Nutrition in early life, immune-programming and allergies: the role of epigenetics

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Summary

Early life nutritional exposures are significant determinants of the development and future health of all organ systems. The dramatic rise in infant immune diseases, most notably allergy, indicates the specific vulnerability of the immune system to early environmental changes. The associated parallel rise in metabolic diseases including obesity, childhood type 2-diabetes and non-alcoholic fatty liver disease highlights the interplay between modern dietary patterns and increasing abnormalities of both immune and metabolic health. The low-grade inflammation that characterize these non-communicable diseases (NCDs) suggests a central role of the immune system in the pathogenesis of these conditions. Understanding how environmental influences disrupt the finely balanced development of immune and metabolic programing is of critical importance. Diet-sensitive pathways are likely to be crucial in these processes. While epigenetic mechanism provides a strong explanation of how nutritional exposures can affect the fetal gene expression and subsequent disease risk, other diet-induced tissue compositional changes may also contribute directly to altered immune and metabolic function. Although modern dietary changes are complex and involve changing patterns of many nutrients, there is also interest in the developmental effects of specific nutrients such as folic acid levels, which have clear epigenetic effects on programming. Here we examine the current knowledge of the nutritional-programming of immune health and how research into nutritional-epigenetics in the context of allergic disease as one of the earliest onset NCDs can expand our knowledge to discover the biological processes sensitive to nutritional exposures in early life to prevent later disease risk. *(Asian Pac J Allergy Immunol 2013;31:175-82)*

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Introduction

Nutrition is the most influential environmental factor during fetal development. Prenatal nutrition influences fetal growth and development of physiological functions of all organ systems.[1] Similarly, postnatal nutritional exposures are critical for the ongoing developmental maturation of many organ systems and optimal physiological functions.[2] It has been shown that environmental exposures including nutritional exposures during these critical and sensitive periods of life can have permanent changes in many physiological processes, which is known as "programing". From a series of studies Barker and colleagues demonstrated how the effects of early programing extend to adult health, and that nutritional patterns in early life are linked to the risk of cardio-vascular and metabolic diseases many decades later.[3] This relatively new field of research has become known as Developmental Origin of Health and Disease (DOHaD).

Complex environmental and lifestyle changes have been implicated in the dramatic increase in cardiovascular, metabolic, some cancers, chronic lung diseases and allergic diseases (collectively known as non-communicable diseases, NCDs). Of all of the potential environmental culprits, modern dietary changes are among the most likely factors implicated in the rising risk of both immune (allergic)[4] and metabolic (obesity and type 2 diabetes)[5] diseases. Although there are wide regional variations, many modern diets typically contain more processed and synthetic foods rich in fats and refined carbohydrates with lower amounts of fibre, fresh fish, fruits and vegetables compared to more traditional diets. These changes have been associated with changes in the gut microbiome, metabolic responses and immune function - all of which may contribute to the rising propensity for chronic low-grade inflammation and altered homeostatic mechanisms which are common risk

factors for virtually all NCDs. Viewed in this way, the immune system can be seen as an integral part of the pathogenesis of these diverse conditions, and may mediate some of the effects of modern environmental changes. This may also explain the epidemiological association between allergic diseases and metabolic diseases.[6,7] Notably in children, Immunoglobulin E (IgE) levels are linearly related to body mass index (BMI) and c-reactive protein (CRP), and childhood obesity is an independent risk factor for food allergy.[8]

While it has been recognised for some time that nutritional changes (and other environmental exposures) could modify patterns of gene expression with lasting influences on phenotype and function, it has been only relatively recently identified that epigenetic mechanisms play a key role in this process. Epigenetic mechanisms can be broadly defined as a network of biological processes that regulate the expression of genes, to produce changes in cellular function without changes in the underlying DNA sequence.[9] These processes include DNA methylation, post-translational modification to histone tails and regulation through non-coding RNAs (ncRNAs).This has provided new insights into how gene expression can be altered by a range of early nutritional and environmental factors, and has become the cornerstone of DOHaD research.[10]

Many mechanisms are involved in diet-induced immune modulation

Although genetic factors can influence individual susceptibility to disease, the dramatic rise in so many NCDs cannot be explained by genetic propensity. Among the NCDs, allergic disease is typically the first to manifest, often presenting within the first few months of life. Moreover, we and others have shown that this is predated by detectable differences in immune function at birth in affected children.[11-13] This is a clear indication that the gene-environmental interactions that lead to allergic disease are already operating in the antenatal period, and this is a critical period in determining subsequent immune ontogeny.[14]

Experimental, epidemiological and intervention studies all indicate that nutritional exposures during this critical period of life can influence the development of immune system[4] and metabolic responses (discussed further below) to modify the future disease susceptibility. Epidemiological studies have revealed how favourable dietary patterns, such as the Mediterranean diet in pregnancy and in early childhood can have a protective effect on persistent wheeze and atopy in children.[15,16] This dietary pattern is also associated with reduced risk of diabetes,[17] cardiovascular disease, some cancers and other NCDs.[18] Notably, these beneficial effects reflect composite dietary patterns, and are difficult to attribute to a single dietary element. On the other hand, there are also a number of studies that have taken a 'component' approach to demonstrate the specific immunomodulatory properties of individual dietary components such as vitamins, minerals and long-chain polyunsaturated fatty acids (LCPUFA).[4]

Modulation of gene expression through epigenetic changes is one important mechanism by which dietary exposures can lead to changes in immune development.[9] The epigenetic program regulates all aspects of mammalian development including developmental timing and expression of immune genes. The relative differences in immune gene expression between neonates and adults are associated with differences in epigenetic profiles reflecting the role of epigenetics in immune development.[19-21] External environmental pressures such as dietary exposures can lead to subtle variations in epigenetic regulation of immune gene expression, which can potentially lead to more profound effects on subsequent immune function, clinical phenotype and disease risk.[22]

The local tissue microenvironment is an important determinant of immune development. Changes in the local tissue milieu can modify the pattern of effector response[23] potentially also through epigenetic changes. Environmental factors which modify the local microenvironment therefore have significant potential to alter immune programing and the propensity for inflammation. Of these, a range of dietary factors (such as LCPUFA and antioxidants)have recognised effects on tissue milieuas a result of their influence as metabolic components, substrates or structural components of cells and tissues, with downstream effects on gene expression through a number of different pathways.[24,25]

Long chain polyunsaturated fatty acids (LCPUFA) are good examples of dietary factors that have multiple metabolic and structural functions that influence the propensity for inflammation. As structural components of cell membranes, they influence membrane fluidity and cell signaling.[26] As substrates for prostanoid production, they influence the level of inflammatory prostaglandins[27] and as substrates for resolvins they influence the local

control of inflammation.[28] Animal studies demonstrate that dietary modulation with LCPUFA (a combination of n-3 and n-6 PUFA) can change the local production of immunomodulatory factors by the skin keratinocytes including interleukin-10 (IL-10) and thymic stromal lymphopoietin (TSLP) thereby influencing skin inflammation.[29] In humans we have previously demonstrated that high dose n-3 PUFA supplementation in pregnancy can modulate fetal oxidative stress,[30] leukotriene metabolism[31] with associated effects on immune function in cord blood.[32] Clinical trials in pregnancy and during the early postnatal period using fish oil supplementation have shown that these immunomodulatory properties of n-3 PUFA have been associated with a reduction in some allergic diseases suggesting that the biological effects have clinical relevance.[32-35] This adds credence to the epidemiological observations that reduced intake of n-3 PUFA and increased intake of nutrients rich in n-6 PUFA such as in margarine has shown to be associated with the rise in allergic disease.[36-38]

Antioxidants (such as selenium, zinc, vitamin C and vitamin E) are other examples of dietary nutrients that have immunomodulatory effects potentially through changes in the local tissue milieu. In vitro human studies have shown that by favourably altering the 'redox' status of cells, antioxidants can enhance IL-12 production by antigen presenting cells to promote Th1 differentiation,[39] although it is not clear if 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[40,41] and early childhood[42,43] 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 two contrary hypotheses around the role of dietary antioxidant intake and immune outcomes.[44] While antioxidants have been suggested to protect against allergic disease, there has also been conjecture that oxidative stress, which increases the production of reactive oxygen species (ROS) by macrophages could favour Th1 immune differentiation. This alternative hypothesis proposes a theoretical concern that antioxidant supplementation could increase the probability of Th2 differentiation (by inhibiting oxidative stress) and favour the development of asthma and allergic disease.[45]

One of the most important environmental changes implicated in early immune dysregulation and the rise in allergic disease, is the changing microbiome associated with progressively more 'hygienic environments'. While this was initially attributed to antibiotic use[46,47] and reduced exposure to infectious agents,[48] it now seems that other changes in dietary profiles are a major determinant of the gut microbiome and biodiversity.[49] There is now good evidence that high-fat, low-fibre diets can alter the gut microbiome leading to the promotion of low-grade chronic inflammation.[50, 51] Experimental mouse models have been used to demonstrate the importance of neonatal colonization with a diversified intestinal microbiota for successful induction of oral tolerance to ovalbumin.[52,53] In humans there is also evidence that infants who go on to develop allergic disease have altered pattern of gut microbiota in early life, again indicating the importance of a balanced gut microbiome in immune programming.[54,55] The use of prebiotics fibre to promote more favourable colonisation in infancy with demonstrable reduction in allergic disease[56,57] further highlights the role of dietary nutrients in modulating immune development through effects on the intestinal microbiota. Furthermore, gut microbiota can interact with immune pathways through their capacity to influence the host epigenetic mechanisms. For an example, short chain fatty acid by-products of microbial fermentation of dietary fibre, have also shown to affect the enzymes involved in post-translational modification of histone proteins and gene expression[58] providing another possible epigenetic mechanism in the nutrition-gut microbiota and immune responses.

A range of other specific nutrients have also been implicated in the rise of allergic conditions and many other inflammatory diseases.[4] Some of these, such as vitamin D, have recognised effects on immune function, as well as epidemiological associations with allergic disease and other NCDs, as reviewed in more detail elsewhere.[59] Others, such as folate, have been of particular interest because of epigenetic effects on immune programming in animal models (below).

Collectively these studies underscore the importance of early events during immune programming and how dietary factors can influence these processes.

Modern dietary changes have wider implications in both immune and metabolic pathways

Dietary change is one of the most significant lifestyle changes experienced in modern industrialised societies, with spiralling rates of obesity and associated chronic inflammatory NCDs.In the USA, rates of obesity have risen from less than 15% in the 1960's, to over 35% in 2010.[60] In Australia, more than 28% adults are now obese (and 63% are either overweight or obese).[61] The same trends are now evident in developing regions of Asia and South America, and if anything, the increase appears to be occurring more rapidly in regions experiencing rapid economic transition. In China, the obesity rates have tripled in the last decade. Global projections by the World Health Organisation estimate that 2.3 billion adults will be overweight and 700 million will be obese by 2015. Of greatest concerns, the rising rates of obesity in childhood have major long-term implications for health through adverse effects on chronic metabolic and immune dysregulation.

Obesity is a chronic inflammatory state. Body compositional changes (visceral obesity) associated with the modern dietary patterns also appear to modify the immune microenvironment of the local tissues. Obesity in mice and humans is associated with infiltration of adipose tissue by macrophages, CD8+ T-cells and CD4+ T-cells expressing inflammatory cytokines and chemokines such as TNF, CCL2, IL-2, IFNγ and IL-17[62-64] in contrast to the accumulation of immunosuppressive regulatory T- cell populations (Tregs) in adipose tissue in lean mice.[65] Alteration of metabolic pathways caused by these nutritional changes can also modulate the immune response. For example mTOR (mammalian target of rapamycin) signalling which integrates many metabolic pathways showed altered activity following a high fat diet in mice[66] and also appears to influence the immune environment by regulating CD8+ T-cell trafficking and dendritic cell differentiation.[67,68] These underlying immunological changes are likely to contribute to the significantly higher CRP levels in overweight and obese individuals (as a marker for systemic inflammation). These correlations between BMI and CRP are also seen in young children.[8]

Chronic inflammation affects many tissues and is likely to contribute to the risk of the diverse range of chronic NCDs that are associated with obesity including type 2-diabetes, cardiovascular disease, neurocognitive disorders, and non-alcoholic fatty liver disease(NAFLD).[69] More recently, obesity in childhood has also been recognised as a risk factor for food allergy, suggesting that the chronic low grade systemic inflammation associated with obesity plays a role in the rising predisposition to an allergic phenotype.[8] Furthermore, the rising rates of maternal obesity in pregnancy, with associated metabolic and inflammatory changes, have additional implications for many aspects of fetal programming. The effects of maternal obesity on fetal metabolic programming are well recognised[70] and the effects on other immune and physiological processes needs to be further investigated.

There are also emerging interactions between metabolic and immune programming and the gut microbiota. The changes in gut immune regulation that have been implicated in the rise in allergy and autoimmunity[54,55] have also been linked to the rise in obesity and metabolic disease.[71,72] The changes in the gut microbiome which are associated with a high-fat, low-fibre diet induces changes in gut permeability, higher systemic antigenic load (low grade endotoxaemia) and higher serum levels of inflammatory cytokines.[50,51] Animal studies demonstrate how gut microbiota can regulate expression of genes that affect fatty acid oxidation and fat deposition in host adipocytes.[73] In humans, obese individuals have documented differences in gut microbiota compared to lean individuals, suggesting a role of gut microbiota in maintaining host metabolic homeostasis.[74] These observations collectively highlight the complex interplay between metabolic and immune programming in the gut and provide new perspectives on how diet-induced alterations to gut microbiome can have multisystem effects.

Although there has been a dominant focus on postnatal microbial effects, changing patterns of maternal diet and colonisation in pregnancy could have multifaceted effects on fetal programming, including direct effects on maternal metabolic and immune function, which modulate fetal programming, as well the more obvious effects on postnatal colonization of the infant. There is emerging evidence of intrauterine effects in animal models, which reveal that maternal exposure to both pathogenic[75] and nonpathogenic[76] bacteria protect offspring from developing an allergic phenotype. This may be mediated in part through epigenetic effects.[77] Accordingly, in humans the use of probiotic bacteria in pregnancy has been associated with both metabolic and immune benefits with a

reduction of infant eczema[78] reduced gestational diabetes,[79] favourable effects on fetal metabolic programming (proinsulin levels)[80] and breast milk adiponectin.[81] Although data are still limited, this is another important avenue of investigation in reducing the risk of NCDs.

Several other specific nutrients that are of interest in the context of immune modulation and allergy prevention also have metabolic and cardio-protective effects, namely n-3PUFA[82] antioxidants[83] and vitamin D,[84] even in childhood.[85] This emphasises the complex multisystem effects of modern dietary changes, and how restoring a more traditional dietary pattern may improve both immune and metabolic homeostasis to reduce the risk of many NCDs, particularly if this is instituted early in development.

Epigenetics at the crossroad between nutritionally mediated immune and metabolic pathways

The discovery of epigenetic programming provided a clear mechanism by which nutritional exposures during critical periods of life can have lasting effects on later health. There are now good examples in animal models[22,86] of how dietary changes can directly influence the epigenetic machinery by inhibiting the enzymes that catalyse DNA methylation or histone modifications or by altering the availability of substrates necessary for enzymatic reactions. These alterations in epigenetic marks lead to either enhanced or supressed gene expression with an altered phenotype depending on the nature of the affected biological pathways.[9]

Methyl donors derived from the diet (such as folate, methionine and choline) are therefore logically of much interest because their role in DNA methylation through 1-carbon metabolism. In a highly cited murine study, Hollingsworth et al, demonstrated 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.[22] Another animal (sheep) model also showed how early dietary intervention during the periconceptional period (restricting the supply of cofactors for the methionine cycle) lead to altered DNA methylation pattern of fetal liver tissue and long term effects on immune and metabolic function in the adult offspring - including altered body composition, altered immune responses and change in insulin-sensitivity.[87] In humans, Fryer et al.[88] analysed the CpG dinucleotide methylation in 12 cord blood samples using a high-resolution genome-wide methylation profiling and found that plasma homocysteine (a metabolite of folate) levels correlated with DNA methylation patterns. Gene ontology analysis revealed genes associated with birth weight centile (also suggesting growth associations) were enriched for terms associated with lipid metabolism. Together, these findings indicate that nutritional changes that influence one-carbon metabolism can lead to wide spread DNA methylation changes in many biological pathways that modify the risk of immune as well as metabolic diseases in later life.

Maternal dietary micronutrients (such as B vitamins, folate, zinc) may also have a role in modifying the methylation status of imprinted genes.[89-92] Imprinted genes are organized in clusters and are expressed from only one of the parental alleles. The H19/IGF2 cluster is an example for such imprinting and the regulation of gene expression involves methylation of differentially methylated regions (DMRs). Alterations to epigenetic marks at these DMRs have been associated with a number of diseases including growth disorders such as Beckwith–Wiedemann and Silver–Russell syndrome[93] and colon cancers.[94] IGF2 is an important regulator of fetal growth which mediates its effects through 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. It has been shown that periconceptional maternal folic acid supplementation can lead to a higher methylation level of IGF2 DMR in children with inverse correlations between DNA methylation and birth weight.[89] This provides further evidence of a link between *in utero* folic acid exposure, aberrant DNA methylation and altered growth pattern. Evidence from these human studies together with the observed effects of maternal folic acid supplementation on immune genes in animal experiments[22] is highly suggestive of global effects of *in utero* folic acid exposure. Of note, genetic factors also appear to play a role in determining the effects of methyl donors on DNA methylation as gene variants encoding for enzymes in folate metabolism appear to influence the DNA methylation in association with methyl donor intake.[90,95] Nevertheless, more studies, specifically epigenome wide association studies are needed to explore the wider epigenetic effects of folic acid supplementation.

Conclusions

There is now good evidence that nutrients can modify immune and metabolic programing during sensitive periods of mammalian development, namely the fetal and early postnatal periods. Modern dietary patterns increase the risk of both immune and metabolic dysregulation with associated increased risk of a broad range of NCDs. In addition to changes in nutrient profiles and caloric burden, diet-induced changes in gut microbiota are also implicated in the pathogenesis and increased propensity for many chronic diseases. In this context allergic disease is highly relevant as one of the earliest onset NCDs and a potential early measure of efficacy in prevention strategies aimed at improving immune and metabolic health.

Much research on nutritional epigenetics to date has taken a candidate gene approach that may not give a complete picture of the nature of the nutritional effects on the epigenetic landscape. The use of a whole genome approach has the potential of unravelling the novel epigenetic targets that are sensitive to a particular dietary modification. As for any other epigenome wide association studies, epigenetics of allergic diseases has a considerable challenge to explore whether epigenetic variation is the cause or the consequence of the disease. However, elucidating the potential relationship between epigenetic variations in allergic diseases offers new opportunities in diagnostics and/or therapeutics. Furthermore, investigation of dietary effects on different epigenetic marks (DNAmethylation, histone acetylation and microRNA) across the genome will advance our understanding of the nutritionally orchestrated epigenetic events. Our greatest challenge is to identify the stages in life-course during which epigenetic machinery is most sensitive for a particular dietary factor in relation to health outcomes so that interventional strategies could be planned to get the maximum benefit from dietary modifications. Understanding how nutritional manipulations alter the epigenetic machinery to affect the key immune and metabolic genes will help us to better identify strategies to modify the risk of many inflammatory diseases. Most importantly, the many complex multisystem interactions highlight the need for interdisciplinary approaches in overcoming the rising burden of so many NCDs.

Conflicts of interest

Dr. Debra Palmer reported no potential conflicts of interest relevant to this article.

Prof. Susan Prescott is on the advisory Boards of Danone and the Nestle Nutrition Institute and has received speaker's fees from these organisations and from ALK Abello. None of these is relevant to this article.

Dr. Manori Amarasekera reported no potential conflicts of interest relevant to this article.

References

1. Harding JE, Johnston BM. Nutrition and fetal growth. Reprod Fertil Dev. 1995;7:539-47.
2. Caron E, Ciofi P, Prevot V, Bouret SG. Alteration in neonatal nutrition causes perturbations in hypothalamic neural circuits controlling reproductive function. J Neurosci. 2012;32:11486-94.
3. Barker DJ. In utero programming of chronic disease. Clin Sci (Lond). 1998;95:115-28.
4. West CE, Videky DJ, Prescott SL. Role of diet in the development of immune tolerance in the context of allergic disease. Curr Opin Pediatr. 2010;22:635-41.
5. Kolb H, Mandrup-Poulsen T. The global diabetes epidemic as a consequence of lifestyle-induced low-grade inflammation. Diabetologia. 2010;53:10-20.
6. Rzehak P, Wijga AH, Keil T, Eller E, Bindslev-Jensen C, Smit HA, et al. Body mass index trajectory classes and incident asthma in childhood: Results from 8 European Birth Cohorts-a Global Allergy and Asthma European Network initiative. J Allergy Clin Immunol. 2013.131:1528-36.
7. Luo X, Xiang J, Dong X, Cai F, Suo J, Wang Z, et al. Association between obesity and atopic disorders in Chinese adults: an individually matched case-control study. BMC Public Health. 2013;13:12.
8. Visness CM, London SJ, Daniels JL, Kaufman JS, Yeatts KB, Siega-Riz AM, et al. Association of obesity with IgE levels and allergy symptoms in children and adolescents: results from the National Health and Nutrition Examination Survey 2005-2006. J Allergy Clin Immunol. 2009;123:1163-9.
9. Waterland RA, Michels KB. Epigenetic epidemiology of the developmental origins hypothesis. Annu Rev Nutr. 2007;27:363-88.
10. Gluckman PD, Hanson MA, Mitchell MD. Developmental origins of health and disease: reducing the burden of chronic disease in the next generation. Genome Med. 2010;2:14.
11. Prescott SL, Noakes P, Chow BW, Breckler L, Thornton CA, Hollams EM, et al. Presymptomatic differences in Toll-like receptor function in infants who have allergy. J Allergy Clin Immunol. 2008;122:391-9.
12. Prescott SL, Taylor A, King B, Dunstan J, Upham JW, Thornton CA, et al. Neonatal interleukin-12 capacity is associated with variations in allergen-specific immune responses in the neonatal and postnatal periods. Clin Exp Allergy. 2003;33:566-72.

13. Tulic MK, Hodder M, Forsberg A, McCarthy S, Richman T, D'Vaz N, et al. Differences in innate immune function between allergic and nonallergic children: new insights into immune ontogeny. J Allergy Clin Immunol. 2011;127:470-8.
14. Prescott SL. Early-life environmental determinants of allergic diseases and the wider pandemic of inflammatory noncommunicable diseases. J Allergy Clin Immunol. 2013;131:23-30.
15. Castro-Rodriguez JA, Garcia-Marcos L, Alfonseda Rojas JD, Valverde-Molina J, Sanchez-Solis M. Mediterranean diet as a protective factor for wheezing in preschool children. J Pediatr. 2008;152:823-8.
16. Chatzi L, Torrent M, Romieu I, Garcia-Esteban R, Ferrer C, Vioque J, et al. Mediterranean diet in pregnancy is protective for wheeze and atopy in childhood. Thorax. 2008;63:507-13.
17. Viscogliosi G, Cipriani E, Liguori ML, Marigliano B, Saliola M, Ettorre E, et al. Mediterranean Dietary Pattern Adherence: Associations with Prediabetes, Metabolic Syndrome, and Related Microinflammation. Metab Syndr Relat Disord. 2013;11:201-6.
18. Sofi F, Abbate R, Gensini GF, Casini A. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. Am J Clin Nutr. 2010;92:1189-96.
19. White GP, Hollams EM, Yerkovich ST, Bosco A, Holt BJ, Bassami MR, et al. CpG methylation patterns in the IFNgamma promoter in naive T cells: variations during Th1 and Th2 differentiation and between atopics and non-atopics. Pediatr Allergy Immunol. 2006;17:557-64.
20. White GP, Watt PM, Holt BJ, Holt PG. Differential patterns of methylation of the IFN-gamma promoter at CpG and non-CpG sites underlie differences in IFN-gamma gene expression between human neonatal and adult CD45RO- T cells. J Immunol. 2002;168:2820-7.
21. Porras A, Kozar S, Russanova V, Salpea P, Hirai T, Sammons N, et al. Developmental and epigenetic regulation of the human TLR3 gene. Mol Immunol. 2008;46:27-36.
22. Hollingsworth JW, Maruoka S, Boon K, Garantziotis S, Li Z, Tomfohr J, et al. In utero supplementation with methyl donors enhances allergic airway disease in mice. J Clin Invest. 2008;118:3462-9.
23. Matzinger P, Kamala T. Tissue-based class control: the other side of tolerance. Nat Rev Immunol. 2011;11:221-30.
24. Prescott SL, Dunstan JA. Prenatal fatty acid status and immune development: the pathways and the evidence. Lipids. 2007;42:801-10.
25. Seifried HE, Anderson DE, Fisher EI, Milner JA. A review of the interaction among dietary antioxidants and reactive oxygen species. J Nutr Biochem. 2007;18:567-79.
26. Stulnig TM, Zeyda M. Immunomodulation by polyunsaturated fatty acids: impact on T-cell signaling. Lipids. 2004;39:1171-5.
27. Bagga D, Wang L, Farias-Eisner R, Glaspy JA, Reddy ST. Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. Proc Natl Acad Sci U S A. 2003;100:1751-6.
28. Ariel A, Serhan CN. Resolvins and protectins in the termination program of acute inflammation. Trends Immunol. 2007;28:176-83.
29. Weise C, Heunemann C, Loddenkemper C, Herz U, van Tol EA, Worm M. Dietary docosahexaenoic acid in combination with arachidonic acid ameliorates allergen-induced dermatitis in mice. Pediatr Allergy Immunol. 2011;22:497-504.
30. Barden AE, Mori TA, Dunstan JA, Taylor AL, Thornton CA, Croft KD, et al. Fish oil supplementation in pregnancy lowers F2-isoprostanes in neonates at high risk of atopy. Free Radic Res. 2004;38:233-9.
31. Prescott SL, Barden AE, Mori TA, Dunstan JA. Maternal fish oil supplementation in pregnancy modifies neonatal leukotriene production by cord-blood-derived neutrophils. Clin Sci (Lond). 2007;113:409-16.
32. Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG, et al. Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial. J Allergy Clin Immunol. 2003;112:1178-84.
33. Palmer DJ, Sullivan T, Gold MS, Prescott SL, Heddle R, Gibson RA, et al. Effect of n-3 long chain polyunsaturated fatty acid supplementation in pregnancy on infants' allergies in first year of life: randomised controlled trial. BMJ. 2012;344:e184.
34. Furuhjelm C, Warstedt K, Larsson J, Fredriksson M, Bottcher MF, Falth-Magnusson K, et al. Fish oil supplementation in pregnancy and lactation may decrease the risk of infant allergy. Acta Paediatr. 2009;98:1461-7.
35. D'Vaz N, Meldrum SJ, Dunstan JA, Martino D, McCarthy S, Metcalfe J, et al. Postnatal fish oil supplementation in high-risk infants to prevent allergy: randomized controlled trial. Pediatrics. 2012;130:674-82.
36. Sausenthaler S, Koletzko S, Schaaf B, Lehmann I, Borte M, Herbarth O, et al. Maternal diet during pregnancy in relation to eczema and allergic sensitization in the offspring at 2 y of age. Am J Clin Nutr. 2007;85:530-7.
37. Oien T, Storro O, Johnsen R. Do early intake of fish and fish oil protect against eczema and doctor-diagnosed asthma at 2 years of age? A cohort study. J Epidemiol Community Health. 2010;64:124-9.
38. Hodge L, Salome CM, Peat JK, Haby MM, Xuan W, Woolcock AJ. Consumption of oily fish and childhood asthma risk. Med J Aust. 1996;164:137-40.
39. Utsugi M, Dobashi K, Ishizuka T, Endou K, Hamuro J, Murata Y, et al. c-Jun N-terminal kinase negatively regulates lipopolysaccharide-induced IL-12 production in human macrophages: role of mitogen-activated protein kinase in glutathione redox regulation of IL-12 production. J Immunol. 2003;171:628-35.
40. Devereux G, Turner SW, Craig LC, McNeill G, Martindale S, Harbour PJ, et al. Low maternal vitamin E intake during pregnancy

is associated with asthma in 5-year-old children. Am J Respir Crit Care Med. 2006;174:499-507.

41. Martindale S, McNeill G, Devereux G, Campbell D, Russell G, Seaton A. Antioxidant intake in pregnancy in relation to wheeze and eczema in the first two years of life. Am J Respir Crit Care Med. 2005;171:121-8.
42. Forastiere F, Pistelli R, Sestini P, Fortes C, Renzoni E, Rusconi F, et al. Consumption of fresh fruit rich in vitamin C and wheezing symptoms in children. SIDRIA Collaborative Group, Italy (Italian Studies on Respiratory Disorders in Children and the Environment). Thorax. 2000;55:283-8.
43. Okoko B, Burney P, Newson R, Potts J, Shaheen S. Childhood asthma and fruit consumption in South London. The European Respiratory Journal. 2007;26:1161-8.
44. Nurmatov U, Devereux G, Sheikh A. Nutrients and foods for the primary prevention of asthma and allergy: systematic review and meta-analysis. J Allergy Clin Immunol. 2011;127:724-33.
45. Murr C, Schroecksnadel K, Winkler C, Ledochowski M, Fuchs D. Antioxidants may increase the probability of developing allergic diseases and asthma. Med Hypotheses. 2005;64:973-7.
46. Alm JS, Swartz J, Lilja G, Scheynius A, Pershagen G. Atopy in children of families with an anthroposophic lifestyle. Lancet. 1999;353:1485-8.
47. Wickens K, Pearce N, Crane J, Beasley R. Antibiotic use in early childhood and the development of asthma. Clin Exp Allergy. 1999;29:766-71.
48. Strachan DP. Hay fever, hygiene, and household size. BMJ. 1989;299:1259-60.
49. Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature. 2011;474:327-36.
50. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56:1761-72.

Reference 51-95 are available online