

Nutritional Influences on Epigenetic Programming

Asthma, Allergy, and Obesity



Debra J. Palmer, PhD^{a,b,*}, Rae-Chi Huang, MD, PhD^{b,c},
Jeffrey M. Craig, PhD^d, Susan L. Prescott, MD, PhD^{a,b,c}

KEYWORDS

• Nutrition • Early life • Epigenetic regulation • Asthma • Allergy • Obesity

KEY POINTS

- Maternal and infant nutrition play a critical role in determining the subsequent risk of asthma, allergic diseases, and obesity.
- Modern dietary patterns (including less consumption of vegetables, legumes, and fish) result in reduced antiinflammatory nutrient intakes, in particular prebiotics, antioxidants, and omega-3 long-chain fatty acids.
- Antiinflammatory nutrients modulate the developmental programming of metabolic and immune pathways, and increase the risk of the chronic inflammation and immune dysregulation seen in association with asthma, allergic diseases, and obesity.
- Epigenetics is providing substantial advances in understanding how early-life nutritional exposures can affect disease development.

INTRODUCTION

Nutrition in early life, especially from conception until 2 years of age (the first 1000 days), has a major influence on later predisposition to many noncommunicable diseases (NCDs), including cardiovascular, metabolic, and allergic diseases. Barker¹ laid the core foundations by showing the relationships between early-life conditions (particularly nutritional status) and the subsequent risk of cardiovascular and

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^a School of Paediatrics and Child Health, University of Western Australia (M561), Roberts Road, Subiaco, Western Australia 6008, Australia; ^b Members of 'In-FLAME' the International Inflammation Network, World Universities Network (WUN); ^c Telethon KIDS Institute, University of Western Australia, Roberts Road, Subiaco, Western Australia 6008, Australia; ^d Department of Paediatrics, University of Melbourne and Early Life Epigenetics Group, Murdoch Children's Research Institute, Royal Children's Hospital, Flemington Road, Parkville, Victoria 3052, Australia

* Corresponding author. School of Paediatrics and Child Health, University of Western Australia (M561), Roberts Road, Subiaco, Western Australia 6008, Australia.

E-mail address: debbie.palmer@uwa.edu.au

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metabolic diseases many decades later. This paradigm of developmental programming, now known as developmental origins of health and disease (DOHaD), has been extended to many other organ systems through a large range of cohort studies, mechanistic studies, and animal models. More recently, these have revealed that one of the main mechanisms through which nutrition can influence these long-term health outcomes is via modulation of epigenetic programming. Epigenetic mechanisms can be broadly defined as a network of biological processes that regulate the expression of genes to produce mitotically heritable changes in cellular function without changes in the underlying DNA sequence.² These processes include DNA methylation, post-translational modification to histone tails, and regulation through noncoding RNAs. This knowledge has provided new insights into how subsequent patterns of gene expression can be changed by a range of early nutritional and environmental factors to alter the risk of both early-onset and late-onset NCDs, and has become the cornerstone of DOHaD research.³

New so-called modern epidemics such as allergic disease, which is an early-onset NCD, provide evidence that the immune system is specifically vulnerable to recent environmental, diet, and lifestyle changes.⁴ Over the past 2 decades there has been a dramatic increase in the incidence of allergic disease, especially food allergy, in the first few years of life.^{5,6} Over the same period there has been a parallel increase in obesity,^{7,8} and there have been suggestions that allergic disease and obesity may be associated.⁹ It is also increasingly clear that both immune and metabolic programming are under epigenetic regulation. This article discusses recent evidence focusing on the influence of nutrition on metabolic and immune pathways that are likely to underpin the increasing rates of allergy, obesity, and other inflammatory diseases, with a particular focus on the epigenetic mechanisms.

EPIGENETICS, OBESITY, AND ASTHMA

Epigenetics has provided a substantial advance in understanding of how the early environment, including early-life nutritional exposures, can have effects on disease propensity much later in life. There are now many examples of how nutritional exposures in utero can induce differential effects on epigenetic machinery. These alterations in epigenetic marks are associated with either enhanced or suppressed gene expression with an altered phenotype depending on the nature of the affected biological pathways.²

In humans, Fryer and colleagues¹⁰ analyzed the cytosine-guanine dinucleotide (CpG) dinucleotide methylation in 12 cord blood samples using high-resolution genomewide methylation profiling, and levels of plasma homocysteine, a metabolite of folate, correlated with infant DNA methylation patterns and birth weight. In addition, it has been shown that periconceptional maternal folic acid supplementation can lead to a higher methylation level of the differentially methylated region in the *IGF2* gene in children.¹¹ In this study, DNA methylation was inversely correlated with birth weight. *IGF2* is an important regulator of fetal growth that mediates its effects through the *IGF1* receptor (*IGF1R*), and observed differential methylation patterns in *IGF2* and *IGF1R* in relation to maternal folic acid intake suggest that maternal folic acid intake influences fetal growth and metabolism.

Obesity and Epigenetics

In the context of the development of obesity, experimental evidence shows a causative link between early-life nutritional challenges and the risk of subsequent obesity and metabolic disease.^{12–14} In animal models, exposure to maternal obesity and

high-fat diets influences the risk of obesity in the offspring. Vucetic and colleagues¹⁵ showed that obese mothers fed a high-fat diet had offspring with epigenetic changes in key genes controlling appetite and metabolism. Borengasser and colleagues¹⁶ recently found hepatic mRNA expression of circadian (*CLOCK*, *BMAL1*, *REV-ERBa*, *CRY*, *PER*) and metabolic (*PPARa*, *SIRT1*) genes were strongly suppressed in offspring exposed to both maternal obesity and a high-fat diet.

Animal studies have also determined that early-life exposure to bisphenol A (BPA), a high-production-volume chemical used in the manufacture of polycarbonate plastic, can change offspring phenotype by stably altering the epigenome. However, this effect can be counteracted by maternal dietary supplements. Using an agouti mouse model, Dolinoy and colleagues¹⁷ showed that in utero exposure to BPA is associated with higher body weight by decreasing CpG methylation. However maternal dietary supplementation, with either methyl donors like folic acid¹⁷ or genistein (the major phytoestrogen in soy),¹⁸ negated the DNA hypomethylating effect of BPA.

Human evidence of altered DNA methylation leading to fetal programming of obesity was first seen at the *IGF2/H19* locus. During the Dutch Hunger Winter in the 1940s, offspring were exposed to maternal famine early in gestation. Sixty years later, these offspring had decreased methylation in a region controlling expression within the *IGF2* locus¹⁹ accompanied by increased obesity.²⁰ Periconceptional folate supplementation has been associated with altered DNA methylation in the imprinting control region of *IGF2/H19*²¹ and overweight status at 12 months of age.²² DNA methylation at the *IGF2/H19* imprinting control region has also been positively associated with subscapular, suprailiac, abdominal, and triceps skin fold thickness and subcutaneous fat thickness in adolescents.²³

Differential methylation in other loci in humans has also been associated with obesity. Methylation of specific CpGs within the promoter of the retinoid X receptor alpha (*RXRa*) gene in umbilical cord tissue at birth is associated with childhood fat mass¹² and neonatal epigenetic marks and sex of the infant explained more than 25% of the variance in childhood obesity.¹² Another example is the finding that DNA methylation in the peroxisomal proliferator gamma coactivator 1-alpha promoter in blood predicted adiposity up to 14 years of age independently of sex, age, pubertal timing, and physical activity.²⁴

Asthma and Epigenetics

Although a family history of asthma and allergic disease is a strong predictor of asthma development, there seem to be many influential environmental, lifestyle, and dietary factors that modify the inheritable risk. In particular, maternal exposure to cigarette smoke and air pollution during pregnancy are known to increase the risk,^{25,26} whereas a maternal diet during pregnancy high in fish, legumes, and vegetables decreases the risk.²⁷

Epigenetic changes are likely to improve future understanding of, and strategies for, asthma prevention. Using an adult cohort of monozygotic twins discordant for asthma, Runyon and colleagues²⁸ showed increased levels of CpG methylation within the *FOXP3* locus, decreased *FOXP3* protein expression, and impaired regulatory T-cell function. In parallel, Runyon and colleagues²⁸ also showed increased methylation of the *IFNG* locus, decreased *IFNG* expression, and reduced effector T-cell function in the asthmatic twin compared with the nonasthmatic twin. Patil and colleagues²⁹ investigated DNA methylation of the interleukin (IL)-13 gene and found interactions with asthma-related lung function. The findings from Soto-Ramirez and colleagues³⁰ also suggest that DNA methylation modulates the risk of asthma in association with genetic variants in the IL-4R gene.

Further to this, a recent pilot study ($n = 32$)³¹ found differences in DNA methylation between children with obesity-associated asthma compared with children with obesity but without asthma, nonobese asthmatic children, and healthy normal-weight controls. This study investigated epigenomewide DNA methylation in peripheral blood mononuclear cells (PBMCs) collected from preadolescent children. PBMCs from 8 obese asthmatic children had decreased promoter methylation of *CCL5*, *IL2RA*, and *TBX21* genes, which encode proteins linked to Th1 polarization, innate immune, and nonatopic patterns of inflammation. This study also found that PBMCs from obese asthmatic children had increased promoter methylation of *FCER2* and *TGFB1*. Together, these differences in methylation profiles were associated with T-cell differentiation and increased macrophage activation, which have previously been linked to the pathophysiology of obesity-associated asthma.^{32,33} Future studies further examining the potential dietary and/or lifestyle factors that may have preceded these DNA methylation effects will be of key importance.

MULTIPLE EPIGENETIC EFFECTS OF NUTRITIONAL EXPOSURES

Methyl donors such as folate, methionine, and choline derived from the diet are of much interest because of their role in DNA methylation through 1-carbon metabolism.³⁴ Furthermore, a range of other nutritional factors, including vitamin D,³⁵ antioxidants (such as selenium; zinc; and vitamins A, C, and E),^{36,37} long-chain polyunsaturated fatty acids (LCPUFAs),³⁸ and short-chain fatty acids (SCFAs),³⁹ also seem to modulate gene expression through epigenetic effects. Diet-induced changes in the microbiome⁴⁰ and even food contaminants such as bisphenol A^{41,42} can also induce epigenetic changes in genes in metabolic and/or immune networks to influence the risk of disease. Although many of these effects have been shown in animal models, more evidence in humans is greatly needed.

FOLATE (FOLIC ACID) AND DNA METHYLATION

In the early 1990s, randomized controlled trials (RCTs)^{43,44} found that folic acid supplementation in the periconceptional period significantly reduced the risk of occurrence of neural tube defects (NTDs). Over the past 20 years, folic acid supplementation of 400 $\mu\text{g}/\text{day}$ at least 1 month before and 3 months after conception has been recommended, in addition to consuming folate from foods in a varied diet. The widespread use of maternal folate supplementation in the periconceptional period has been effective at reducing the risk of NTDs. However, long-term effects of maternal folate supplementation, particularly when continued at high doses after the window of risk for NTDs has passed (beyond 3 months after conception), on other health outcomes are less clear.

In a highly cited murine study, Hollingsworth and colleagues⁴⁵ showed that maternal folic acid supplementation modifies the expression of immune genes in the offspring through changes in DNA methylation, with associated development of an allergic phenotype; specifically, an enhanced severity of allergic airway disease in mice offspring. Furthermore, evidence from an Australian human prospective birth cohort study⁴⁶ shows that folic acid taken in supplement form in late pregnancy was associated with an increased risk of childhood asthma at 3.5 years. Another Australian study found that infants exposed in utero to more than 500 $\mu\text{g}/\text{day}$ of maternal folic acid supplementation during late pregnancy (third trimester) were more likely to develop eczema during infancy.⁴⁷ A recent meta-analysis⁴⁸ (cohort and case-control studies; no RCTs), found no evidence of an association between maternal folic acid supplementation use in the periconceptional period (before and during the first trimester of

pregnancy) and asthma during childhood (0–8 years) in the offspring. Thus it seems that the timing of exposure to folate supplementation in utero may be critical with regard to childhood allergic disease outcomes.

An interesting recent study using Wistar rats⁴⁹ also provides new evidence on metabolic effects related to the timing and duration of a high-folate diet in early life. This study found improved metabolic outcomes in pups exposed to a high-folate diet both in utero as well as a high-folate postnatal diet, compared with a normal postnatal diet. These improved metabolic outcomes included lower food intake, reduced weight gain, and improved glucose response to both a glucose load and an insulin load. This new animal study evidence supports the hypothesis that folate can modify hypothalamic feeding pathways in rat offspring, possibly because of epigenetic alterations in DNA methylation. The results from this study suggest that in utero programming can be further modified by early postnatal diets. Further research in this field is needed to better define the effects of timing and duration of folate supplementation during early life on early and later health outcomes. More studies, specifically epigenome-wide association studies, are also needed to explore the wider epigenetic effects of folic acid supplementation.

BEYOND FOLATE: EPIGENETICS AND OTHER NUTRIENTS

In recent years, several other key nutrients, including antioxidants, vitamin D, LCPUFAs, and SCFAs, have been linked to multiple health outcomes, including asthma and allergic diseases, as well as obesity. Research is currently underway investigating possible epigenetic effects of changing the dietary intake of these nutrients, especially in the maternal diet during pregnancy and the early postnatal period.

Antioxidants

In vitro human studies have shown that antioxidants (such as selenium; zinc; vitamins A, C, and E) favorably alter the redox status of cells by enhancing IL-12 production by antigen-presenting cells to promote Th1 differentiation,⁵⁰ although it is not clear whether this can be extrapolated to the in vivo setting. Observational studies suggest that higher dietary intakes of antioxidant-rich foods (such as fresh fruits and vegetables) or higher antioxidant levels measured in pregnancy^{51,52} and early childhood^{53,54} may reduce the risk of wheezing, asthma, and/or eczema. As yet there are no intervention studies in early life to directly examine potential preventive effects. In part, this has been because there are 2 contrary hypotheses around the role of dietary antioxidant intake and immune outcomes. Although antioxidants have been suggested to protect against allergic disease, there is also an alternative hypothesis that proposes a theoretic concern that antioxidant supplementation could increase the probability of Th2 differentiation (by inhibiting oxidative stress) and favor the development of asthma and allergic disease.⁵⁵ As for other nutrients, the dose, timing, and duration in early life of antioxidant exposures are likely to be critical.

Long Chain Polyunsaturated Fatty Acids

Dietary omega-3 LCPUFAs have been shown to have multisystem antiinflammatory benefits on both immune and metabolic outcomes. Several RCTs investigating maternal fish oil supplementation during pregnancy have found beneficial effects on immunomodulatory effects in cord blood,^{56–58} with reduced allergen sensitization and allergic disease outcomes in the offspring.^{57,59–61} Maternal fish oil supplementation in pregnancy lowers neonatal oxidative stress⁵⁶ and neutrophil production of leukotriene B4, which correlated with reduced toll-like receptor 4 (TLR 4) mediated

inflammatory responses.⁵⁸ Other studies have also found benefits for metabolic programming⁶² and reducing cardiovascular risk,^{63,64} with higher dietary intakes of omega-3 LCPUFAs in early life.

Recent studies in mice and rats^{38,65} have found some evidence that maternal fat intake (both quantity and fatty acid composition) during pregnancy and lactation can cause changes in the epigenetic regulation of the offspring. Niculescu and colleagues⁶⁵ showed higher DNA methylation of the *Fads2* promoter after mice were given a postnatal flaxseed oil containing 50% alpha-linolenic acid (mothers or pups). Hoile and colleagues³⁸ found decreased *Fads2* mRNA expression associated with increased methylation of CpG loci in the *Fads2* promoter in the rat offspring after increasing maternal dietary fat content. This effect on epigenetic regulation was observed to continue into adulthood. Human studies are needed and currently this field of research is still in its infancy, but it will be important to interpret some of the findings of human RCTs investigating the role of omega-3 LCPUFA supplementation during pregnancy and early life on multiple health outcomes.

Vitamin D

A particular challenge will be investigating the possible role of vitamin D in epigenetic regulation. It has been estimated that around 2000 genes may be directly or indirectly regulated by various vitamin D metabolites, and possible epigenetic effects are currently thought to be involved with histone modifications, especially acetylation, and mediated by the nuclear vitamin D receptor.⁶⁶ Vitamin D status is influenced by environmental factors through sunlight exposure as well as dietary sources, through foods naturally rich in vitamin D (including fatty fish and cod liver oil), foods fortified with vitamin D in some countries (including milk and margarine), as well as the use of oral vitamin D supplementation in some populations.⁶⁶ It will be important to study the possible effects of changes to vitamin D status during early life. In particular, will there be epigenetic regulation effects in which a child experiences an in utero environment high in vitamin D from maternal oral vitamin D supplementation during pregnancy, but low vitamin D status during infancy? In the future, some of the current controversies, especially surrounding the effects of vitamin D status or use of supplementation in early life on allergic disease development, may be explained by epigenetic regulation effects.

GUT MICROBIOTA BIODIVERSITY AND SHORT-CHAIN FATTY ACIDS

Modern diets typically contain more processed, low-fiber foods than more traditional diets.^{67,68} In particular, diets are generally containing less fruit, vegetables, unprocessed grains, nuts, and seeds. This dietary pattern with reduced fiber content is associated with changes in gut microbiota biodiversity, which is another common risk factor now strongly linked with both allergy and obesity.⁴⁰ The gut microbiota and its collective genetic material (the microbiome) have emerged as important factors in normal immune development. Although the original focus of the so-called hygiene hypothesis was on declining infectious exposures, disruption of the nonpathogenic commensal gut microbiota is now recognized as a potentially greater risk for early immune and metabolic dysregulation, and is implicated in the increasing risk of obesity and many associated inflammatory NCDs.⁴⁰ Changing composition of gut microflora has been linked to associated inflammation and disruptions in gut homeostasis and immune maturation, with altered gut barrier function, increased systemic endotoxin, and low-grade TLR-mediated systemic inflammation with increased C-reactive protein, IL-1 β , tumor necrosis factor, and IL-6.^{69–71}

Animal models provide evidence that the gut microbiota modulate immune programming, and that manipulation of the microbiome can prevent not only allergic disease^{72,73} but also the risk of obesity, cardiovascular disease, and metabolic disease through well-described metabolic effects.⁷⁴ The use of soluble prebiotic fiber (oligosaccharides) has similarly been shown to have beneficial effects on both immune⁷⁵ and metabolic homeostasis.⁷⁶ Prebiotic fermentation products, SCFAs, have antiinflammatory effects⁷⁷ that promote intestinal integrity and reduce systemic endotoxin and antigenic load in experimental models. SCFAs play a critical role in local and systemic metabolic function and stimulate regulatory immune responses.⁷⁸ There is newly emerging evidence of effects of SCFA-producing bacteria on epigenetic regulation of metabolic genes, notably free fatty acid receptor in type 2 diabetes and obesity.³⁹ Our collaborators are also currently examining the epigenetic effects of SCFAs on immune genes.

The Importance of Antenatal Nutrition and Maternal Biodiversity

Most studies investigating the early immune-modulatory mechanisms have focused on postnatal microbial diversity.^{79,80} However, it is increasingly clear that the maternal environment, nutrition, and gut biodiversity during pregnancy are also important in early immune programming.^{40,81} We have shown emergent differences in immune function at birth in newborns destined to develop allergy,^{82,83} indicating that the scene is set to some extent by birth. Experimental animal models show that maternal treatment with commensal bacteria such as *Lactobacillus rhamnosus*⁸⁴ or other apathogenic bacteria such as *Acinetobacter lwoffii*⁸⁵ during pregnancy attenuate allergic sensitization and inflammation in the offspring. These effects are mediated by activation of maternal innate immune pathways, namely TLR signaling,⁸⁵ with associated epigenetic effects in immune genes (*IFNG*) in the newborn offspring.⁸⁶ This finding is consistent with human evidence that maternal probiotics in late pregnancy significantly modulate the expression of TLR-related genes both in placenta and the fetal gut.⁸⁷

Studies of specific prebiotic oligosaccharides in pregnancy are still limited. In animal models prebiotics alter colonization and metabolic homeostasis,⁸⁸ and reduce eczema-like inflammation in offspring.⁸⁹ To our knowledge, the only human RCT to use prebiotics in pregnancy was too small (n = 48) to reliably assess immune effects on the fetus or clinical effects, but did achieve favorable changes in maternal gut microbiota.⁹⁰ Larger human trials of prebiotics in pregnancy are needed to examine these effects further, including the interplay between metabolic and immune effects.

IMPORTANCE OF A WHOLE DIETARY COMPOSITION APPROACH RATHER THAN INDIVIDUAL NUTRIENTS

Research in this topic is not straightforward and inconsistencies in the human evidence base to date are likely to reflect the inherent complexity of each individual's diet, difficulty in accurately measuring it, and interplay with many other segregating environmental and lifestyle factors. Clinicians must not just consider or focus too heavily on 1 or 2 foods or nutrients, but should remember that the total diet and lifestyle combination is what is most important. For example, dietary patterns such as a Mediterranean diet have been repeatedly shown in many studies over the past decade (as reviewed in Ref.⁹¹) to have a protective effect against the development and progression of NCDs, including obesity. The Mediterranean diet describes the common foods and resulting dietary intake pattern that was characteristic of several countries in the Mediterranean Basin geographic region during the early 1960s.⁹² The Mediterranean diet during pregnancy, in particular higher consumption of vegetables, legumes, and fish, has also been associated with protection from childhood

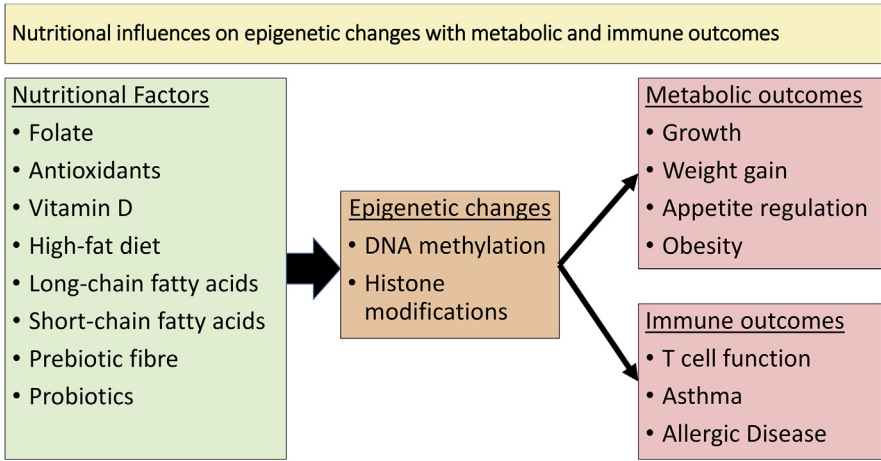


Fig. 1. Nutritional influences on epigenetic changes with metabolic and immune outcomes.

wheeze and atopy.²⁷ Given the evidence presented in this article, vegetables, legumes, and fish in particular are consistently shown to be beneficial dietary components, because they are good sources of many antioxidants, soluble prebiotic fiber, and omega-3 LCPUFAs. However, modern diets tend to be much lower in fish, vegetables, and legumes compared with more traditional diets.^{67,68} Encouraging a healthy balanced diet, including fish, vegetables, and legumes, especially in early life (the first 1000 days) seems an obvious and logical approach to reducing the risk of asthma, allergic diseases, and obesity. However, reversing current modern dietary habits, with their increased reliance on processed and convenience foods and often with low-fiber and poor nutritional quality, is a major challenge.

SUMMARY

It is now apparent that many NCDs, including asthma and obesity, share the same common early-life nutritional risk factors. Moreover, most of the modern dietary risk factors modulate developmental programming of metabolic and immune pathways to increase the risk of the chronic inflammation and immune dysregulation seen in association with many NCDs. Changing modern dietary intakes and resulting nutritional effects on these pathways provide an obvious common element contributing to the risk and prevention of these conditions, which underscores the need to take a whole-diet approach to overcoming the increasing burden of many NCDs (Fig. 1).

There is no longer any doubt that maternal and infant nutrition play critical roles in determining the subsequent risk of asthma, allergic diseases, and obesity. The increasing reliance on dietary supplementation and fortification of foods (eg, with folate) may be causing more harm than benefit. Effective strategies are needed to increase dietary intakes of particular foods (eg, fish, vegetables, and legumes) and nutrients (eg, antioxidants, LCPUFAs, and SCFAs) that have many properties that protect against certain NCDs. Further research on changing nutritional intakes and epigenetic consequences will improve the evidence base.

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