

REVIEW

Maternal diet and its influence on the development of allergic disease

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Summary

The early presentation of childhood allergies and the rise in their prevalence suggest that changes in early-life exposures may increase the predisposition. Very early-life exposures may act upon the developing foetal immune system and include infection, environmental tobacco smoke, other pollutants and nutrients provided via the mother. Three nutrients have come under close scrutiny: vitamin D, omega 3 polyunsaturated fatty acids (PUFAs) and folate (or the synthetic form, folic acid). Much of the data on these nutrients are observational although some randomised, placebo-controlled trials have been conducted with omega 3 PUFAs and one with vitamin D. Some studies with omega 3 PUFA supplements in pregnancy have demonstrated immunomodulatory effects on the neonate and a reduction in risk of early sensitisation to allergens. A few studies with omega 3 polyunsaturated fatty acid supplements in pregnancy have shown a reduction in proportion of children affected by allergic symptoms (food allergy) or in symptom severity (atopic dermatitis). Observational studies investigating the association of maternal vitamin D intake or maternal or neonatal vitamin D status have been inconsistent. One randomised, controlled trial of vitamin D supplementation during pregnancy did not show any significant effect on allergic outcome in the offspring. Studies investigating the association between maternal folic acid or folate intake or maternal or neonatal folate status and offspring risk of allergic disease have been equivocal. Further evidence is required to clarify whether increased intake of these nutrients during pregnancy influences allergic disease in the offspring. In the light of current evidence, mothers should not either increase or avoid consuming these nutrients to prevent or ameliorate allergic disease in their offspring. However, these essential nutrients each have important roles in foetal development. This is reflected in current government recommendations for intake of these nutrients by pregnant women.

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Introduction

Allergic diseases including non-IgE and IgE-mediated allergies often manifest very early in infant life. Thus, to influence primary development of allergy through diet, any effect of nutrition would need to be exerted in very early life, perhaps upon the foetus, and thus via dietary influences on the mother during pregnancy or even before conception. The influence of nutrition on allergic disease has been broadly reviewed elsewhere [1]. More specifically, the influence of maternal nutrition during pregnancy on the primary development of early allergic disease in the offspring has come under scrutiny. There have been a number of areas of interest including dietary avoidance of food allergens and the

influence of intake of certain foods and specific nutrients such as antioxidants, vitamins and minerals [2]. The dietary avoidance of particularly allergenic food (such as fish, cows' milk, hens' egg or peanuts) by the pregnant mother was investigated in a number of studies in the 1980s and 1990s. The milk or eggs come from more than one animal thus not the milk from one cow but milk from the species. This proved to be an extremely difficult strategy to implement, did not appear to confer any protective or ameliorating effect on infant allergies, and carried the risk for avoidance of certain allergens to cause nutrient deficiencies in the mothers (reviewed in ref. 3). Indeed, in a recent report, the incidence of peanut or tree nut allergy in the offspring of 8059 non-allergic mothers was significantly lower

among those children born to mothers with a higher peanut and tree nut intake during pregnancy (≥ 5 times per month) when compared with the incidence among children born to mothers with a lower peanut and tree nut intake (< 1 time per month) during pregnancy [4]. More recently, interest has been focussed on a number of essential nutrients, vitamin B9 or folate (and folic acid – the synthetic form used in supplements), vitamin D and omega 3 long-chain polyunsaturated fatty acids (LC PUFAs). This review article will be confined to these latter nutrients and will examine data that have emerged from studies of altered intake or status during pregnancy on allergic outcomes in the offspring. Where available, outcomes from intervention studies are included.

Omega 3 long-chain polyunsaturated fatty acids

Originally termed 'vitamin F' when discovered, long-chain polyunsaturated fatty acids (LC PUFAs) are essential for human health. They consist of a long hydrocarbon chain (typically 20 or 22 carbons in length) with a carboxyl group (acid) at one end and a methyl group at the other. Within the hydrocarbon chain, there are a number double bonds linking carbon atoms; this gives rise to the term polyunsaturated. The position of the first of these double bonds counting from the methyl end of the hydrocarbon chain influences the structure and function of the fatty acid molecule and is dictated by the enzyme which inserts the double bond (these enzymes are termed desaturases). For omega 3 LC PUFAs, this first double bond is between the third and fourth carbons from the methyl end of the molecule. The desaturase enzyme required to insert the 'n-3 double bond' is not expressed by mammals but is present in plants and in lower organisms. However, mammals do produce the enzymes that metabolise and interconvert LC PUFAs. Nevertheless, omega 3 LC PUFAs must be provided in the diet. Oily fish (mackerel, sardines, pilchards, herring, fresh tuna, salmon) are rich dietary sources of omega 3 LC PUFAs because organisms at the start of the marine food chain like phytoplankton synthesise the omega 3 LC PUFAs. The omega 3 LC PUFAs that are most abundant in oily fish and in supplements based on fish oil are the twenty-carbon eicosapentaenoic acid (EPA) and the twenty-two-carbon docosahexaenoic acid (DHA). Omega 3 LC PUFAs are essential for foetal development, particularly for neurological and retinal tissue [5]. The current UK government guidelines recommend that pregnant and lactating women should consume 1–2 portions (~140 g each) of fish per week, at least one of which should be oily fish [6]. This is intended to provide the required omega 3 LC PUFAs, and particularly DHA during pregnancy (which would equate to approximately 450 mg EPA and DHA

daily from 1 to 2 portions of oily fish per week). This intake of fish is capped at 2 portions per week to avoid consuming levels of contaminants (e.g. methyl mercury) which may be harmful to the developing foetus. The European Food Safety Authority have recommended that pregnant women should consume 100–200 mg per day DHA in addition to the recommended daily intake for healthy adults (250 mg EPA plus DHA/day) [7]. In the United Kingdom, fish and oily fish intake, and consequently omega 3 LC PUFA intake, generally falls well below these recommendations as demonstrated in recent data on fish consumption from the National Diet and Nutrition Survey [8].

Rationale for the role of omega 3 LC PUFAs

Omega 3 LC PUFAs consumed in the diet are well absorbed in the gut and are transported in the bloodstream being made available to tissues. They are incorporated into cell membranes throughout the body, including into cells of the immune system. Healthy volunteers supplemented with fish oil providing 3.2 g/day omega 3 LC PUFAs showed a 12-fold increase in the EPA content of their peripheral blood mononuclear cells [9]. When healthy women consume fish oil or oily fish during pregnancy, there is an increase in foetal omega 3 LC PUFA status, as demonstrated by neonatal plasma, erythrocyte and blood mononuclear cell EPA and DHA concentrations when compared with matched control groups [10, 11]. Incorporation of omega 3 LC PUFAs into cell membranes has functional consequences. Because they are highly unsaturated, these fatty acids may increase the fluidity of the cell membrane which influences receptor molecule movement and cell signalling [12]. EPA and DHA are substrates for synthesis of a number of different lipid mediators including eicosanoids [e.g. prostaglandins (PGs), leukotrienes (LTs)] that are closely related to those known to have roles in the allergic response (Fig. 1) [12]. The 2-series PGs such as PGE₂ and the 4-series LTs such as LTB₄ are known to have roles in allergic disease and inflammation [13, 14]. Indeed, LT antagonists are used to treat allergic asthma by blocking the actions of LTs. These pro-allergic eicosanoids are synthesised from arachidonic acid, a twenty-carbon LC PUFA from the omega 6 family (Fig. 1). Arachidonic acid is relatively abundant in immune cell membranes of healthy volunteers, for example representing around 23% of the fatty acids as compared to the 0.7% of its n-3 LC PUFA equivalent, EPA [9]. An increase in the proportion of EPA in the cell membrane reduces PGE₂ and LTB₄ production both by replacing the substrate (arachidonic acid) and by inhibiting the actions of the enzymes responsible for eicosanoid synthesis (cyclooxygenase and lipoxygenase) [15]. *In vitro* immune cell PGE₂ pro-

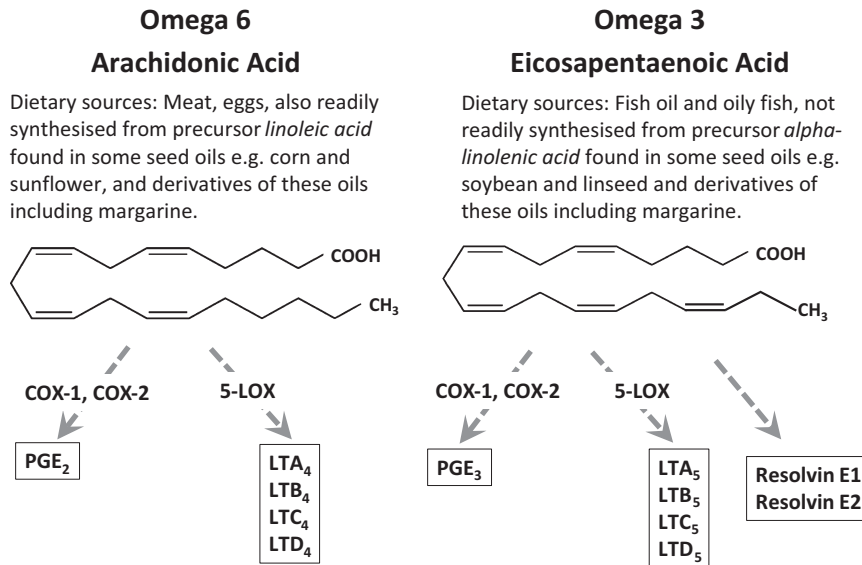


Fig. 1. Polyunsaturated fatty acids from the omega 6 family, for example arachidonic acid (AA), and the omega 3 family, for example eicosapentaenoic acid (EPA), are precursor molecules for a range of lipid mediators. The 2-series prostaglandins and 4-series leukotrienes are derived from AA which is relatively abundant in the modern Western diet and can be readily synthesised from other commonly consumed omega 6 polyunsaturated fatty acids. These eicosanoids have established roles in inflammation and allergic disease. Increasing EPA may reduce the actions of the 2-series prostaglandins and 4-series leukotrienes by several means. EPA is acted upon by the same enzymes, for example cyclooxygenases (COX) and lipoxygenases (LOX) as AA to synthesise the 3-series prostaglandins and 5-series leukotrienes which are less potent than the AA-derived counterparts. EPA also acts upon these enzymes to inhibit the synthesis of the 2-series prostaglandins and 4-series leukotrienes. EPA is a precursor for lipid mediators (resolvin E1 and E2) which are involved in reducing inflammation and controlling eicosanoid synthesis.

duction by cells from healthy volunteers or pregnant women supplemented with omega 3 LC PUFAs is lower than that of immune cells from matched controls [16, 17]. Eicosanoids derived from arachidonic acid and EPA appear not to be equipotent, with the EPA-derived molecules often being less active [18]. EPA and DHA also give rise to newly discovered lipid mediators (e.g. resolvins, protectins and maresins) involved in resolving inflammation and controlling eicosanoid synthesis [19]. Thus, through influences on membrane fluidity and cell signalling, eicosanoid production and other mechanisms, omega 3 LC PUFAs can affect the function of immune cells and could influence development of allergic responses.

Evidence for a role of omega 3 LC PUFAs

There are a number of observational studies demonstrating that higher consumption of fish and oily fish during pregnancy reduces the risk of allergic outcomes in the offspring (reviewed elsewhere ref. 20).

There have been several studies with fish oil supplements in pregnancy investigating the influence on early atopy in the offspring. The first of these was based in Perth, Australia, and examined the effects of fish oil supplements consumed from week twenty to the end of pregnancy compared with a control group consuming olive oil capsules [10]. There has been a single study of

increased intake of oily fish (salmon) during pregnancy [11, 21]. Such studies have shown an increase in omega 3 LC PUFAs in tissues (plasma, erythrocytes and mononuclear cells) of the babies born to mothers in the active arm (e.g. consuming fish oil or oily fish) compared with the control group [10, 11]. In these studies, the increase in neonatal omega 3 LC PUFA status was accompanied by a decrease in mononuclear cell PGE₂ production [21] and in neutrophil LTB₄ production [22]. These studies have demonstrated a number of other interesting immunological observations. In addition to changes in eicosanoid production, studies have demonstrated changes in cytokine production by mononuclear cells. A suggestion that foetal cytokine production is altered by supplementation with omega 3 LC PUFAs in pregnancy comes from results showing that cord blood plasma interleukin (IL)-13 concentration was reduced and messenger RNA coding for IL-4 and IL-13 were also reduced in cord blood immune cells in neonates whose mothers were supplemented with omega 3 LC PUFAs in pregnancy [10, 23]. In the study by Krauss-Etschmann and colleagues, there was an increased messenger RNA coding for transforming growth factor beta (TGF- β) in neonates whose mothers were supplemented with omega 3 LC PUFAs in pregnancy [23]. The increase in mRNA coding for TGF- β is interesting as TGF- β plays a role in induction of tolerance via effects on generation of T regulatory cells and maintenance of low-affinity T cells and B-cell secretory

IgA production [24, 25]. In the study by Dunstan and colleagues, no differences were seen between the intervention and control group for neonatal ex vivo IL-5, IL-13 and interferon-gamma (IFN- γ) production, but a decreased IL-10 in response to stimulation with cat allergen was seen in the fish oil group [26]. In the same study, Prescott and colleagues reported that T-cell protein kinase C (PKC) zeta, a kinase isoform inversely associated with increased risk of developing atopic dermatitis and atopic sensitisation, was significantly higher in cord blood cells from the babies whose mothers were supplemented with fish oil [27].

Two studies have shown a decrease in the numbers of infants sensitised to hens' egg in the first year of life in babies born to mothers supplemented with omega 3 LC PUFAs during pregnancy compared with babies born to mothers in the control group [28, 29]. One of these studies showed a significant reduction in IgE-associated eczema in the first year of life and cumulative IgE-mediated food allergy in the first 2 years of life in babies born to mothers supplemented with fish oil [28, 30]. A reduction in severity of eczema, but not incidence, in the babies born to mothers who were supplemented with omega 3 LC PUFAs during pregnancy was reported in one study [26]. Another study found no significant impact of increasing maternal n-3 LC PUFA intake in pregnancy on the development of sensitisation and allergic disease in infancy [21].

Summary of studies of omega 3 LC PUFAs

A small number of controlled studies investigating the effects of antenatal supplementation with omega 3 LC PUFAs on manifestations of atopy in the offspring have reported some interesting changes in immune function and sensitisation. However, they have demonstrated relatively limited effects on early symptoms of atopic disease. Larger, longer, carefully designed and well-controlled studies would help to make the evidence base more robust. The study design needs to take into account the proportionally large intake of omega 6 LC PUFAs which will vary in different populations and may have a significant bearing on the possible influence of prenatal omega 3 LC PUFAs on the development of atopy in the offspring [31, 32]. Thus, the dose of omega 3 LC PUFAs, the background diet fatty acid composition and the characteristics of the women studied (at high risk of having a baby affected by atopy etc.) need to be taken into account in future studies.

Vitamin D

Vitamin D is not truly essential in the diet as it can be synthesised in the human dermis using the action of UVB light (Fig. 2). There are two vitamin D isoforms: that synthesised in humans (and other animals) is cholecalciferol (vitamin D₃) and that synthesised in some fungi and plants, ergocalciferol (vitamin D₂). Vitamin D supplements

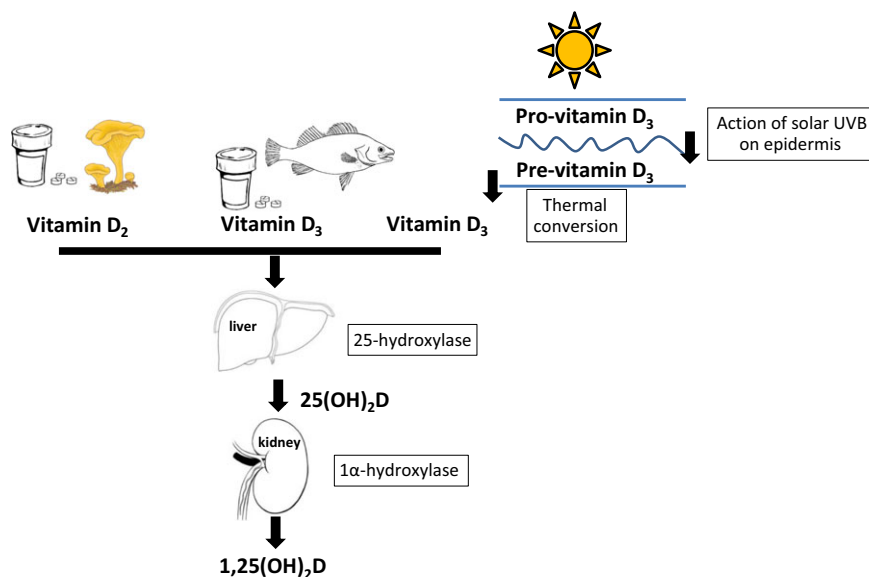


Fig. 2. Vitamin D can be consumed in the diet as ergocalciferol (from some fungi and plants) or cholecalciferol (from some animal sources, e.g. oily fish, fish oil) or in either form in supplements or supplemented foods. It can also be synthesised in the human epidermis from the action of solar UVB on the pro-vitamin, 7-dehydrocholesterol and a further thermal conversion of the pre-vitamin to vitamin D₃. Two successive hydroxylation reactions, the first in the liver and the second in the kidney or in many other tissues of the body, convert the vitamin D to the active metabolite 1,25-dihydroxyvitamin D (1,25(OH)₂D). This has a short half-life in the blood of ~ 6 h. The intermediate metabolite 25-hydroxyvitamin D (25(OH)D) has a half-life of 2 weeks in the blood and is used to assess vitamin D status.

may be vitamin D2 or D3. The two isomers may not be equivalent in their ability to increase vitamin D status, with vitamin D3 being significantly more effective [33, 34]. The major circulating form of vitamin D is 25-hydroxyvitamin D3 (25(OH)D), commonly used as a marker of vitamin D status, while the biologically active form is 1,25-dihydroxyvitamin D3 (1,25(OH)₂D). 25(OH)D is the precursor for synthesis of 1,25(OH)₂D. The 25(OH)D is synthesised in the liver by the hydroxylation of vitamin D. A second hydroxylation reaction synthesises 1,25(OH)₂D from 25(OH)D (Fig. 2). This takes place in the kidney but also extra-renally in many other cells and tissues in the body including monocytes, macrophages, dendritic cells and some epithelial cells [35].

Historically, the definition of vitamin D deficiency was based upon serum 25(OH)D concentrations of patients with calcium deficiency disorders such as bone pain and fragility fractures (< 10 ng/mL or < 25 nmol/L). However, subsequent investigations revealed that calcium absorption was positively responsive in serum 25(OH)D concentrations of between 10 and 30 ng/mL (25–75 nmol/L) but with no apparent symptoms. Thus, vitamin D insufficiency was defined as a serum 25(OH)D concentration of between 10 and 30 ng/mL (25–75 nmol/L), and a normal vitamin D status defined as a serum 25(OH)D concentration of 30–76 ng/mL (75–190 nmol/L) [36].

Dietary sources of vitamin D are limited and current concern regarding vitamin D status within the population has resulted in some governments (e.g. the United States and Canada) opting to fortify foods such as milk to address this. In the United Kingdom, there is no current policy to fortify foods with vitamin D, although some foods are fortified by the manufacturers. Studies in the UK report that around half of pregnant women are vitamin D insufficient, defined according to blood concentration of 25(OH)D [37, 38]. Currently, the UK Department of Health recommends that women consume a daily vitamin D supplement providing 10 micrograms (400 IU) throughout pregnancy and lactation. The recommendation for consumption of a vitamin D supplement during pregnancy is broadly in keeping with or a little below recommended levels of supplementation by other nations such as the United States, Canada, Australia, New Zealand and Japan [39–41]. There is concern that in the United Kingdom only a very small proportion of women use vitamin D supplements during pregnancy; for example, recent survey results from EuroPrevall showed that 1.4% of UK respondents consumed vitamin D supplements during pregnancy [42].

Rationale for the role of Vitamin D

Vitamin D receptors are present in the vast majority of cells in the body, including immune cells [43]. Consequently, vitamin D can influence the actions of a

number of different cells of the immune system including macrophages, monocytes, B cells and T cells [44, 45]. Furthermore, cells of the immune system are able to convert the pro-hormone 25(OH)D into the active metabolite 1,25 (OH)₂D [46]. Vitamin D acts upon macrophages and monocytes upregulating proliferation and immune mediator production [44]. The actions of vitamin D on T cells are complex. Actions on T effector cells include decreasing proliferation, cytotoxicity, production of IL-2 and IFN- γ and increasing production of IL-4 and IL-10 [45]. Vitamin D has also been shown to induce differentiation and expansion of T regulatory cells and to increase TGF- β production [47]. Thus, vitamin D may enhance innate immunity and Th2 cell differentiation and suppress Th1 cell responses as well as increase T regulatory cell populations. Taken together, these effects do not suggest a clear and simple role for vitamin D in preventing or ameliorating allergic disease, although the induction of T regulatory cells and increased TGF- β production could suggest an influence via induction of peripheral tolerance.

Evidence for a role of Vitamin D

Epidemiological data negatively correlating anaphylaxis with level of UV exposure (as a proxy of vitamin D status) were the forerunners of the interest in vitamin D and allergy. These studies showed that residing in areas with higher UVB intensity (Northern United States or Southern Australia) was correlated with proportionally fewer prescribed adrenaline auto injectors [48, 49] and paediatric hospital admission for anaphylaxis [50]. However, such geographical variations in these outcomes could be influenced by many other socio-economic, biological and biogeographical factors. Existing evidence for the role of vitamin D in regulating the development of allergic disease is observational and comes from three sources: season of birth studies, studies assessing dietary intake of vitamin D during pregnancy and those analysing antenatal maternal and neonatal vitamin D status. Season of birth studies looked for relationships between early allergic disease and whether the birth took place in the 'light' or 'dark' season. These include large studies based in North America, Australia and Japan, suggesting that being born in the 'light' relative to the 'dark' half of the year results in reduced incidence of early food allergy and eczema [51–53]. However, whether this is related to vitamin D status or a myriad of other seasonal factors cannot be ascertained.

Studies assessing vitamin D intake have shown some relationships with allergic outcomes. Devereux and colleagues found a negative association between maternal vitamin D intake during pregnancy and risk of wheeze in the offspring at 5 years of age [54]. Camargo and

colleagues reported that a higher maternal vitamin D intake (> 400 IU/day) during pregnancy was associated with a lower risk of wheeze in the children at 3 years of age [55]. When they looked at the influence of infant vitamin D intake at age 2 years in this study, it did not influence the risk of wheeze in the children at 3 years of age [55]. More recently, in a study looking at the association between intake of a range of different vitamins and minerals during pregnancy and the development of atopy in the offspring, vitamin D was the only one which was significantly associated with atopic markers [56]: maternal vitamin D intake in pregnancy was associated with a reduced risk of detection of specific IgE to food allergens in the offspring at 5 years of age [56]. In a prospective study of 44 825 Danish mother-child pairs of vitamin D intake in pregnancy, mothers with the highest vs. lowest intake of vitamin D were significantly less likely to have children with asthma at 7 years of age (defined as lifetime asthma diagnosis and wheeze in the last 12 months) and significantly less likely to have children admitted to hospital due to asthma [57].

Other observational studies have focussed on maternal and foetal vitamin D status by measuring 25(OH)D in plasma or serum samples. The data from these studies are far from clear, with some studies suggesting that a low 25(OH)D status is associated with an increased risk of subsequently developing atopic sensitisation or disease [58, 59], some showing the opposite [37, 60] and others demonstrating a 'u'-shaped curve in which both low and high vitamin D status in pregnancy precedes increased incidence of allergic sensitisation in the offspring [61].

Studies reporting maternal status have found no or positive relationships between maternal vitamin D status and atopic manifestations in the offspring. A UK study found that those children born to mothers in the highest quartile of the cohort for maternal serum 25(OH)D concentration in late pregnancy had an increased risk of eczema at 9 months of age and asthma at 9 years of age [62]. In a UK study of 860 mother-infant pairs, there were no significant associations between maternal late-pregnancy 25(OH)D and either asthma or wheeze in the children at age 6 years [63]. In the Avon Longitudinal Study of Parents and Children, maternal serum 25(OH)D concentration was measured at any stage of pregnancy [64]. There was no association between maternal serum 25(OH)D in pregnancy and parentally reported allergic disease (wheeze, asthma, eczema or hay fever) or sensitisation to three common aeroallergens [64]. In a study based in Germany (LINA cohort), higher maternal 25(OH)D was associated with a higher risk of diagnosed food allergy and food allergen-specific IgE in the offspring at 2 years of age [60].

Studies reporting neonatal vitamin D status have also been ambiguous in relation to development of atopy. A study of 922 New Zealand neonates showed no associations between cord blood 25(OH)D and asthma by 5 years of age, although there was a significant inverse relationship between 25(OH)D and risk of wheezing at 15 months, 3 and 5 years of age [65]. In a multicentre study in the United States, cord blood 25(OH)D was measured for 568 neonates, 520 of whom had at least one atopic parent [66]. There was a weak but significant positive correlation between cord plasma 25(OH)D concentration and *in vitro* cord blood immune mononuclear cell IFN- γ production, but this was not seen for IL-4, IL-10, IL-12, IL-13 or tumour necrosis factor-alpha. In this study, the proportions of cord blood T regulatory cells (identified as CD4⁺CD25⁺ cells expressing FoxP3) to total T helper cells (CD4⁺ cells) was inversely correlated with cord blood 25(OH)D. The authors concluded that higher vitamin D concentrations at birth may be associated with a lower number of T regulatory cells [66]. Rothers found that the highest and lowest cord vitamin D status within their cohort was associated with higher total and aeroallergen-specific IgE over first 5 years of life [61]. In a third study based in the United States (Boston Birth Cohort), cord blood 25(OH)D was measured in 649 neonates [67]. Vitamin D deficiency (defined as cord blood 25(OH)D < 11 ng/mL) was not associated with sensitisation to a panel of eight common food allergens [67]. In a study of 115 children with IgE-mediated peanut allergy and matched controls, cord blood 25(OH)D in the upper third quartile was associated with a significantly lower risk of peanut allergy [68]. Cord blood 25(OH)D concentration was found to have no significant associations with allergen sensitisation, IgE-mediated food allergy or eczema severity in an Australian cohort of 321 infants [58]. Jones reported that infants who developed eczema had a significantly lower cord blood 25(OH)D concentration compared with the infants who did not develop eczema [58]. In the study of the LINA cohort (see above), higher cord vitamin D status was associated with a higher risk of diagnosed food allergy at 2 years of age [60]. Baiz reported results of cord blood 25(OH)D and allergic outcome to 5 years of age in 259 French children [59]. They reported that cord serum 25(OH)D was significantly inversely associated with risk of transient early wheezing and early- and late-onset atopic dermatitis by the ages of 1, 2, 3 and 5 years [59]. The Copenhagen Prospective Studies of Asthma in Childhood showed no significant association between cord blood 25(OH)D and asthma, respiratory infections, lung function, sensitisation to 16 common food and inhalant allergens, allergic rhinitis or eczema assessed from birth to seven years of age for 257 children [69].

A single vitamin D intervention during pregnancy and allergic outcomes in the offspring has been published to date. In this UK study, 180 women were randomised at 27 weeks' gestation to receive no supplement, 800 IU per day ergocalciferol (vitamin D2) or a single bolus dose of 200 000 IU cholecalciferol (vitamin D3) [70]. Skin test and specific IgE were measured at 3 years of age for 95 and 86 of the children, respectively. There were no significant differences between the control group vs. intervention groups for atopic sensitisation or risk of eczema [70].

Summary of studies of vitamin D

There is limited and conflicting evidence for the role of vitamin D in the development of early allergic disease. The few studies investigating maternal intake have been in concordance with each other, suggesting a preventative or ameliorating effect of higher maternal vitamin D intake on atopic outcomes in the offspring. However, vitamin D status data are not in agreement with this intake data.

Thus, current data suggest that mothers with a higher vitamin D intake might have infants with a reduced risk of wheezing and possibly of developing allergic diseases (sensitisation and asthma). Studies investigating maternal or neonatal vitamin D status using blood concentrations of 25(OH)D in relation to offspring risk of allergic diseases are less consistent. The lack of concordance between vitamin D intake and status data might suggest that the intake data are a surrogate marker for increased intake of other important nutrients. One of the few dietary sources of vitamin D is oily fish. Thus, perhaps vitamin D intake is largely dictated by consumption of oily fish which is also a rich source of other nutrients which may themselves have effects (e.g. omega 3 LC PUFAs and other nutrients). However, when considering the status data, it is unclear how well a single 25(OH)D measurement represents vitamin D status throughout foetal life and whether there is a time window in which the vitamin D status may be particularly influential. As season has major impact on maternal vitamin D status in pregnancy [37], a high 25(OH)D concentration at birth or in the last trimester suggests that at about 12 weeks of gestation the foetus would be experiencing the seasonal nadir for vitamin D. Furthermore, the measurement of serum 25(OH)D is the method of choice for assessing vitamin D status but is not without issues. Although serum 25(OH)D concentration does reflect dietary intake and UVB exposure, a number of other factors influence circulating 25(OH)D. Genetic variation in a number of genes including the vitamin D binding protein, the carrier molecule which delivers all vitamin D and vitamin D metabolites to tissues, influence serum 25(OH)D [71]. Variation near genes involved

in cholesterol synthesis and hydroxylase enzymes has been related to low circulating concentration of serum 25(OH)D [72].

To date, the single intervention study has not shown an effect of vitamin D supplementation during pregnancy on atopic outcomes in the offspring [70]. However, the design of the study has some weaknesses. The intervention was started relatively late in pregnancy (27 weeks) and the vitamin D given as either a modest dose of vitamin D2 or a single very large bolus dose of vitamin D3. More double-blind placebo-controlled vitamin D intervention studies are required. These need to consider the supplement type (vitamin D3 vs. D2), timing (a highly developed immune system is already present by 20 weeks of pregnancy) and dose. There are controlled vitamin D intervention studies underway which may help to answer the question of whether vitamin D status during pregnancy influences risk of offspring allergic disease [73–75].

Folic acid and folate

Vitamin B9 (folate) is essential for cell division, nucleic acid synthesis and is a methyl donor for methylation reactions within the body. Folic acid is an oxidised synthetic form of folate. Rich dietary sources of folate are liver, yeast extract and green leafy vegetables. Folic acid is used as a supplement as it is more stable and better absorbed compared with folate. Folic acid is added to some foods such as breakfast cereals. In the 1960s, evidence was published indicating that supplemental folic acid given during pregnancy reduced the risk of primary or secondary occurrence of foetal neural tube defects. Foetal neural tube defects result in such conditions as anencephaly, spina bifida and myelomeningocele conferring high risk of morbidity and mortality. The closure of the neural tube during embryonic development is an early event, occurring by day 28 of gestation in humans. Interventions aimed at modulating neural tube defects are thus aimed at the very early stages of pregnancy or preconception. Currently, the UK Department of Health recommends that pregnant women should consume 400 micrograms of folic acid per day for the first 12 weeks of pregnancy and preferably before conception. A substantial proportion of pregnancies in the United Kingdom are unplanned, pregnant women in the United Kingdom have folate intakes below the recommended intake with only a proportion using folic acid supplements as recommended [42, 76]. In many other countries including the United States and Canada, a decision has been made to fortify staple foodstuffs with folic acid to improve folate status. After recommendations for folic acid fortification of food in the United Kingdom by the Scientific Advisory Committee on Nutrition which date back to

2006 [77, 78], it is probable that folic acid fortification of wheat flour will be adopted in the United Kingdom [79].

Rationale for the role of folic acid

The role of folic acid as a methyl donor indicates that it might influence the methylation of DNA carried out by DNA methyl transferase enzymes. The methylation of nucleic acids and of transcriptional machinery (e.g. histones) is able to influence transcriptional activity. Hypermethylation of specific regulatory regions of genes can silence the expression of the gene. During polarisation of naive T helper cells to Th2 cells, methylation of the promoter region of the IFN- γ gene prevents transcription factor binding and thus expression of the IFN- γ gene [80]. Thus, increased folic acid intake could influence the expression of genes which may be involved in T-cell differentiation. The influence on T-cell differentiation during gestation may, in turn, influence the allergic predisposition in the neonate. Indeed, infants born with a reduced immune cell IFN- γ production do appear to have an increased risk of atopy [81, 82]. The ability to influence the phenotype of an organism in a long-term, heritable fashion without influencing the sequence of the DNA is the definition of epigenetics. The stable differentiation of T regulatory cells which have tolerogenic roles is also under epigenetic control [83, 84]. Maternal environmental factors during pregnancy have been shown to influence neonatal T regulatory cell numbers which in turn was associated with a change in offspring risk of developing sensitisation and atopic dermatitis [85]. Thus, folic acid consumed prior to or during pregnancy could influence epigenetic programming of the foetus influencing the allergic predisposition of the neonate.

Evidence for a role of folic acid

Interest in the influence of maternal folic acid supplementation on allergic manifestation in the offspring was sparked by a paper investigating the effects of a maternal diet high in methyl donors (including folic acid) in a murine model of ovalbumin-induced atopic asthma [86]. The authors showed that feeding pregnant mice a diet high in methyl donors resulted in increased offspring atopy (e.g. higher total IgE, specific IgE and broncho-alveolar lavage fluid eosinophilia and IL-13) compared with matched controls [86]. There followed a series of papers looking at folic acid intake in humans in pregnancy and allergic outcomes. The first, from Haberg *et al.*, showed that in a large Norwegian cohort of over 32 000 mothers, taking folic acid in the first 12 weeks of pregnancy increased the risk of wheezing in children from 6–18 months of age and of having an

upper respiratory tract infection and being hospitalised for an upper respiratory tract infection in the first 18 months of life [87]. This study showed that these risks were not influenced significantly by having the folic acid only after the first 12 weeks of pregnancy. In the same year, a second paper looking at a smaller Australian cohort ($n = 557$) showed that consuming folic acid in late pregnancy increased risk of asthma and persistent asthma at 3.5 years of age, but at 5 years, the trend for this was no longer significant [88].

A Dutch study of folic acid intake reported no significant association between maternal folic acid supplement use during pregnancy and risk of wheeze, lung function, asthma or related atopic outcomes in the offspring [89]. However, in this study, maternal intracellular folic acid concentration was found to be significantly inversely associated with asthma risk at age 6–7 years in a dose-dependent manner [89]. In another study from the Netherlands, the use of maternal folic acid supplements was not associated with offspring risk for asthma symptoms, wheeze, lower respiratory tract infection, frequent respiratory tract infection and eczema from 1 to 8 years of age [90]. Martinussen investigated the use of maternal folic acid supplements during pregnancy in 1499 women with asthma risk in the offspring by 6 years of age and found no associations [91]. Miyake and colleagues investigated maternal intake of folate during pregnancy and found no significant association with infant symptoms of wheeze and eczema for 763 Japanese mother–child pairs [92].

More recently, studies looking at folate status in the mother and neonate and allergy in the offspring have been published. Kiefte-de Jong found that higher maternal folate status (> 16.2 nmol/L) was associated with a higher risk of atopic dermatitis in the offspring up to the age of 4 years [93]. Dunstan *et al.* investigated the relationship between maternal folic acid and folate intake and maternal and cord folate status in relation to atopic outcomes at 1 year of age. They reported that the folate concentrations of cord or maternal blood serum were not different for infants who did or did not develop eczema by 1 year. Maternal folate intake from all foods was also found to be not different for infants who did or did not develop eczema by 1 year. They did report that maternal folate intake from supplements was higher for infants with subsequent eczema. Mothers who had an intake of folic acid > 500 μ g/day as a supplement had infants who were more likely to develop eczema by 1 year of age than those born to mothers who had a folic acid intake of < 200 μ g/day. They also reported that both a relatively low and high neonatal folate status (cord folate < 50 or > 75 nmol/L) was associated with a greater risk of sensitisation [94].

Summary of studies of folate/folic acid

Evidence for the role of folic acid or folate during pregnancy in the primary development of atopy in the offspring is far from compelling. There are a number of studies suggesting that increased intake of folic acid supplements may increase the risk of wheeze in early life, but this is not a consistent finding. Furthermore, these studies are not in agreement whether this is associated with use of folic acid supplements during early or late pregnancy. Studies investigating maternal or neonatal folate status with atopic outcome have been inconsistent. They have demonstrated that neonatal folate status is positively or not related to risk of infant atopic dermatitis [93, 94]. Risk of infant sensitisation was associated with both higher and lower neonatal folate status [94]. One study has reported a positive dose-dependent association between maternal folate status and asthma at six to seven years of age [89]. A recent meta-analysis of the effect of prenatal folic acid and risk of asthma in the offspring found no evidence of an association [95]. Given the proven effects of antenatal folic acid supplements in primary and secondary prevention of foetal neural tube defects, it is neither ethical nor desirable to conduct blinded, placebo-controlled studies to clarify whether folic acid supplements or folate status prior to or during pregnancy have a bearing in primary development of atopic disease. However, the continuing stream of observational studies may shed further light on this question. In particular, in larger cohort studies in which dietary intake of folate and folic acid is well documented, maternal folate status is measured and prevalence of allergic disease in the offspring investigated over a longer period (at least to 5–7 years of age) may give clearer answers to this question.

Conclusions

During the last decade, a number of nutrients were identified as having putative influence via the pregnant mother on the risk of developing allergic disease in the offspring. These included omega 3 LC PUFAs, vitamin D and folic acid. Since this time, a wealth of data have been published to confirm or refute these links. To date, omega 3 LC PUFAs have been investigated in a limited number of randomised placebo-controlled trials. These have demonstrated neonatal immunomodulatory effects and some reduction in early allergen sensitisation. However, few have shown effects of supplementation with omega 3 LC PUFAs during pregnancy on allergic symptoms in the offspring. Evidence for the effects of vitamin D during pregnancy and risk of allergic disease in the offspring is to date mostly observational and inconsistent. There are randomised placebo-controlled

trials of maternal vitamin D supplementation underway, particularly focussing on the influence on symptoms of asthma in the offspring [73–75]. It is to be hoped that these data will help to clarify whether vitamin D during pregnancy influences risk of allergic disease in the offspring. Finally, the evidence published to date relating antenatal folate status or supplementation with folic acid with offspring risk of allergic disease is equivocal. This cannot be answered by randomised placebo-controlled trials, but further observational studies may give a clearer picture.

So, what advice can be given to a mother who wants to know what she can eat or avoid during her pregnancy to protect her future children from allergic disease? The answer is to eat a balanced diet and to abide by the recommendations for increased intake of specific nutrients during (and for folic acid prior to) the pregnancy. In the United Kingdom, this is currently

Omega 3 LC PUFAs - eat 2 portions of fish per week at least one of which should be oily fish

Vitamin D - 10 micrograms (400 IU) per day during pregnancy and lactation

Folic acid - 400 micrograms per day preconception and up to 12 weeks' gestation

These nutrients have proven important roles supporting optimal foetal development and health. Mothers should also follow guidelines for foods to avoid during pregnancy including those which might contain pathogens (e.g. listeria found in foods such as uncooked soft cheeses and pates, salmonella found in foods containing raw or undercooked, e.g. or unpasteurised dairy products), those with significant concentrations of vitamin A (e.g. liver or vitamin supplements containing high concentrations of vitamin A) and consumption of alcohol. It is clear that foetal exposure to environmental factors via the mother has the potential to influence allergic predisposition of the child. However, current data do not give sufficient evidence to advocate the reduced or increased intake of any specific nutrient during pregnancy to reduce risk of atopic disease in the offspring. In the context of the specific nutrients reviewed here, there are many valid, proven reasons for ensuring an adequate intake to maintain optimal foetal and maternal health. Future results from well-designed, controlled studies should bring greater clarity to the question of prenatal nutrient influence on offspring risk of allergic disease.

Conflict of interest

PCC serves on the Danone Nutricia Research Scientific Advisory Board on Immunity and Allergy and has received research funding from Abbott Nutrition.

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