Nutrition During Pregnancy Impacts Offspring's Epigenetic Status—Evidence from Human and Animal Studies

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Supplementary Isuue: Parental Nutritional Metabolism and Health and Disease of Offspring

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ABSTRACT: Pregnancy is a vital time of growth and development during which maternal nutrition significantly influences the future health of both mother and baby. During pregnancy, the fetus experiences a critical period of plasticity. Epigenetics, specifically DNA methylation, plays an important role here. As nutrition is influential for DNA methylation, this review aims to determine if maternal nutrition during pregnancy can modify the offspring's epigenome at birth. Research focuses on micronutrients and methyl donors such as folate and B vitamins. Evidence suggests that maternal nutrition does not largely influence global methylation patterns, particularly in nutrient-replete populations; however, an important impact on gene-specific methylation is observed. A link is shown between maternal nutrition and the methylome of the offspring; however, there remains a paucity of research. With the potential to use DNA methylation patterns at birth to predict health of the child in later life, it is vital that further research be carried out.

KEYWORDS: epigenetics, pregnancy, nutrition, programing, offspring

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Introduction

Pregnancy is a critical period of plasticity whereby fetal development may be significantly influenced by environmental factors, such as maternal nutrients and hormones,[1–4] as well as the inherited genetic profile.[5,6] There is a strong potential for these factors to exert a long-lasting impact on the offspring's growth and health into adulthood. This concept of *fetal programing* is well established in the literature.[6,7] It was described among the offspring of mothers who were exposed to famine during the Dutch Hunger Winter, such that babies were born with lower birth weights and were subsequently at increased risk of cardiovascular diseases and other adverse health outcomes in adulthood.[8–10]

Epigenetics has been found to play a role in fetal programing.[6,11] The term "epigenetics" was first coined by Waddington in the 1940s.[12] It refers to the changes to an individual's genetic code that can alter gene expression, without changing the DNA sequence, and are passed from one cell generation to the next.[13] Effectively controlling which genes are expressed can enable the developing fetus to adapt to its environment at birth. One of the key mechanisms in epigenetic modification is DNA methylation.[14] In brief, this involves the addition of a methyl group along the DNA strand where a cytosine base is located beside a guanine base (CpG site). This addition affects the expression of the gene[15] and has been shown to prevent protein coding and decrease the expression levels of the gene.[14,16] For an in-depth review of DNA methylation, see the articles by Nakao and Bird.[17,18] There is considerable interest in the impact that epigenetic mechanisms in utero may have on fetal programing. It has been shown that during early development, the fetal epigenome is much more susceptible to environmental stimuli.[11,19–22]

Many factors during pregnancy can impact the child's epigenetic status, including the health of the mother.[23–25] A study carried out in the UK identified particular locations and CpG sites within the genome, where methylation patterns of the offspring were altered by mothers' gestational diabetes status.[23] Maternal weight during pregnancy was also associated with altered methylation patterns in the child's DNA and later infant adiposity.[24] Offspring in both underweight and overweight mothers were also reported to have altered DNA methylation patterns. This later influenced the adiposity levels

of the offspring.[24] A further study reported that excessive gestational weight gain was associated with increased DNA methylation levels affecting relevant pathways implicated in developmental programing of the offspring.[25] In addition, it has been shown that the effects of such particular epigenetic stimuli occur individually with each pregnancy.[22,26] A study conducted among mothers, who had bariatric gastrointestinal bypass surgery and subsequently improved their weight and cardiovascular profile, demonstrated a change in the methylation status of more than 5,500 genes, particularly those relating to cardiometabolic pathways, in infants born after the surgery compared to those born before the surgery.[26]

Particular nutrients are known to impact DNA methylation due to their interaction with the one-carbon metabolism cycle.[16] This cycle results in the formation of methyl groups that are required for the methylation of DNA. Folate feeds into this cycle and has been shown to alter the levels of DNA methylation in women of childbearing age.[27] A decrease in the level of dietary folate has been found to decrease genomic DNA methylation levels.[28] Other nutrients, including vitamins B12, B6, and B2, choline, and betaine, are required to provide the cofactors that are used to make the methyl groups.[29]

This review aims to determine if maternal nutrient intakes modify the epigenome of the offspring at birth in both human and animal studies. Studying this specific time point controls for the influence of other factors, such as the environment and diet, after the child is born. Methylation patterns are known to be tissue specific, and as the umbilical cord tissue contains mesenchymal cells and vascular tissue, this may be considered useful when looking for associations with later life anthropometry.[30] For this reason, the human studies included in this review will focus on DNA methylation patterns measured in cord blood, where possible. Due to the limited number of published human studies, animal models are also included.

Methods

For the purpose of this review, the primary search engine used was the online database PubMed (http://www.ncbi.nlm.nih.gov/pubmed). The following keywords were searched: "epigenetics," "DNA methylation," "pregnancy," "nutrition," "offspring," and "cord blood." Relevant free-access abstracts were identified and reviewed to determine appropriate studies. Suitable published articles in the English language were included, and no restrictions were applied to the dates of articles. Both animal and human studies were included as, due to the ethical nature of this area of research, nutrient deficiency studies were primarily available in animal models utilizing DNA methylation patterns from various tissue types. Human studies were restricted to those involving cord bloods for DNA methylation analysis only to try and control for the variation in methylation status found in different human tissues. Manual searches of reference lists were carried out on the selected papers to identify further eligible studies. In total, 17 papers were selected and summarized in Tables 1–3.

Results

Seventeen papers fit the criteria for inclusion. Table 1 summarizes nine animal studies that are primarily made up of mouse and rat models, examining the effect of maternal nutrient intake on various tissue types. Tables 2 and 3 summarize eight human studies that look at the impact of particular nutrients on the offspring's pattern of DNA methylation in cord blood; both genome-wide methylation and gene-specific methylation were examined.

Discussion

A limited number of studies have been carried out examining the impact of maternal diet in pregnancy on the offspring's epigenetic profile at birth. These studies have primarily focused on nutrients known to interact with the one-carbon metabolism cycle, namely, folate, vitamin B12, B6, choline, and betaine.

Animal Models

Animal studies examining maternal nutritional impact on DNA methylation patterns focused mainly on the combinations of methyl donor nutrients, such as folate, B vitamins, choline, and betaine[31–34] (see Table 1). While few studies examined the impact of these nutrients on global methylation, overall evidence shows a relationship between the two. Reduced levels of methyl donors lead to global hypomethylation, as shown in a bovine study in 2007, where 88% of the altered CpG sites had decreased levels of DNA methylation due to experimental methyl donor deficiency.[31] Similarly, in agouti mice, it was shown that diets high in methyl donors resulted in higher DNA methylation levels of the offspring's agouti gene, which altered the phenotype.[33,34] In another mouse study, which examined allergic air disease, it was also found that high levels of these methyl donor nutrients increased the levels of DNA methylation in the offspring, which impacted the disease severity.[32]

There are few studies examining the individual impact of these methyl donor nutrients. One such study in rats looked at the effect of choline supplements and their deficiencies in relation to controls.[35] Surprisingly, it was found that choline deficiency resulted in an increase in global methylation levels in the offspring when compared to controls. However, this was as a result of hypomethylation of regulatory CpG sites on the *DNMT1* gene (that encodes the enzyme DNA methyltransferase), which is thought to lead to subsequent overexpression of this gene and an increase in global DNA methylation levels.[36] This was suggested as a mechanism by which the mother's diet could provide important feedback for the offspring, allowing them to compensate where maternal diet is lacking. This programing can occur even in the early stages of pregnancy; a study in rats by Maloney et al showed how levels of methyl donor nutrients in the mother's diet in the first five days of gestation alone changed how the offspring metabolized glucose.[37] The effect of the methyl-deficient diet

Table 1. Summary of animal studies examining changes in offspring epigenome in response to maternal nutritional alterations during gestation.

NUTRIENT	MODEL	ALTERATION	GENE/CpG INFLUENCED	EFFECT ON OFFSPRING	STUDY SIZE (n)	REFERENCE
1. Folic Acid, Vitamin B12, Methionine	Sheep: liver	Reduced levels of B12, folic acid, methionine compared to control	57 CpG loci	4% of the 1,400 CpG islands examined had altered methylation status. 88% of altered CpG sites were hypomethylated relative to controls.	37	31
2. Folic Acid, Vitamin B12, Choline, L-methionine, Zinc, and Betaine	Mouse: lung	High-methyl diet compared to a low-methyl diet	82 CpG loci on *Zdhhc5*, *Vldlr*, *Spock2*, *Cited4*, *Cnnm1*, *Mpp5*, *Dguok*, *A3galt2*, *Zfp503*, *Rcor3*, *Rnd3*, *Cdc42ep1*, *Runx3*, *Nfatc1*, *Jak2* genes	No alteration in global methylation between diets; however, 82 CpG loci were differentially methylated. The high-methyl diet significantly increased severity of allergic air disease in the mice.	105	32
3. Folic Acid, Vitamin B12, L-Methionine, Choline, Betaine, Zinc	Mouse: liver/ kidney	Medium and high levels of methyl donor supplementation	Long terminal repeat of agouti gene	Significant increase in DNA methylation of LTR and expression of Agouti gene with high-methyl diet. Subsequent alteration in offspring to a healthier phenotype.	759	33, 34
4. Choline	Rat: liver	Choline supplemented, control and deficient diets	Global Methylation, *IGF2* gene	Choline deficiency resulted in significant global and *IGF2* hypermethylation in liver samples when compared to controls.		35
5. Protein, Folic Acid	Rat: liver	Low protein intake (9% of diet) and Folic Acid supplements	*GR*, *PPAR* genes	GR and PPARα CpG methylation was lower with protein restricted diet alone. Folic Acid supplements prevented this decrease.	30	38
6. Protein, Folic Acid	Rat: liver	Low protein intake (9% of diet) and Folic Acid supplements	*IGF2*, *H19* genes	Significant increase in DNA methylation in imprinting control region of *IGF2* and *H19* with low-protein diet only. Supplementation with folic acid prevented this hypermethylation.	9	39
7. Fat	Mouse: adipose tissue	High-fat diet (62% fat, 20% carbohydrate, 18% protein)	Histones H3K9 (adiponectin), H4K20 (leptin)	The high-fat diet increased methylation of H4K20 in the promoter region of the leptin gene. The control mice had lower H3K9 methylation at 2, 12, and 24 weeks of age.	48	40
8. Fat	Mouse: brain tissue	High-fat diet (45% fat) during pregnancy of grandmother	*GHSR* gene	Significantly decreased methylation status at *GHSR* promoter of second generation offspring.	6	41

Abbreviations: CpG, site where a cytosine nucleotide occurs next to a guanine nucleotide common area for DNA methylation; Zdhhc5, zinc finger DHHC domain containing 5; Vldlr, very low-density lipoprotein receptor; Spock2, sparc/osteonectin; Cited 4, Cbp/p300 interacting transactivator; Cnnm1, cyclin M1; Mpp5, palmitoylated 5; Dguok, deoxyguanosine kinase; A3galt2, α-1,3-galactosyltransferase 2; Zfp503, zinc finger protein NOLZ1; Rcor3, REST corepressor 3; Rnd3, Rho GTPase; Cdc42ep1, CDC42 effector protein; Runx3, runt-related transcription factor 3; Nfact1, nuclear factor of activated T cells; Jak2, Janus kinase 2; GR, glucocorticoid receptor; PPAR, peroxisomal proliferator-activated receptor; *IGF2*, insulin-like growth factor II; *H19*, imprinted maternally expressed noncoding transcript; H3K9, histone H3 lysine 9; H4K20, histone H4 lysine 20; *GHSR*, growth hormone secretagogue receptor.

Table 2. Summary of human intervention studies examining changes in offspring epigenome in response to maternal nutritional alterations during pregnancy.

NUTRIENT	ALTERATION	GENE/CpG INFLUENCED	EFFECT ON OFFSPRING	STUDY SIZE (n)	REFERENCE
1. Folic Acid	Folic acid supplementation	Genome-wide methylation/ LINE-1	Folic acid supplements during pregnancy had no significant associations with mean LINE-1 methylation. Plasma homocysteine levels had an inverse correlation with LINE-1 methylation.	24	43
2. Folic Acid	Folic acid supplementation (doses >400 μg/day)	*IGF2*	Folic acid supplements, taken during pregnancy, were associated with significantly lower methylation levels at DNA sequences that are associated with deregulation of *IGF2* expression (particularly in males).	438	49
3. Folic Acid	Folic acid supplementation (400 ug)	*IGF2*	Children of mothers who took folic acid supplements had a 4.5% higher methylation level of IGF2 DMR at 17 months of age.	120	50

Abbreviations: CpG, site where a cytosine nucleotide occurs next to a guanine nucleotide common area for DNA methylation; *IGF2*, insulin-like growth factor II; LINE-1, long interspersed element-1; RXRA, retinoid X receptor alpha.

was found to be sex dependent, as this alteration was only observed in male and not female offspring.

Studies linking global methylation patterns with nutrition primarily focus on micronutrients. Limited studies are carried out on macronutrients, such as protein and fat, and they tend to focus on particular genes or sites. Interestingly, two rat studies looked at the effect of protein restriction on genes relating to cell differentiation and growth.[38,39] In both studies, it was found that low protein intake negatively altered the DNA methylation status of these genes. However, when the diet was supplemented with folic acid, no change was observed. These results highlight the potential importance that folic acid may have on methylation status above other dietary components. In mice, maternal dietary fat had a negative impact where a high intake was found to significantly increase the DNA methylation status of the leptin gene, which is associated with the control of energy balance and satiety.[40] In animal models, maternal fat intake has been shown to influence the following generation of offspring through epigenetic mechanisms. For instance, in mouse models, Dunn and Bale found that a high-fat diet resulted in reduced DNA methylation at the growth hormone secretagogue receptor (*GHSR*) promoter in the second-generation offspring.[41] This resulted in an increase in *GHSR* expression that is hypothesized to influence body length and adiposity.[41] Through similar mechanisms, a follow-on study from the work of Lillycrop et al on protein restriction with rodents demonstrated how the decrease in DNA methylation status that resulted in an increase in peroxisomal proliferator-activated receptor (*PPAR*) alpha, which is beneficial for insulin sensitivity, was maintained in the next generation.[42]

Human Studies

See summaries of papers in Tables 2 and 3. In human studies, research to date has found no association between folic acid intake during pregnancy and global methylation or long interspersed nucleotide element-1 (LINE-1) methylation status in the offspring.[43–45] LINE-1 sequences are frequently used as a surrogate for global methylation.[43] Fryer et al found that neither folic acid intake nor serum folate levels in the mother were associated with the infant's LINE-1 methylation at birth.[43] Another study also found that dietary folate intake along with other methyl donors had no impact on LINE-1 methylation status of the offspring.[45] However, Fryer et al did report that homocysteine levels in cord plasma were inversely correlated with LINE-1 methylation,[43] indicating that an offspring's methylation status is susceptible to modulation via folate-associated intermediates. Another study by the same research group showed that plasma homocysteine, LINE-1 methylation, and birth weight were associated with CpG methylation patterns in cord blood, providing further evidence that folate-associated intermediates in the mother's diet can influence not only pregnancy outcomes but also the offspring's global methylation status.[46]

Other important nutrients involved in the one-carbon metabolism cycle are vitamins B12, B2, B6, choline, and betaine. Maternal serum vitamin B12 was shown to be inversely correlated with offspring's global methylation status at birth.[47] Another study found that early pregnancy intakes of methyl donors, including vitamins B12, B2, and B6, did not impact infants' global methylation status. However, they did find that intake of choline and betaine in early pregnancy was inversely associated with global cord blood methylation among male infants only.[45] Azzi et al noted that folic acid supplementation and the use of a combination of micronutrients before or during pregnancy had no impact on methylation status of the *ZAC1* gene. However, maternal dietary B2 intake was positively correlated with *ZAC1* methylation status. Loss of methylation at the differentially methylated region of *ZAC1* is associated with infant growth retardation and diabetes development in the first weeks of life, and thus, intake of vitamin B2 could play a vital preventative role.[48] An important point made by Crider et al regarding folate, methyl donor intake, and levels of DNA methylation is the need to consider the

Table 3. Summary of human observation studies examining changes in offspring epigenome associated with maternal nutrition pregnancy.

NUTRIENT	OBSERVATION	GENE/CPG INFLUENCED	EFFECT ON OFFSPRING	STUDY SIZE (n)	REFERENCE
1. Folate	High and low serum folate (1st and 3rd quartile-extremes of exposure)	Global DNA Methylation, LINE-1, *ZFP57* gene	In CD4+ cells there was no association of serum folate with LINE-1 or global DNA methylation status. High folate group had lower levels of DNA methylation at the *ZFP57* DMR (which is associated with transient neonatal diabetes mellitus type 1).	23	44
2. B12	Serum vitamin B12 levels	Global DNA methylation, *IGBP3* gene	Higher maternal serum vitamin B12 was associated with lower global DNA methylation. Higher cord serum vitamin B12 levels were associated with reduced methylation across the *IGFBP3* locus.	121	47
3. B2 Folate, B9 Alcohol	Dietary intake in last three months of pregnancy	*ZAC1* gene	Positive correlation of dietary Vitamin B2 levels and *ZAC1* DMR methylation level. No impact of taking folic acid supplements or vitamin B9 on *ZAC1* methylation. Positive correlation of alcohol levels prior to and during pregnancy with *ZAC1* DMR methylation level.	254	48
4. B12, B2, B6, Methionine, Iron, Zinc, Cadmium, Folate Choline, Betaine	Dietary intake during weeks 0–4 and in second trimester of pregnancy	Global methylation, LINE-1	No associations of any of the nutrients in early or mid pregnancy with LINE-1 methylation levels. In males only, betaine and choline intakes were inversely associated with LINE-1 methylation levels in early pregnancy.	516	45
5. Protein, Fat Carbohydrate	Dietary intake in early pregnancy	*RXRA* gene	Protein and fat intake in early pregnancy had no association with cord *RXRA*. Low intakes of carbohydrate in early pregnancy were associated with significantly higher methylation of *RXRA* methylation.	239	30

Abbreviations: CpG, site where a cytosine nucleotide occurs next to a guanine nucleotide common area for DNA methylation; DMR, differentially methylated region; LINE-1, long interspersed element-1; *ZFP57*, zinc finger protein 57 homolog; *IGBP3*, insulin-like growth factor binding protein-3; *ZAC1*, pleomorphic adenoma gene-like 1; *RXRA*, retinoid X receptor alpha.

characteristics of a given study population, as baseline folate levels may be an important factor.[27] A study in the US looking at a population with sufficient folate intakes found no association between the intake of methyl donor nutrients during pregnancy and DNA methylation levels.[45] Their findings suggest that in a folate-replete population, excess dietary intake of folate or other nutrients has little impact on the global methylation status of the infants.

With respect to specific genes, folate intake during pregnancy has been shown to have an impact on the infant (see Table 2). One study conducted in the US reported that women who took folic acid supplements during pregnancy gave birth to infants with lower methylation levels at DNA sequences that regulate insulin-like growth factor II (*IGF2*), an imprinted gene associated with fetal growth.[49] They found that folic acid had an impact on the regulation of *IGF2* expression in this way. Similarly, another study in the Netherlands found that folic acid supplementation directly impacted the methylation status of the *IGF2* gene in the infants up to 17 months of age.[50] This group also found an association of higher *IGF2* methylation with lower birth weight, which highlights the importance of this change. There is a lack of research relating to macronutrients during pregnancy and their impact on the offspring's DNA methylation patterns. Godfrey et al found that low carbohydrate intake in early pregnancy was associated with higher methylation of the retinoid X receptor alpha (*RXRA*) gene.[30] This increase in methylation was associated with an increase in child body mass index and child fat mass. The potential mechanism for this may be through the *RXRA* gene that has been shown to interact with adipogenesis, insulin sensitivity, and fat metabolism.[30,51] However, early intakes of protein or fat had no associations with the methylation status of this gene.

Another important point to note in this area of research is the gender of the offspring. Many studies have reported gender differences in relation to DNA methylation patterns.[45,49,52,53] Boeke et al found that cord blood methylation levels are usually higher for males than females.[45] For future studies, it is advisable that gender-specific analysis be conducted and considered when interpreting results. Understanding the role of gender and how males versus females respond to environmental perturbations particularly at this basic epigenetic level could help physicians and patients to anticipate disease susceptibility. There is emerging evidence that particular patterns of DNA methylation in cord blood are associated with children's body size and composition in later years.[54] Furthermore, DNA methylation patterns at birth may predict the risk of developing particular diseases later in life, such as metabolic disorders. Given the growing childhood obesity epidemic and associated metabolic diseases, advancing our understanding of factors that influence DNA methylation during pregnancy and early life, and how to correctly interpret these patterns, may offer crucial insight into effective measures for future obesity prevention.

Conclusion

Current epigenetic studies suggest an association between maternal nutrient intake during pregnancy and the epigenetic patterns of the offspring at birth. Folate and other methyl donor nutrients appear to primarily affect the offspring's pattern of DNA methylation; however, macronutrient composition of the maternal diet can also exert an influence. In human beings, the impact of nutrients is more clearly seen when examining gene-specific methylation levels rather than overall global methylation levels. It is important that the characteristics of the study cohort, particularly current folate status and offspring gender, should be considered when interpreting results, as these have been shown to influence the impact of particular nutrients. While these results can be used to explain fetal programing in pregnancy, there remains a paucity of research in this area, particularly in human studies. The first years of life are a critical period of development, and advancements in this area of research could influence advice and guidelines regarding maternal nutrition during pregnancy and lactation. With the potential to use DNA methylation patterns at birth to predict health and growth of the child in later life, further epigenetic research is urgently required.

Author Contributions

Conceived and designed the experiments: AAG, ERG, KLL, and FMMA. Analyzed the data: AAG. Wrote the first draft of the manuscript: AAG. Contributed to the writing of the manuscript: AAG and ERG. Agree with manuscript results and conclusions: AAG, ERG, FMMA, KLL, and GA. Jointly developed the structure and arguments for the paper: AAG and ERG. Made critical revisions and approved final version: AAG, ERG, FMMA, KLL, and GA. All authors reviewed and approved of the final manuscript.

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