global methylation in circulating cord blood cells (by LUMA) but not with specific sites at 3 genes of high interest for fetal growth and

Table 1 Main genes of interest investigated in more than one report regarding maternal nutritional status and epigenetic profile in the offspring

Gene	Undernutrition	Ref	Over-nutrition	Ref	Dietary composition	Ref
<i>IGF2/H19</i>	- Lower in severe caloric restriction	Heijmans BT 2008	- Not associated with maternal BMI or maternal glycemia	St-Pierre J 2012	- Not associated with circulating maternal B12	McKay JA 2012
	- Not associated in seasonal exposure	Waterland RA 2010	- Association with maternal BMI detected only if paternal BMI taken into account	Soubry A 2013	- Not associated with folic acid in early pregnancy or periconception	Hoyo C 2011; Steegers-Theunissen RP 2009
<i>IL10</i>	- Higher in severe caloric restriction	Tobi EW 2009			- Not associated with higher choline supplementation	Jiang X 2012
	- Not associated in seasonal exposure	Waterland RA 2010			- Not associated with folic acid intake at 12 weeks, but higher in mother taking folic acid after 12 weeks	Haggarty P 2013
<i>LEP</i>	- Higher in severe caloric restriction	Tobi EW 2009	- Associated with maternal hyperglycemia	Bouchard L 2010	- Not associated with higher choline supplementation	Jiang X 2012
<i>GNAS4S</i>	- Higher in severe caloric restriction	Tobi EW 2009			- Differentially methylated in RCT of multivitamins supplementation	Khulan B 2012
<i>IGF2R</i>	- Not associated in seasonal exposure	Waterland RA 2010			- Differentially methylated in RCT of multivitamins supplementation (only in female offspring)	Cooper WN 2012
<i>CRH</i>	- Not associated in severe caloric restriction	Tobi EW 2009			- Associated with higher choline supplementation	Jiang X 2012
<i>NR3C1</i>	- Not associated in severe caloric restriction	Tobi EW 2009			- Associated with higher choline supplementation	Jiang X 2012
<i>ADIPOQ</i>			- Associated with maternal hyperglycemia	Bouchard L 2012	- Associated with consumption of red meat, fish, vegetable, bread, and potatoes	Drake AJ 2012
					- Differentially methylated in RCT of multivitamins supplementation	Khulan B 2012

development (*IGF2*, *IGFBP3*, *ZNT5*) [36]. The different methods of estimating maternal micronutrient status (self-reported intake vs. circulating levels) and of measuring global methylation might partly explain the different findings between these studies. This also highlights the challenges in detecting locus-specific variations related to exposure to diverse maternal nutritional characteristics, complicated by the many subtleties of dietary intake and the difficulties in measuring the nutritional exposures.

Using the candidate gene approach, several observational studies have found significant associations between maternal micronutrient consumption and DNA methylation levels at specific loci in offspring. Methylation levels at *IGF2* were higher in 17-month-old infants of women who reported taking supplemental folic acid around the time of conception compared with infants of women who were not taking supplements during the same period (49.5% vs. 47.4% respectively; $P = 0.01$) [37]. In contrast, another study found that maternal folic acid intake (both preconceptional and during pregnancy) was not associated with methylation levels at *IGF2* but was associated with lower methylation levels at *H19* DMR in cord blood cells [38]. In a large pregnancy cohort study in United Kingdom, folic acid intake before 12 weeks of pregnancy was not associated with methylation levels in circulating cord blood cells globally (based on LINE1) or at three maternally imprinted candidate genes (*IGF2*, *PEG3*, *SNRPN*) [39]. In the same study, folic acid intake after 12 weeks gestation was associated with higher methylation levels at *IGF2* but lower methylation levels at *PEG3* [39]. The inconsistent results between these observational studies might be due to population-specific effects (given the differences in folate deficiency at baseline), and the specific locus analysed at each candidate gene. Importantly, the inconsistent results emphasize the need for well-powered replication studies, which are still too rarely performed in human epigenetics research.

A few studies have taken advantage of intervention paradigms designed to evaluate the impact of supplementation of micronutrients in pre-pregnancy or periconception on neonatal outcomes. In a population living in rural Gambia, circulating blood cells were collected at birth and at 9 months of age in offspring born to women included in a randomized controlled trial of micronutrient supplementation pre- and periconception. In a first preliminary analysis using a candidate genes approach, very few loci were found to be differentially methylated, except in subgroup analyses (*IGF2R* in girls and *GLT2* in boys at birth) [40]. Then, using a genome-wide array (27k Illumina), 14 loci in boys and 21 loci in girls were differentially methylated (more than 10% difference; FDR $p < 0.001$) at birth according to treatment group (receiving multivitamin vs. placebo starting in preconception) [41•]. More than 50% of the differentially methylated loci at birth remained so in infancy (aged 9 months). Interestingly, a larger number of loci (108 in boys, and 106 in girls) were found to be

differentially methylated at 9 months of age according to multivitamin supplementation or not. Among the differentially methylated loci in infancy, many of the genes near the identified probes have been previously implicated in immune function (a finding to interpret with caution since methylation levels were measured in circulating blood cells). In addition, genes located in metabolic pathways included *AHSG* (encoding for fetuin, suggested to regulate insulin action at the insulin receptor tyrosine kinase level), *PROX1* (associated with T2D); *SLC22A18* and *MKRN3* (implicated in Beckwith-Wiedemann and Prader-Willi syndromes respectively) in boys, as well as *ADIPOQ* (encoding for adiponectin, an adipokine with putative insulin sensitivity properties) and *GNAS* (imprinted locus associated with various endocrine disorders) in girls [41•].

The effects of choline during the third trimester of pregnancy on epigenetic regulation of cortisol-related genes and other candidates were assessed in a randomized, controlled trial of choline supplementation [42]. In placenta samples collected at birth, high choline intake was associated with higher methylation levels globally, and specifically at *CHR* and *NR3C1* (glucocorticoid receptor), but not at *IL10*, *LEP*, or *IGF2*. The higher methylation levels at *CHR* also were associated with lower *CHR* expression in placenta, suggesting that the epigenetic modulation induced by high maternal choline intake may be functional [42].

Maternal Macronutrient Imbalance

Some studies have suggested that the offspring's epigenetic profile also could be influenced by the macronutrient composition of the maternal diet. For example, Godfrey et al. demonstrated that the low carbohydrate intake (defined as the lowest quartile of the carbohydrate intake distribution for the population) measured by a food frequency questionnaire at 15 weeks of gestation was associated with higher methylation levels at *RXR α* measured in circulating cord blood cells [43•]. *RXR α* encodes retinoid X receptor A, which regulates the transcription of many genes involved in energy metabolism, adipocyte differentiation, and adipogenesis to a large extent via heterodimerization with the nuclear receptor *PPARG*. Godfrey et al. also observed a dose-dependent relationship between methylation levels at *RXR α* in cord blood and adiposity in childhood in two independent cohorts, suggesting that epigenetic profile at birth can predict metabolic outcomes in childhood and providing evidence that DNA methylation could be one of the molecular mechanisms linking maternal diet during pregnancy with childhood obesity [43•].

Drake et al. studied children of mothers who were advised to eat high amounts of red meat and to avoid carbohydrate-rich food during pregnancy (in 1967–1968 in Scotland) [44]. At age 40 years, the offspring were invited for a medical

assessment during which circulating blood cells were collected to perform epigenetic analyses in candidate genes. The authors demonstrated that higher methylation levels at *NR3C1* (glucocorticoid receptor) in offspring was associated with higher intake of red meat, fish, and vegetables and with lower intake of bread and potatoes by their mother most adherent to the “high meat/low carb” diet during pregnancy compared with the offspring from mothers who were less adherent. In addition, higher methylation at *NR3C1* was correlated with higher adult BMI and waist circumference, but lower blood pressure (all measured at 40 years old, cross-sectionally with the methylation levels). This suggests that low carbohydrate intake/high protein intake during pregnancy affects epigenetic regulation of glucocorticoid pathway in the offspring and might be one of the mechanism leading to higher risk of obesity in later life. A key limitation of this study is that methylation was only assessed in samples collected in adulthood; hence, it is unknown whether these epigenetic marks were present from birth or whether postpartum events that correlate with maternal diet caused these marks to emerge (offspring’s weight trajectory, metabolic health, dietary preferences, etc.).

Maternal “Overnutrition”

Obesity and Gestational Weight Gain

Historically, much more attention has always been devoted to risk from maternal undernutrition and nutrient deficiencies. With the recent rise in obesity, maternal overweight status, and excessive gestational weight gain are now considered risk factors for many adverse offspring outcomes over the short- and long-term. Children born to women who were obese in pregnancy are themselves at increased risk of obesity, insulin resistance, and many other metabolic diseases throughout their lives [45]. It is postulated that maternal obesity induces adverse environmental milieu for the developing fetus by alteration of lipid and glucose metabolism and by promoting a proinflammatory response, overall influencing placenta biology and nutrients exchange and consequently fetal development.

Therefore, a few studies have used maternal BMI as a proxy for overnourishment of the placenta and fetus. Prepregnancy BMI does not seem to be associated with global methylation assessed by LINE1 [46]. Given its strong status as a candidate gene for fetal growth and development, several studies have focused on the impact of maternal BMI on the *IGF2/H19* locus. In our study based on a French-Canadian population (50 dyads of mothers-infants), maternal BMI or gestational weight gain was not associated with methylation levels at the *IGF2/H19* locus in the placenta or cord blood cells (supplementary analyses derived from published report

from our group [47]). In line with these results, in 300 mother-newborn dyads from the Newborn Epigenetic Study (NEST) cohort, methylation levels at *IGF2* or *H19* DMRs were not associated with prepregnancy BMI or excessive gestational weight gain [48]. Interestingly, using a smaller sample from the same cohort (NEST), the investigators reported that higher methylation levels at *IGF2* DMR were associated with higher maternal BMI but lower paternal BMI when both were taken into account in multivariable models [49]. The association between paternal BMI and methylation levels in offspring suggests an effect of obesity on gamete formation prior to conception, a hypothesis that warrants testing in future studies.

Maternal Glycemic Regulation During Pregnancy

Maternal hyperglycemia is also considered as a state of placental-fetal overnutrition. A few candidate gene studies have been conducted to investigate the impact of gestational diabetes mellitus (GDM) exposure on offspring DNA methylation in placenta and cord blood cells. With a focus on adipokines, our group demonstrated that higher levels of blood glucose at 2 h during a standard second trimester oral glucose tolerance test were associated with lower DNA methylation levels at *ADIPOQ* (encoding for adiponectin) [50] and *LEP* (encoding for leptin) [51] in the fetal side of the placenta. Our analyses revealed that at the *ADIPOQ* promoter CpG sites, methylation levels were correlated with maternal glycemia across the whole spectrum (from normoglycemia to GDM) [50], whereas at the *LEP* promoter, the correlation was observable only in women with impaired glucose regulation [51]. Our group also conducted an epigenome-wide association study (EWAS; using the 450k Illumina platform) to compare offspring DNA methylation profile (both in placenta tissue and cord blood cells) according to exposition to GDM or not during *in utero* development. Using pathways analyses, we noted that the ‘metabolic diseases’ pathway was among the top pathways differentially methylated according to GDM exposure in both tissues, in line with the fetal metabolic programming hypothesis [52•]. Another group investigated 16 candidate loci in a GDM case–control study [53]. They found that exposure to GDM was associated with lower methylation levels at the *MEST* locus in both the placenta and cord blood cells [53]. *MEST* is a maternally imprinted gene that might have a role in fetal development.

Taken together, these results suggest that maternal glycemia can affect DNA methylation in the offspring; this seems to be locus and/or site specific and also might vary according to the degree of hyperglycemic exposure [50, 51]. The type of treatment (e.g., diet only, insulin, oral hypoglycemic agents) to control maternal blood glucose concentrations also might have an effect on epigenetic regulation of specific locus, but this has not been reported yet.

Perspectives and Challenges

Although there are relatively few human studies of epigenetic variation in pregnancy and early life, recent results indicate that maternal nutrition and the metabolic *in utero* milieu can affect the offspring epigenetic profile. It is likely that variability in nutritional status *in utero* and during infancy will have different effects on epigenetic variation that are gene/locus specific (or even CpG site-specific) and that the effect might differ depending on the degree of the nutritional exposure (e.g., degree of caloric deprivation or hyperglycemia) or the level of a specific nutrient given other nutrients (e.g., B12/folates).

Many challenges will need to be overcome before a detailed mechanistic understanding of fetal metabolic programming is achieved. Key challenges are that: 1) epigenetic variations are most often tissue-specific, hindering the direct assessment of metabolic programming in humans for tissues of interest in the fields of obesity, diabetes, and CVD (beta cells, adipose tissue, liver, muscles, vessels, heart, etc.); 2) DNA methylation is the most studied epigenetic variation but other epigenetic phenomena (e.g., histone modifications, DNA hydroxymethylation, miRNA) influence gene expression, which also are biologically meaningful but are much harder to assess; 3) epigenetic marks are fairly stable but some are modifiable, so longitudinal studies will be necessary to address the question of whether epigenetic marks present at birth are still present later in life, and whether these marks are causally related with adverse outcomes or merely correlates of other causal exposures and processes; and 4) many potential interactions likely exist between genetic variants, epigenetic variations, and environmental/metabolic factors that can influence the DNA methylation levels at one site (a possibility that has not been taken into account in observational studies so far, largely due to the complexities of such analyses). Until now, the published candidate genes studies provide some elements of evidence supporting the concepts that epigenetics is involved in the molecular mechanisms of developmental origins of adult diseases (when positive findings are significant) but most studies are limited by fairly small sample sizes, test only a handful of candidate genes, lack replication, and focus on cross-sectional methylation analyses (often at birth or much later in life).

Nevertheless, we feel that the field of fetal epigenetic programming is one of the most promising in the current state of investigations of the early determinants of chronic metabolic diseases. Efficient epigenome-wide technologies are now available and allow us to investigate the impact of the maternal metabolic health, nutritional status, or dietary intake on the offspring's epigenetic profile across the whole genome without a priori candidate genes targeted hypotheses – similar to the recent genome-wide association studies – and hopefully reveal new relevant biologic pathways. To achieve this, we will need well phenotyped, adequately powered pregnancy cohorts that are large enough to observe realistic epigenetic effects, whilst accounting for the statistical burden of testing

multiple hypotheses in epigenome-wide experiments. Another important aspect will be to have the opportunity to measure epigenetic variations at more than one time over childhood adolescence and adulthood to test the stability of the “programmed” epigenetic imprints to evaluate the impact of other events and lifestyle over the life course on these epigenetic variations; and to investigate their association with clinically relevant outcomes (e.g., obesity, diabetes, CVD events).

Finally, causal inference in observational studies is rarely possible, even when conducted under the best design (prospective, longitudinal, detailed phenotypes, etc.), and so, intervention studies will be needed to address causality and lead to clinically meaningful recommendations. The impact on epigenetic modelling of different dietary approaches (including variations in macro- and micronutrient balance), appropriate gestational weight gain, and intensity and type of treatment for hyperglycemia during pregnancy should be compared with determining how to optimize metabolic programming and positively affect the cardiometabolic risk trajectory during the early stages of life. As outlined, epigenetic analyses performed in longitudinal cohort studies, in which repeated biosampling has been performed throughout the developmental years, will prove valuable in determining the impact of modifiable environmental risk factors on cardiometabolic health.

Conclusions

Human studies investigating the role of epigenetics in developmental origins of health and adults diseases are still few, but the emerging data strongly suggest that site-specific DNA methylation levels in the offspring are influenced by maternal nutritional status at both end of the spectrum (under- and overnutrition) or micro- and macronutrients balance. Current literature clearly shows that the effect of various *in utero* exposures to maternal nutritional status has different impact at any specific studied locus. This means that future studies will need to investigate epigenetic phenomenon using locus specific technologies and to collect detailed information on maternal diet and metabolic status. High-quality observational and intervention studies will be needed to pursue primordial prevention of obesity and diabetes and slow down the actual epidemic in future generations.

Compliance with ethics Guidelines

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References

Papers of particular interest, published recently, have been highlighted as

- Of importance
- Of major importance

1. Veenendaal MV, Painter RC, de Rooij SR, Bossuyt PM, van der Post JA, Gluckman PD, et al. Transgenerational effects of prenatal exposure to the 1944–45 Dutch famine. *BJOG: An International Journal of Obstetrics & Gynaecology*. 2013;120:548–53.
2. van Abeelen AF, Elias SG, Bossuyt PM, Grobbee DE, van der Schouw YT, Roseboom TJ, et al. Famine exposure in the young and the risk of type 2 diabetes in adulthood. *Diabetes*. 2012;61:2255–60.
3. Li Y, He Y, Qi L, Jaddoe VW, Feskens EJ, Yang X, et al. Exposure to the Chinese famine in early life and the risk of hyperglycemia and type 2 diabetes in adulthood. *Diabetes*. 2010;59:2400–6.
4. Li Y, Jaddoe VW, Qi L, He Y, Wang D, Lai J, et al. Exposure to the Chinese famine in early life and the risk of metabolic syndrome in adulthood. *Diabetes Care*. 2011;34:1014–8.
5. Kaati G, Bygren LO, Pembrey M, Sjöström M. Transgenerational response to nutrition, early life circumstances and longevity. *Eur J Hum Genet*. 2007;15:784–90.
6. Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjöström M, et al. Sex-specific, male-line transgenerational responses in humans. *Eur J Hum Genet*. 2006;14:159–66.
7. Pettitt DJ, Baird HR, Aleck KA, Bennett PH, Knowler WC. Excessive obesity in offspring of Pima Indian women with diabetes during pregnancy. *N Engl J Med*. 1983;308:242–5.
8. Franks PW, Looker HC, Kobes S, Touger L, Tataranni PA, Hanson RL, et al. Gestational glucose tolerance and risk of type 2 diabetes in young Pima Indian offspring. *Diabetes*. 2006;55:460–5.
9. Stoger R. In vivo methylation patterns of the leptin promoter in human and mouse. *Epigenetics: Official Journal of the DNA Methylation Society*. 2006;1:155–62.
10. Yajnik CS, Deshmukh US. Fetal programming: maternal nutrition and role of one-carbon metabolism. *Rev Endocr Metab Disord*. 2012. doi:10.1007/s11154-012-9214-8.
11. Heijmans BT, Tobi EW, Lumey LH, Slagboom PE. The epigenome: archive of the prenatal environment. *Epigenetics: Official Journal of the DNA Methylation Society*. 2009;4:526–31.
12. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet*. 2003;33:245–54.
13. Kangaspeska S, Stride B, Metivier R, Polycarpou-Schwarz M, Ibberson D, Carmouche RP, Benes V, Gannon F, Reid G. Transient cyclical methylation of promoter DNA. *Nature*. 2008
14. Gabory A, Attig L, Junien C. Developmental programming and epigenetics. *Am J Clin Nutr*. 2011;94:1943S–52S.
15. Talens RP, Boomsma DI, Tobi EW, Kremer D, Jukema JW, Willemsen G, Putter H, Slagboom PE, Heijmans BT. Variation, patterns, and temporal stability of DNA methylation: considerations for epigenetic epidemiology. *FASEB J*. 2010
16. Talens RP, Christensen K, Putter H, Willemsen G, Christiansen L, Kremer D, et al. Epigenetic variation during the adult lifespan: cross-sectional and longitudinal data on monozygotic twin pairs. *Aging Cell*. 2012;11:694–703.
17. Wong CC, Caspi A, Williams B, Craig IW, Houts R, Ambler A, Moffitt TE, Mill J. A longitudinal study of epigenetic variation in twins. *Epigenetics*. 2010
18. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci U S A*. 2008;105:17046–9.
19. •• Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD, et al. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum Mol Genet*. 2009;18:4046–53. *This study reports the results of investigation of in utero famine exposure on DNA methylation levels at 15 candidate genes. DNA methylation was measured in circulating blood cells at 58 years old in individuals exposed to the Dutch Hunger and compared with their same-sex sibling who was not exposed.*
20. Thompson RF, Atzmon G, Gheorghe C, Liang HQ, Lowes C, Greally JM, et al. Tissue-specific dysregulation of DNA methylation in aging. *Aging Cell*. 2010;9:506–18.
21. Parle-McDermott A, Ozaki M. The impact of nutrition on differential methylated regions of the genome. *Advances in Nutrition*. 2011;2:463–71.
22. Tobi EW, Slagboom PE, van Dongen J, Kremer D, Stein AD, Putter H, et al. Prenatal famine and genetic variation are independently and additively associated with DNA methylation at regulatory loci within IGF2/H19. *PLoS ONE*. 2012;7:e37933 [Electronic Resource].
23. Le Stunff C, Fallin D, Bougneres P. Paternal transmission of the very common class I INS VNTR alleles predisposes to childhood obesity. *Nat Genet*. 2001;29:96–9.
24. Rodriguez S, Gaunt TR, O'Dell SD, Chen XH, Gu D, Hawe E, et al. Haplotypic analyses of the IGF2-INS-TH gene cluster in relation to cardiovascular risk traits. *Hum Mol Genet*. 2004;13:715–25.
25. Guay SP, Voisin G, Brisson D, Munger J, Lamarche B, Gaudet D, et al. Epigenome-wide analysis in familial hypercholesterolemia identified new loci associated with high-density lipoprotein cholesterol concentration. *Epigenomics*. 2012;4:623–39.
26. Lumey LH, Terry MB, Delgado-Cruzata L, Liao Y, Wang Q, Susser E, et al. Adult global DNA methylation in relation to pre-natal nutrition. *Int J Epidemiol*. 2012;41:116–23.
27. •• Waterland RA, Kellermayer R, Laritsky E, Rayco-Solon P, Harris RA, Travisano M, et al. Season of conception in rural gambia affects DNA methylation at putative human metastable epialleles. *PLoS Genetics*. 2010;6:e1001252. *This study reports the association between season of conception in Gambia and DNA methylation levels at candidate genes and putative metastable epialleles. Metastable epialleles' (ME) are characterized by stochastic and systemic interindividual epigenetic variation likely determined very early in fetal development. This is one key study demonstrates this phenomenon in humans.*
28. Dominguez-Salas P, Cox SE, Prentice AM, Hennig BJ, Moore SE. Maternal nutritional status, C(1) metabolism and offspring DNA methylation: a review of current evidence in human subjects. *Proc Nutr Soc*. 2012;71:154–65.
29. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet*. 1991;338:131–7.
30. Persad VL, Van den Hof MC, Dube JM, Zimmer P. Incidence of open neural tube defects in Nova Scotia after folic acid fortification. *CMAJ Canadian Medical Association Journal*. 2002;167:241–5.
31. Shaw GM, Carmichael SL, Yang W, Selvin S, Schaffer DM. Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. *Am J Epidemiol*. 2004;160:102–9.
32. Yajnik CS, Deshpande SS, Jackson AA, Refsum H, Rao S, Fisher DJ, et al. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia*. 2008;51:29–38.

33. Boeke CE, Baccarelli A, Kleinman KP, Burris HH, Litonjua AA, Rifas-Shiman SL, et al. Gestational intake of methyl donors and global LINE-1 DNA methylation in maternal and cord blood: prospective results from a folate-replete population. *Epigenetics: Official Journal of the DNA Methylation Society*. 2012;7:253–60.
34. Fryer AA, Nafee TM, Ismail KM, Carroll WD, Emes RD, Farrell WE. LINE-1 DNA methylation is inversely correlated with cord plasma homocysteine in man: a preliminary study. *Epigenetics: Official Journal of the DNA Methylation Society*. 2009;4:394–8.
35. Wilhelm-Benartzi CS, Houseman EA, Maccani MA, Poage GM, Koestler DC, Langevin SM, et al. In utero exposures, infant growth, and DNA methylation of repetitive elements and developmentally related genes in human placenta. *Environ Health Perspect*. 2012;120:296–302.
36. McKay JA, Groom A, Potter C, Coneyworth LJ, Ford D, Mathers JC, et al. Genetic and non-genetic influences during pregnancy on infant global and site specific DNA methylation: role for folate gene variants and vitamin B12. *PLoS ONE*. 2012;7:e33290 [Electronic Resource].
37. Steegers-Theunissen RP, Obermann-Borst SA, Kremer D, Lindemans J, Siebel C, Steegers EA, et al. Periconceptional maternal folic acid use of 400 microg per day is related to increased methylation of the IGF2 gene in the very young child. *PLoS ONE*. 2009;4:e7845 [Electronic Resource].
38. Hoyo C, Murtha AP, Schildkraut JM, Jirtle RL, Demark-Wahnefried W, Forman MR, et al. Methylation variation at IGF2 differentially methylated regions and maternal folic acid use before and during pregnancy. *Epigenetics: Official Journal of the DNA Methylation Society*. 2011;6:928–36.
39. Haggarty P, Hoad G, Campbell DM, Horgan GW, Piyathilake C, McNeill G. Folate in pregnancy and imprinted gene and repeat element methylation in the offspring. *Am J Clin Nutr*. 2013;97:94–9.
40. Cooper WN, Khulan B, Owens S, Elks CE, Seidel V, Prentice AM, et al. DNA methylation profiling at imprinted loci after periconceptional micronutrient supplementation in humans: results of a pilot randomized controlled trial. *FASEB Journal*. 2012;26:1782–90.
41. • Khulan B, Cooper WN, Skinner BM, Bauer J, Owens S, Prentice AM, et al. Periconceptional maternal micronutrient supplementation is associated with widespread gender related changes in the epigenome: a study of a unique resource in the Gambia. *Hum Mol Genet*. 2012;21:2086–101. *This study reports the results of the epigenome-wide analyses (based on 27k platform) comparing offspring from women included in a RCT and allocated to receive micronutrient supplementation or not; despite limited samples size and the use of the 27k platform (and not the most recent 450k). Results are of interest but will necessitate replication.*
42. Jiang X, Yan J, West AA, Perry CA, Malysheva OV, Devapatla S, et al. Maternal choline intake alters the epigenetic state of fetal cortisol-regulating genes in humans. *FASEB Journal*. 2012;26:3563–74.
43. • Godfrey KM, Sheppard A, Gluckman PD, Lillycrop KA, Burdge GC, McLean C, et al. Epigenetic gene promoter methylation at birth is associated with child's later adiposity. *Diabetes*. 2011;60:1528–34. *This study reports first the association between maternal macronutrient intake (low carbohydrates) and DNA methylation levels at RXRA locus in cord blood cells; then it shows the association between the DNA methylation levels at the same locus and childhood adiposity in two independent cohorts providing a very strong argument for epigenetics linking maternal diet and the risk of childhood obesity.*
44. Drake AJ, McPherson RC, Godfrey KM, Cooper C, Lillycrop KA, Hanson MA, et al. An unbalanced maternal diet in pregnancy associates with offspring epigenetic changes in genes controlling glucocorticoid action and foetal growth. *Clin Endocrinol (Oxf)*. 2012;77:808–15.
45. O'Reilly JR, Reynolds RM. The risk of maternal obesity to the long-term health of the offspring. *Clin Endocrinol (Oxf)*. 2013;78:9–16.
46. Michels KB, Harris HR, Barault L. Birthweight, maternal weight trajectories and global DNA methylation of LINE-1 repetitive elements. *PLoS ONE*. 2011;6:e25254 [Electronic Resource].
47. St-Pierre J, Hivert MF, Perron P, Poirier P, Guay SP, Brisson D, et al. IGF2 DNA methylation is a modulator of newborn's fetal growth and development. *Epigenetics*. 2012. doi:10.4161/epi.21855.
48. Hoyo C, Fortner K, Murtha AP, Schildkraut JM, Soubry A, Demark-Wahnefried W, et al. Association of cord blood methylation fractions at imprinted insulin-like growth factor 2 (IGF2), plasma IGF2, and birth weight. *Cancer Causes & Control*. 2012;23:635–45.
49. Soubry A, Schildkraut JM, Murtha A, Wang F, Huang Z, Bernal A, et al. Paternal obesity is associated with IGF2 hypomethylation in newborns: results from a Newborn Epigenetics Study (NEST) cohort. *BMC Medicine*. 2013;11:29.
50. Bouchard L, Hivert MF, Guay SP, St-Pierre J, Perron P, Brisson D. Placental adiponectin gene DNA methylation levels are associated with mothers' blood glucose concentration. *Diabetes*. 2012;61:1272–80.
51. Bouchard L, Thibault S, Guay SP, Santure M, Monpetit A, St-Pierre J, Perron P, Brisson D. Leptin Gene Epigenetic Adaptation to Impaired Glucose Metabolism during Pregnancy. *Diabetes Care*. 2010;Aug 19.
52. • Ruchat SM, Houde AA, Voisin G, St-Pierre J, Perron P, Baillargeon JP, Gaudet D, Hivert MF, Brisson D, Bouchard L. Gestational Diabetes Mellitus Epigenetically Affects Genes Predominantly Involved in Metabolic Diseases. *Epigenetics: Official Journal of the DNA Methylation Society*. 2013;(in press)*This is the first study to report the impact of GDM exposure on the offspring DNA methylation levels across the epigenome using the most recent technology (450k Illumina); no single locus reached "epigenome-wide" significance levels after correcting for multiple testing, arguing for the need for larger samples size and replication studies.*
53. El Hajj N, Plushch G, Schneider E, Dittrich M, Muller T, Korenkov M, et al. Metabolic programming of MEST DNA methylation by intrauterine exposure to gestational diabetes mellitus. *Diabetes*. 2013;62:1320–8.