

REVIEW

Antioxidants and allergic disease: a case of too little or too much?

K. Allan¹, F. J. Kelly² and G. Devereux¹

¹Department of Environmental and Occupational Medicine, University of Aberdeen, Aberdeen, UK and ²MRC-Asthma UK Centre in Allergic Mechanism of Asthma, King's College London, London, UK.

Clinical & Experimental Allergy

Summary

Speculation persists as to the possible role, if any, of dietary antioxidants in allergic disease. While it has been hypothesized that the recent increase in allergic disease is a consequence of declining dietary antioxidant intake, an alternative hypothesis proposes that the increase in allergic disease is due to increasing antioxidant intake. Dietary trends are conflicting; the intake of some antioxidants has declined, for others intakes are likely to have increased. Animal model studies demonstrate that antioxidant supplementation at the time of primary and subsequent allergen exposure attenuates allergic inflammatory responses. The data from human studies are less clear. Observational epidemiological studies of humans are beset by several methodological limitations associated with the assessment of diet and predominantly focus on asthma. Most observational studies report potentially beneficial associations between dietary antioxidants and allergic outcomes, but a small minority report potentially adverse associations. Human intervention studies suggest that single antioxidant supplements confer minimal, if any clinical benefit in adults with asthma, however, there is still scope for studies in children, atopic dermatitis, allergic rhinitis (AR) and of antioxidant combinations. More recently, it has been suggested that dietary antioxidants in the developmental context of fetal and infant development influence the development childhood asthma and atopic sensitization possibly by affecting the first interactions between the neonatal immune system and allergens. While a small number of birth cohort studies have reported potentially beneficial associations between maternal intake of some antioxidants during pregnancy and childhood asthma, there is very limited data suggesting associations between maternal antioxidant intake and childhood atopic dermatitis and AR. The available epidemiological, animal, molecular and immunological data suggest that there are associations between antioxidants and asthma and to a much lesser extent, atopic dermatitis and AR. However, the exact nature of the relationships and the potential for therapeutic intervention remain unclear.

Correspondence:

Prof. Graham Devereux, Department of Environmental and Occupational Medicine, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZB, UK.
E mail: g.devereux@abdn.ac.uk

Introduction

The utilization of oxygen as an electron acceptor in metabolic processes is a characteristic of aerobic organisms, however, the addition of electrons to oxygen creates cytotoxic reactive oxygen species (ROS). To counter the toxic effects of ROS, organisms have evolved complex antioxidant defences, however, when these are insufficient, oxidative stress occurs and such stress is a feature of many inflammatory conditions such as allergic disease [1–4]. There is increasing interest in the role of dietary antioxidants in allergic disease, particularly the therapeutic potential of dietary antioxidants as adjuncts to conventional therapies and their possible use in the primary prevention of allergic disease.

Pulmonary antioxidant defences

The lung, owing to its function and large surface area is regularly exposed to a range of substances many of which involve direct or indirect oxidant challenge. Consequently, it is not surprising that it has developed robust antioxidant defence systems to protect the pulmonary epithelial cells from injury. When an oxidant gas or particle enters the lung, the first interface it encounters is the lung lining fluid. Human lung lining fluid is a complex and regionally heterogeneous compartment ranging in depth from between 1 and 10 µm in the proximal airways to 0.2 and 0.5 µm in the distal airways and alveoli [5]. Lung lining fluid consists of secretions from underlying lung and resident immune cells, as well as plasma-derived

exudates. In the nasal and proximal airways lung lining fluid exists as a two-phase structure consisting of gel and sol phases, the former consisting of thiol-rich mucopolypeptide glycoprotein or mucins. This contrasts with the distal airway and alveolar lining fluids, which are devoid of mucins, but contain surfactant lipids and proteins. In addition to the mucin and surfactant components lung lining fluid also contains a broad spectrum of low molecular weight antioxidants, as well as small concentrations of antioxidant enzymes [6].

The low molecular weight antioxidants are present in similar concentrations to those found in blood plasma, including ascorbic acid (vitamin C), uric acid and α -tocopherol (vitamin E). Lung lining fluid obtained from the lower respiratory tract contains abundant amounts of reduced glutathione and ascorbate but low concentrations of uric acid and α -tocopherol [7]. In contrast, lining fluid from the nasal cavity contains uric acid in large quantities, with much smaller amounts of reduced glutathione and vitamin C. Lung lining fluid also contains antioxidant enzymes such as superoxide dismutase and catalase, as well as the metal binding proteins caeruloplasmin and transferrin. It is not currently clear which, if any, of these antioxidant defences play the most key roles protecting the delicate blood/air barrier. It is likely that, like antioxidant networks in other cellular and non-cellular locations, there is little redundancy in the system and each has a key role in defending a particular micro environment from a particular radical species.

The influx of inflammatory cells to the lung is a common response to both oxidant and non-oxidant challenges. This highly orchestrated series of events usually leads to a second wave of oxidative stress, because activated inflammatory cells also generate and release large quantities of ROS. In the absence of any invading organisms to kill, these free radicals attack local tissue components and cause cell injury. As different respiratory diseases are characterized by varying types of airway inflammation (i.e. asthma eosinophils; COPD neutrophils) there are likely to be subtle differences in airway antioxidant defences requirements in these situations.

Redox balance at the lung surface

The rules that govern the balance between beneficial and detrimental interactions in the lung lining fluid compartment are not well established, but these may contribute in part, to the sensitivity of individuals to oxidant challenge [6]. A simplistic viewpoint would be that the greater the range and concentration of antioxidant defences on the lung surface, the better the level of protection from oxidants. If this were the case, however, subgroups of the population recognized to be susceptible to oxidant challenge should have decreased lung lining fluid antioxidant defences. This was indeed found in asthmatics – a suscep-

tible subgroup in that they have markedly decreased concentrations of ascorbic acid in lung lining fluid compared with healthy control subjects [7]. However, the same was not found to be true of those healthy subjects who happen to show the largest decrease in lung function following ozone challenge [8].

It is not yet clear to what extent lung antioxidants perform a critical role in reducing the toxic consequences of oxidant challenges to the lung or indeed the exacerbations in allergic respiratory disease. Undoubtedly, however, they do play a role and therefore therapeutic options include either decreasing the rate of generation of ROS or augmenting lung antioxidant defences. As low molecular weight antioxidants within human lung lining fluid have been proposed to confer protection against oxidant challenge there is considerable interest in augmenting the concentrations of these defences at the air–lung interface to protect against injury to the airway epithelium and the induction of inflammation. Antioxidant supplementation studies in Mexico City in street workers [9] and children with asthma [10], as well as in cyclists in the Netherlands [11], have demonstrated small, but measurable protection against ozone-induced lung function decrements. These studies, however, failed to address the fate of the supplemental vitamins. It was assumed that the protection observed reflects an enhanced concentration of these antioxidants within the lung lining fluid and pulmonary epithelium, but this was not demonstrated experimentally. This view is based on the widely held contention that lung lining fluid ascorbate is derived from the plasma pool, though there are few experimental data to support this notion. Indeed, those studies which have examined the plasma–lung fluid ascorbate relationship have failed to demonstrate a simple association [12, 13], suggesting that regulation of ascorbate at the air–lung interface may be more complicated than envisaged. Recently, it was shown that following supplementation at a high vitamin C dose, there is a rapid, but transient increase in nasal lung lining fluid [14]. It is therefore possible to modify the antioxidant status of the lung lining fluid through supplementation, but the response is transient and does not persist beyond 24 h. The absence of a simple association between basal plasma and lung fluid ascorbate concentrations and response implies that ascorbate regulation at the lung–air barrier, at least within the nose, is more complex than has previously been envisaged. If cellular sequestration of supplemental ascorbate is occurring this raises a number of intriguing questions regarding the mechanism of its uptake into airway cells, its capacity to protect these cells from oxidative stress, and the possibility that these cells may subsequently release their augmented intracellular ascorbate stores onto the lung surface upon appropriate stimulation to confer protection from oxidant challenge.

Previously, it was thought that the concentration of antioxidants within the lung lining fluid determined an individual's responsiveness to oxidant challenge. Ongoing research now suggests that inter-individual variations in antioxidant transport and metabolism within the lung fluid compartment is a more likely explanation of differing abilities to deal with oxidant challenge. For example, GST polymorphisms have been associated with the asthma phenotype. GSTM1 and GSTP1 polymorphisms in asthmatic children exposed to ozone demonstrated increased respiratory symptoms in children with GSTM1 or GSTP1 Val/Val genotypes [15].

Hypotheses relating antioxidants to allergic disease

Two somewhat contradictory hypotheses relate antioxidants to allergic disease. The first proposes that the recent increase in asthma in westernized countries has, in part been a consequence of declining dietary antioxidant intake that has occurred because of changes in dietary habit, methods of cultivation, and increased transportation, storage and processing of food [16]. The hypothesis proposed that declining antioxidant intake compromised lung antioxidant defences, increasing airway vulnerability to oxidant stress with ensuing airway inflammation and asthma. Although originally focused on asthma, this hypothesis has been broadened to encompass allergic diseases [17]. The hypothesis was based on the observation that asthma had increased at the same time as declining dietary intake of some antioxidant rich foods. European selenium intake and blood selenium concentrations have declined, probably because of increased use of European grain and changes in bread-making technology [18]. In the United Kingdom, average selenium intake has fallen from was 60 µg/day in 1975 to 30–40 µg/day [18–20] currently. In the UK, vitamin C intake has been relatively stable between 1980 and 2000 [21]. The limited data on UK vitamin E intake suggest little change from 10.82 mg/day in 1994 to 10.66 mg/day in 2004/2005 [22]. However, extrapolation of the 2000/2001 National Diet and Nutrition Survey using the UK National Food Surveys suggests that vitamin E intake in the early 1950s was probably higher at about 13–15 mg/day, perhaps because of increased consumption of green vegetables and bread/cereals [21, 23]. The second antioxidant hypothesis proposes the recent increase in allergic disease to be a consequence of increased antioxidant intake because of the increased availability of functional and antioxidant enriched foods. This hypothesis was based on *in vitro* observations that some antioxidant rich foods and extracts of traditional Vietnamese and Chinese herbal medicines suppress secretion of the T-helper type 1 (Th1) cytokine, IFN-γ [24–26]. It is postulated that increased antioxidant intake 'antioxidant stress' suppresses Th1 differentiation and because of inherent cross-regulatory

mechanisms, this promotes development of the Th2 phenotype. While this hypothesis is primarily based on *in vitro* immunological observations, there is some evidence that the intake of some antioxidant (principally vitamin C) rich food groups has increased, for example in the UK fruit juice consumption increased from 7 mL/person/week in 1950 to 303 mL/person/week in 1996 [27].

Observational studies

There are now many observational epidemiological studies relating dietary antioxidants to allergic disease, and these have been comprehensively reviewed elsewhere [28]. The majority have related antioxidants to respiratory outcomes, principally ventilatory function and parameters of asthma, a minority have investigated antioxidants and atopic dermatitis/allergic rhinitis (AR).

Methodological considerations

A notable feature of the observational literature relating antioxidants to allergic disease are the inconsistencies in reported associations (if any) between studies and while this may reflect more complex associations than originally hypothesized, methodological issues have undoubtedly contributed. This is clearly illustrated by two analyses of the Third National Health and Nutrition Examination Survey (NHANES III) relating diagnosed asthma in children to serum antioxidant levels [29, 30]. Using the same database one study reported childhood asthma to be associated with serum β-carotene [odds ratio (OR) 1 SD increase 0.87, 95% confidence interval (95% CI) 0.7–1.0] [30], whereas the second reported no association [29].

Assessment of diet remains problematic and because there is no perfect way of assessing diet, methods of assessment differ between studies. Most have used food frequency questionnaires (FFQ) to quantify dietary intake, but the level of dietary detail collected ranges from a few food groups (10–20) to those asking about >150 food groups and portion sizes; consequently some FFQ provide basic information on the frequency of consumption of major food groups, while others when used in conjunction with food composition tables can be used to derive semi-quantitative estimates of individual nutrient intakes. Although the estimates of nutrient intake derived from the more detailed FFQs correlate with weighed dietary intakes, the strength of the associations suggests that the inherent accuracy of this method is relatively low [31, 32]. The precision of dietary data is compromised by factors such as incomplete food-nutrient databases, variability in the nutrient composition of food, methods of preparation, eating habits, and the tendency of subjects to over- or under-report some food items. The misclassification of diet with respect to disease outcome is usually random, thus reducing the magnitude of any association between

FFQ-derived estimates of dietary intake and disease outcome. FFQ-derived nutrient intakes are not reliable estimates of absolute nutrient intake, instead they are useful for comparing and ranking individuals measured by the same tool. This is sufficient for many epidemiological studies [32] but the use of differing instruments to estimate nutrient intake between studies, limits between-study comparisons and the combining of datasets. FFQs that fail to comprehensively capture participants' dietary intake are unable to provide the necessary information needed to adjust for total energy intake. This adjustment is critical to control for confounding by energy intake that is often related to disease risk because of associations between physical activity and/or body size and the likelihood of disease. Adjustment for energy intake has not been universal in studies of antioxidants and allergic disease; studies failing to do so are more likely to report false negative results and run the risk of reporting associations that are in the opposite direction to those actually present [33].

Epidemiological studies of dietary antioxidants are also beset by the problem of confounding because dietary intakes can be associated with age, gender, region, socioeconomic status, culture, lifestyle, smoking habit ethnicity; furthermore, foods and diets rich in one antioxidant tend to be rich in others [34]. Adjustment for potential confounding factors in studies of antioxidants and allergic disease ranges from minimal (if any) to large numbers of factors. Multiple testing of numerous nutrients with several outcomes is a feature of many studies of antioxidants and allergic outcomes, few studies report adjustments for multiple testing. The use of nutritional biomarkers in biological samples, especially those that reflect integrated nutrient exposure over time is an alternative, but expensive, approach to quantifying nutrient status that avoids many of the weakness of the traditional methods of assessing dietary intake. Some studies have related allergic outcomes to serum antioxidant levels, however, such measurements may reflect recent rather than long-term nutrient status, and very few studies have quantified longer term nutrient biomarkers, e.g. erythrocytes, leucocytes, hair nail clippings. Fat soluble α -tocopherol is transported in the lipoprotein fraction of blood and it is accepted that α -tocopherol values must be adjusted for cholesterol [35]; however, such adjustment is not universal in studies relating vitamin E to allergic outcomes. Nearly all studies quantifying blood antioxidant levels have been of cross-sectional or case-control design and because allergic diseases are associated with oxidant stress it is not possible to determine whether low antioxidant levels preceded and/or resulted from disease. As an illustration, an adult case-control study reported that whole blood levels of total carotenoids, lycopene, lutein, β -cryptoxanthin, α -carotene and β -carotene were lower in asthmatics than controls. However, with the

exception of lutein there were no significant differences in the dietary intakes of these nutrients between asthmatics and controls; the conclusion being that the reduced blood antioxidant levels were most likely a consequence of the depletion of blood antioxidants by oxidative stress associated with asthma [36].

Studies of allergic diseases

Although a substantial minority (30–40%) of observational studies report no associations between antioxidants and asthma, the majority (60–70%) report potentially beneficial associations between one, two or occasionally three nutrients (vitamin E, vitamin C, selenium, α -carotene, β -carotene, lycopene, lutein and β -cryptoxanthin) or antioxidant containing food groups (fresh fruit, various fruits, fruit juices, Mediterranean diet and vegetables) and one or more parameters of asthma such as diagnosed asthma, wheezing symptoms, airway reactivity and ventilatory function (reviewed in [28]). The beneficial associations with hard fruits, independent of the nutrients outlined above, have led to the proposition that dietary flavonoids might be important in asthma because of their potent antioxidant properties [28].

In contrast to asthma, the literature relating dietary antioxidants with atopic dermatitis, AR and IgE is limited; most report potentially beneficial associations, some report none and a few report potentially adverse associations. In a cross-sectional study of 2633 UK adults aged 18–70 with a mean vitamin E intake of 6.2 mg/day (SD 2.2) serum IgE and atopic sensitization increased by approximately 5% per milligram decrease in vitamin E intake [37]. In NHANES III serum levels of α -carotene, β -cryptoxanthin and vitamin E in 5742 adults were beneficially associated with atopic sensitization (OR 1 SD difference 95% CI); 0.95 (0.91–0.99), 0.89 (0.83–0.95) and 0.93 (0.87–0.99), respectively; however, atopic sensitization was adversely associated with serum lycopene [1.08 (1.03–1.14)]. There were no associations in 4271 children [38]. Atopic dermatitis has been reported to be associated with reduced vitamin E status: in a small ($n = 43$) case-control study of children aged 1–9, atopic dermatitis was associated with reduced erythrocyte α -tocopherol concentrations 2.14 vs. 3.17 mmol/L, $P < 0.001$ [39], and in a Korean case-control study, the vitamin E intake of 50 adults with atopic dermatitis was significantly lower than that of 50 controls, however, there was some evidence that this may have been a consequence of patients with atopic dermatitis changing their diet [40]. The Mediterranean diet has a high antioxidant content because of the preponderance of fruit, vegetables, legumes, nuts and wholegrain cereals and has been associated with a reduced likelihood of asthma, wheezing and AR in cross-sectional studies of children living in Greece, Spain and Mexico [41–44]. A study of 537 children aged 7–18 living in Crete

reported an association between reduced lifetime prevalence of AR and the frequent (≥ 1 portion/day) consumption of grapes, (OR 0.31 95% CI 0.14–0.64), oranges (0.29; 0.11–0.80), kiwi fruit (0.37; 0.16–0.86) and a high Mediterranean diet score (0.34; 0.18–0.64) [41]. The consumption of fruit ≥ 3 times a week has been reported to be associated with a reduced likelihood of rhinoconjunctivitis (OR vs. never 0.71, 0.57–0.88) in 17 145 Spanish children aged 6–7 [44] and with AR in 2387 Turkish children aged 6–12 (0.64; 0.45–0.51) [45].

Although the majority of studies report beneficial associations between dietary antioxidants and allergic outcomes a few (~5%) have reported potentially harmful associations particularly with some carotenoids. Follow-up of a German cohort for a median of 2.1 years after dietary assessment identified 344 adults who developed hayfever and 1336 who did not [46]. Participants in the highest quartile of β -carotene intake had an increased risk of developing hayfever (OR 1.69, 95% CI 1.09–2.63) when compared with the lowest quartile, whereas those in the highest quartile of vitamin E intake were less likely to develop hayfever (0.38, 0.17–0.85). The Dutch MORGEN cross-sectional investigation of 5744 adults aged 20–59 reported conflicting associations between dietary β -carotene intake and parameters of airway disease [47]. Dietary β -carotene intake was positively associated with the 12-month period prevalence of wheeze in the absence of colds/flu (OR 90th vs. 10th intake centiles 1.27, 95% CI 1.04–1.55), however, β -carotene was not associated with symptoms of cough, phlegm or dyspnoea and was beneficially associated with ventilatory function; the forced expiratory volume in 1 s (FEV₁) of subjects in the 90th centile of β -carotene intake being 60 mL (95% CI 31–89) greater than that of subjects in the 10th centile. Similarly, the difference in FVC between these two levels of β -carotene intake was 75 mL (95% CI 40–110). Dietary intake of vitamin C was similarly positively associated with ventilatory function. There were no associations between dietary vitamin E intake and ventilatory function or wheezing symptoms. As mentioned above in NHANES III, serum lycopene concentrations were adversely associated with the likelihood of atopic sensitization [38].

On balance the observational epidemiological data suggest that while asthma, atopic dermatitis and AR are associated with reduced blood levels and/or reduced dietary intake of the antioxidants vitamin C, vitamin E, some carotenoids, selenium, Mediterranean diet and antioxidant rich fruits, the evidence suggests that antioxidants are more closely associated with asthma than for AR and atopic dermatitis. Interpretation of the observational literature of cross-sectional and case-control studies is limited because allergic diseases are associated with oxidant stress, moreover, there is some evidence suggesting that dietary habits may change as asthma develops [48]. Thus it is not possible to ascertain whether the

reduced antioxidant status observed in allergic disease is causal or consequential. To investigate these issues intervention studies are required. Intervention in animal models has been used to investigate the potential role of antioxidants in established allergic disease and in its aetiology. The majority of antioxidant intervention studies in humans have been in adult asthmatics, we are not aware of any human intervention studies investigating antioxidants in the primary prevention of human allergic disease.

Intervention studies

Animal models

Antioxidant supplementation studies in animal models of allergic disease fall into two types: Supplementation of allergen sensitized animals that are subsequently challenged with allergen is analogous to intervention studies in established allergic disease. Supplementation before primary allergen sensitization provides insight into the potential of antioxidants in the primary prevention of allergic disease.

The administration of γ -tocopherol to ovalbumin (OVA)-sensitized rats attenuated nasal and pulmonary eosinophilic inflammatory responses induced by subsequent intranasal challenge. γ -tocopherol supplementation was associated with reduced levels of leukotriene (LT)B₄ and prostaglandin (PG)E₂ in bronchoalveolar lavage fluid (BALF) and reduced nasal expression of IFN- γ , IL-4, IL-5 and IL-13 [49]. Similar results have been observed in mouse models supplemented with vitamin E before primary sensitization [50]. Supplementation with vitamin E before primary sensitization in the murine model of toluene-diisocyanate (TDI) induced nasal allergy, significantly attenuated the outwardly observable indicators of nasal allergy, and reduced mitogen-induced lymphoproliferative, IL-4 and IL-5 responses [51]. Similar results have been reported in nasally TDI sensitized guinea-pigs supplemented with vitamin E and C [52].

In OVA-sensitized mice selenium supplementation before rechallenge reduces eosinophilic airway inflammation by reducing activation of the redox dependent transcription factor NF- κ B and expression of NF- κ B-dependent inflammatory proteins [53]. Similar experiments using mice established on low, medium or high selenium diets before primary sensitization suggested that while selenium inhibits allergic airway inflammation the association is not a simple linear dose-response. This may account for the inconsistencies between human observational studies because mice on the medium intake diet had the most florid allergic inflammatory response [54].

Supplementation of sensitized mice with lycopene before rechallenge significantly reduced allergic cellular influx into the airways and was also associated with

reduced expression of IL-4 and the Th2 transcription factor GATA-3 and increased expression of IFN- γ and the Th1 transcription factor T-bet [55]. Similar experiments in mice and guinea-pigs supplementing with flavonoids before, and after primary sensitization have shown allergic airway inflammation, serum IgE and BALF IL-4 and IL-5 responses to be inhibited by supplementation with luteolin, quercetin, isoquercitrin and nairutin [56–60].

These animal studies suggest that the antioxidants vitamin E, selenium, flavonoids and carotenoids ameliorate allergic airway and nasal inflammation in models of established allergic sensitization. Furthermore, these studies also suggest that the antioxidants vitamin E, carotenoids, flavonoids and possibly selenium have the potential to reduce the likelihood of primary allergic sensitization.

Humans

The therapeutic potential of a limited number of antioxidants (vitamin C, vitamin E, selenium) as adjuncts to conventional treatment of allergic disease has been investigated by intervention studies. The majority have supplemented asthmatics with vitamin C, and a Cochrane review of these studies highlighted the variability in the duration and dosing of vitamin C supplementation. The review concluded that there is insufficient evidence to recommend vitamin C in asthma treatment. There is still a need for well-designed randomized-controlled trials (RCT) of vitamin C supplementation, particularly in children [61].

The literature of non-vitamin C intervention studies is smaller and limited to vitamin E and selenium. An RCT supplementing 197 mild/moderately severe asthmatics with 100 μ g/day selenium or placebo for 24 weeks reported no beneficial effects on any parameter measured; however, the baseline values of the parameters suggested mild disease with little scope for improvement. The authors suggested that, with hindsight, it may have been more instructive to have investigated whether selenium supplementation facilitated greater controlled reduction in steroid dosage than placebo group [62]. An RCT supplementing 40 adults with atopic dermatitis with 600 μ g/day selenium or placebo for 12 weeks was not associated with any clinical improvement, [63]. At present there is little evidence to recommend selenium supplementation in asthmatic adults; however, there is still scope for further studies in children and atopic dermatitis and AR.

There are several studies of vitamin E supplementation in allergic disease. An RCT in which 72 mild/moderately severe adult asthmatics were supplemented with 500 mg/day vitamin E or placebo for 6 weeks reported no beneficial effects on any parameter of asthma control [64]. The 6-week duration may have been insufficient

and the scope for improvement was limited because baseline values suggested mild disease. Studies of vitamin E supplementation in established atopic dermatitis and AR suggest that vitamin E supplementation is associated with minimal, if any clinical benefit, however, most of the studies have either been small or supplemented for short periods. A single-blind study of 96 adults with atopic dermatitis after withdrawal of non-emollient medications supplemented with 268 mg/day vitamin E or placebo for 8 months. Vitamin E supplementation was reported to be associated with reduced serum IgE and clinical improvement, however, the statistical significance of the findings was not documented [65]. A similar RCT supplementing 40 adults with atopic dermatitis with 400 mg/day vitamin E or placebo for 12 weeks was not associated with any clinical improvement, [63]. An RCT supplementing 112 people with AR with 800 mg/day vitamin E or placebo during the pollen season was associated with modest improvements in patient reported nasal symptoms; however, there were no improvements in the many other AR outcomes assessed [66]. A shorter, 4-week RCT of 63 subjects (mean age 12 years) with perennial allergic rhinitis supplemented with either 268 mg/day vitamin E or placebo and reported no improvement in AR symptoms or specific IgE [67].

While these studies fail to support the use of single antioxidant supplements as adjuncts to conventional therapy for asthma there is still scope for studies in children, atopic dermatitis, AR and of combinations of antioxidants. The beneficial effects of antioxidant supplementation in animal models of established allergic disease contrast with the negative studies in humans. The animal data suggest antioxidants have the potential to inhibit Th2 and/or promote Th1 differentiation. However, this is not observed in adult humans possibly because compartmentalization of allergen-specific Th cell immunity into adult-equivalent Th1 and Th2 patterns occurs in most children before 5 years [68] and it is unlikely that dietary antioxidants are sufficiently potent to reverse established Th cell phenotypes. Indeed, whereas serum antioxidant levels in atopic adults are not associated with peripheral blood mononuclear cell cytokine responses [69], neonatal cord blood mononuclear cell (CBMC) responses have been associated with maternal dietary antioxidant intakes during pregnancy [70, 71].

Antioxidants and the aetiology of allergic disease

There is increasing interest in the concept that early life antioxidant status might influence the development of allergic disease. This hypothesis combines current thoughts on the early life origins of allergic disease [72–76] and the original antioxidant hypothesis [16]. As discussed above, antioxidant supplementation of animal models before primary allergen sensitization suggests that

antioxidants have the potential to influence the development of allergic disease. In humans these initial interactions between the immune system and the common perennial and seasonal allergens occur during infancy and possibly *in utero*. It has even been suggested that the associations between antioxidants and allergic disease in older children and adults are indirect reflections of associations with maternal diet during pregnancy. Habitual dietary patterns are primarily established in early childhood and are influenced by parental dietary habits such that there are correlations between the diets of parents and their children and within individuals, between adult and childhood diet [77, 78]. In recent years associations have been reported between maternal antioxidant intake during pregnancy and childhood allergic disease.

Studies of maternal antioxidant intake during pregnancy

Two UK birth cohort studies have reported associations between early childhood wheezing and reduced neonatal and fetal selenium status [79, 80]; however, no associations have been reported with childhood asthma, atopic dermatitis or AR. Moreover, the associations with early childhood wheezing appear to be transient, being absent by age 5 years, possibly reflecting short-lived effects of selenium on early life responses to viral infection [80].

The US Project Viva cohort study has reported low maternal intake of vitamin E, and some carotenoids (β -carotene, lutein+zeaxanthin) during pregnancy to be associated with increased CBMC proliferative responses [71]. This study has also reported low maternal vitamin E intake during pregnancy to be associated with an increased likelihood of wheeze outcomes in 1290 two-year-old children [81]. A Scottish birth cohort study has also reported low maternal vitamin E intake during pregnancy to be associated with increased neonatal CBMC proliferative responses [70] and an increased likelihood of asthma in 1253 children aged 5 years, and with increased levels of exhaled nitric oxide [82]. In addition, low maternal plasma α -tocopherol levels during pregnancy were associated with an increased likelihood of atopic sensitization and reduced post-bronchodilator FEV₁ [82] at age 5 years. Although maternal plasma α -tocopherol concentration during early pregnancy was associated with atopic sensitization at age 5 there were no associations with atopic dermatitis nor AR. There was also an association between reduced maternal apple intake during pregnancy and an increased likelihood of wheeze and asthma outcomes in children at 5 years; similar associations in children and adults have been attributed to their phytochemical content, e.g. flavonoids [83]. Further follow-up of the United States and Scottish birth cohorts when the children are older and their asthmatic/allergic phenotypes more established and clinically meaningful are required.

The Irish Life-ways Cross-Generation Cohort Study reported an association between high maternal fruit and vegetable intake during pregnancy and reduced likelihood of asthma in 631 three-year-old children [84]. A Menorcan birth cohort study has reported wheeze and atopic sensitization in 460 children aged 6–7 years is less frequent if mothers consumed a Mediterranean diet during pregnancy [85]. The Menorcan and the Scottish studies also reported that while maternal and child diets were correlated there were no associations with children's diets [82, 85].

The results of these cohort studies suggest that reduced maternal intake of some antioxidants (vitamin E, but not selenium) and antioxidant rich foods during pregnancy are associated with an increased risk of childhood respiratory outcomes (wheeze and asthma) and atopic sensitization but not with atopic dermatitis or AR. The associations between maternal antioxidant intake during pregnancy and CBMC responses suggest a mechanism whereby maternal antioxidant intake during pregnancy influences the first interactions between the neonatal immune system and allergens [70, 71].

Possible mechanisms

The failure of birth cohort studies to demonstrate associations with some antioxidants, e.g. vitamin C suggests that vitamin E may be acting through biological mechanisms unrelated to antioxidant properties [86]. Vitamin E has been reported to have complex effects on immunological and inflammatory pathways pertinent to the aetiology of allergic disease [86, 87]. The anti-inflammatory effects of vitamin E appear to be mediated by inhibition of protein kinase B and C activity and the subsequent inhibition of the activation and DNA binding of the transcription factor NF- κ B with consequent effects on the regulation of inflammatory cytokines, adhesion molecules and other pro-inflammatory molecules. Vitamin E has many effects on the immune system with reduced vitamin E status being associated with the Th2 phenotype, either by reducing Th cell secretion of IFN- γ and/or increasing secretion of IL-4 or IL-5 [50, 88–92]. Several mechanisms have been proposed for these immunomodulatory effects. Low vitamin E status is associated with increased macrophage cyclo-oxygenase-2 activity and increased production of PGE₂ [92] that has been shown to promote the Th2 phenotype [93–95]. Vitamin E also inhibits the binding of NF- κ B and AP-1 to the IL-4 promoter region, down-regulating IL-4 mRNA expression in human Th cells [88]. Vitamin E may also influence the development of regulatory T cells [96]. The effects of vitamin E on the immune system appear to be age related, enhancing the IFN- γ responses of young but not old mice [97] and in humans, naïve Th cells are more responsive to vitamin E than memory Th cells [50, 90–92]. These age-dependent

associations may partly explain the ineffectiveness of vitamin E supplementation in adult asthmatics, conversely any putative effects of vitamin E on allergic disease may be most potent in early infancy during initial encounters with allergens rather than after disease has been established.

Conclusions

Despite the notable inconsistencies and gaps in the evidence base, it would seem reasonable to conclude that there are associations between antioxidant status and asthma, however, the exact nature of the relationships and the potential for therapeutic intervention is far from clear. From the limited data available it is not possible to exclude an association between antioxidants and atopic dermatitis and AR. Currently, there appears to be little clinical indication for antioxidant supplementation in asthma, but there is a need for intervention studies in atopic dermatitis and AR, in children, and with combinations of antioxidants, ideally in the form of antioxidant-rich foods because they contain natural mixtures of micronutrients that may contribute more than the sum of their parts. While antioxidant supplementation of all patients with allergic disease may provide little clinical benefit, it is possible that targeted antioxidant supplementation of individuals who are genetically susceptible to enzymatic antioxidant deficiencies may be clinically beneficial. Future intervention studies should ideally include investigation of genetic variation in the components of the anti-oxidant defence systems (e.g. GSTM1, GSTP1) and cellular immune response (both Th and Tr cell). Although antioxidants are naturally available and consumed, therapeutic antioxidant supplementation is not without risk: a meta-analysis of randomized trials of antioxidant supplementation in adults for primary and secondary prevention reported an increased mortality (OR 1.05; 95% CI, 1.02–1.08) particularly in studies of β -carotene and vitamin E, but not vitamin C or selenium [98].

Animal models suggest that antioxidants have the potential to influence the development of allergic disease and this is supported by recent reports of potentially beneficial associations between childhood asthma and maternal dietary antioxidant intake during pregnancy, particularly vitamin E. Although some studies have reported associations between maternal dietary antioxidant intake during pregnancy and the likelihood of childhood atopic sensitization, there appear to be few, if any, associations with atopic dermatitis or AR. Before any recommendations can be made to pregnant women further research is required: The epidemiological observations should ideally be replicated in pregnant animal models and most importantly, trials of dietary intervention in pregnant women are required to confirm the

effectiveness and efficacy of any antioxidant intervention and whether any beneficial effects are limited to asthma. The issue of safety is particularly important in trials involving pregnant women and it would seem reasonable that instead of supplementing pregnant women with supraphysiological doses of antioxidants, intervention studies should instead aim to optimize antioxidant intake, preferably by dietary means in order to replicate in food the natural form and intake of antioxidants (and co-existing nutrients) that the lung has evolved to incorporate into antioxidant defences.

Acknowledgement

Keith Allan is supported by an MRC Capacity Building PhD Studentship.

References

- 1 Bowler RP, Crapo JD. Oxidative stress in allergic respiratory diseases. *J Allergy Clin Immunol* 2002; 110:349–56.
- 2 de Luis DA, Armentia A, Aller R *et al*. Dietary intake in patients with asthma: a case control study. *Nutrition* 2005; 21:320–24.
- 3 Emelyanov A, Fedoseev G, Abulimity A *et al*. Elevated concentrations of exhaled hydrogen peroxide in asthmatic patients. *Chest* 2001; 120:1136–39.
- 4 Gannas K, Loukides S, Papatheodorou G, Panagou P, Kalogepoulos N. Total nitrite/nitrate in expired breath condensate of patients with asthma. *Respir Med* 2001; 95:564–649.
- 5 Cross CE, van der Vleit A, Louie S, Thiele JJ, Halliwell B. Oxidative stress and antioxidants at biosurfaces: plants, skin and respiratory tract surfaces. *Environ Health Perspect* 1998; 106:1241–51.
- 6 Kelly FJ, Mudway IS, Krishna MT, Holgate ST. The free radical basis of air pollution: focus on ozone. *Respir Med* 1995; 89:647–56.
- 7 Kelly FJ, Mudway I, Blomberg A, Frew A, Sandstrom T. Altered lung antioxidant status in patients with mild asthma. *Lancet* 1999; 354:482–3.
- 8 Mudway IS, Stenfors N, Blomberg A *et al*. Differences in basal airway antioxidant concentrations are not predictive of individual responsiveness to ozone: a comparison of healthy and mild asthmatic subjects. *Free Radic Biol Med* 2001; 31:962–74.
- 9 Romieu I, Meneses F, Ramirez M *et al*. Antioxidant supplementation and respiratory functions among workers exposed to high levels of ozone. *Am J Respir Crit Care Med* 1998; 158:226–32.
- 10 Romieu I, Sienra-Monge JJ, Ramirez-Aguilar M *et al*. Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. *Am J Respir Crit Care Med* 2002; 166:703–9.
- 11 Grievink L, Jansen SM, van't Veer P, Brunekreef B. Acute effects of ozone on pulmonary function of cyclists receiving antioxidant supplements. *Occup Environ Med* 1998; 55:13–7.
- 12 Blomberg A, Krishna MT, Helleday R *et al*. Persistent airway inflammation but accommodated antioxidant and lung function responses after repeated daily exposure to nitrogen dioxide. *Am J Respir Crit Care Med* 1999; 159:536–43.

- 13 Mudway IS, Stenfors N, Duggan ST *et al.* An in vitro and in vivo investigation of the effects of diesel exhaust on human airway lining fluid antioxidants. *Arch Biochem Biophys* 2004; 423:200–12.
- 14 Behndig AF, Blomberg A, Helleday R, Duggan ST, Kelly FJ, Mudway IS. Antioxidant responses to acute ozone challenge in the healthy human airway. *Inhal Toxicol* 2009; 21:933–42.
- 15 Romieu I, Ramirez-Aguilar M, Sienra-Monge JJ *et al.* GSTM1 and GSTP1 and respiratory health in asthmatic children exposed to ozone. *Eur Respir J* 2006; 28:953–9.
- 16 Seaton A, Godden DJ, Brown K. Increase in asthma: a more toxic environment or a more susceptible population? *Thorax* 1994; 49:171–4.
- 17 Devereux G, Seaton A. Diet as a risk factor for atopy and asthma. *J Allergy Clin Immunol* 2005; 115:1109–17.
- 18 Rayman MP. Dietary selenium: time to act. *Br Med J* 1997; 314:387–8.
- 19 MacPherson A, Barclay MNI, Dixon J, Groden BM, Scott R, Kesson E. Decline in dietary selenium intake in Scotland and effect on plasma concentrations. In: Anke M, Meissner D, Mills CF, eds. *Proceedings of the eighth international symposium on trace elements in man and animals 1993*. Jena, Germany: Verlag Media Touristik, 1993: 269–70.
- 20 Shortt CT, Duthie GG, Robertson JD, Morrice PC, Nicol F, Arthur JR. Selenium status of a group of Scottish adults. *Eur J Clin Nutr* 1997; 51:400–4.
- 21 Department of Health. *Dietary reference values for food energy and nutrients for the United Kingdom. Report on health and social subjects 41*. London: HMSO, 1991.
- 22 Department of Environmental Farming and Rural Affairs., National Food Survey. Trends in household nutrient intake. Available at <http://statistics.defra.gov.uk/esg/publications/efs/2005/chapter5.pdf> (accessed November 2009).
- 23 Henderson L, Irving K, Gregory J *et al.* The National Diet and Nutrition Survey: adults aged 19 to 64 years. HMSO 2003. Available at <http://www.food.gov.uk/multimedia/pdfs/ndnsv3.pdf> (accessed November 2009).
- 24 Zvetkova E, Wirleitner B, Tram NT, Schennach H, Fuchs D. Aqueous extracts of *Crinum latifolium* (L.) and *Camellia sinensis* show immunomodulatory properties in human peripheral blood mononuclear cells. *Int Immunopharmacol* 2001; 1:2143–50.
- 25 Neurauter G, Wirleitner B, Schroecksnadel K, Schennach H, Fuchs D. Wine and grape juice modulate biochemical pathways in stimulated human peripheral blood mononuclear cells. *Pteridines* 2004; 15:1–6.
- 26 Winkler C, Wirleitner B, Schroecksnadel K, Schennach H, Mur E, Fuchs D. In vitro effects of two extracts and two pure alkaloid preparations of *Uncaria tomentosa* on peripheral blood mononuclear cells. *Planta Med* 2004; 70:205–10.
- 27 . Consumption of selected household foods, 1942 to 1996. Available at <https://statistics.defra.gov.uk/esg/publications/nfs/datasets/allfood.xls> (accessed November 2009).
- 28 Tricon S, Willers S, Smit HA *et al.* Nutrition and allergic disease. *Clin Exp Allergy Reviews* 2006; 6:117–88.
- 29 Harik-Khan RI, Muller DC, Wise RA. Serum vitamin levels and the risk of asthma in children. *Am J Epidemiol* 2004; 159:351–7.
- 30 Rubin RN, Navon L, Cassano PA. Relationship of serum antioxidants to asthma prevalence in youth. *Am J Respir Crit Care Med* 2004; 169:393–8.
- 31 Sempos CT. Invited commentary: some limitations of semiquantitative food frequency questionnaires. *Am J Epidemiol* 1992; 135:1127–35.
- 32 Block G. A review of validations of dietary assessment methods. *Am J Epidemiol* 1982; 115:492–505.
- 33 Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997; 65 (Suppl.):1220S–8S.
- 34 Britton JR, Pavord ID, Richards KA *et al.* Dietary antioxidant vitamin intake and lung function in the general population. *Am J Respir Crit Care Med* 1995; 151:1383–7.
- 35 Traber MG. Measurement of lipid-soluble vitamins – further adjustment needed? *Lancet* 2000; 355:2013–4.
- 36 Wood LG, Garg ML, Blake RJ, Garcia-Caraballo S, Gibson PG. Airway and circulating levels of carotenoids in asthma and healthy controls. *J Am Coll Nutr* 2005; 24:448–55.
- 37 Fogarty A, Lewis S, Weiss S, Britton J. Dietary vitamin E, IgE concentrations, and atopy. *Lancet* 2000; 356:1573–4.
- 38 McKeever TM, Lewis SA, Smit H, Burney P, Britton J, Cassano PA. Serum nutrient markers and skin prick testing using data from the Third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 2004; 114:1398–402.
- 39 Hozyasz KK, Chelchowska M, Laskowska-Klita T, Ruskowska L, Milanowski A. Low concentration of alpha-tocopherol in erythrocytes of atopic dermatitis patients. *Med Wieku Rozwoj* 2004; 8:963–9.
- 40 Chung YM, Kim BS, Kim NI, Lee EY, Choue R. Study of nutritional status, dietary patterns, and dietary quality of atopic dermatitis patients. *Korean J Nutr* 2005; 38:419–31.
- 41 Chatzi L, Apostolaki G, Bibakis I *et al.* Protective effect of fruits, vegetables and the Mediterranean diet on asthma and allergies among children in Crete. *Thorax* 2007; 62:677–83.
- 42 De Batlle J, Garcia-Aymerich J, Barraza-Villarreal A, Antó JM, Romieu I. Mediterranean diet is associated with reduced asthma and rhinitis in Mexican children. *Allergy* 2008; 63:1310–6.
- 43 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.
- 44 Garcia-Marcos L, Canflanca IM, Garrido JB *et al.* Relationship of asthma and rhinoconjunctivitis with obesity, exercise and Mediterranean diet in Spanish schoolchildren. *Thorax* 2007; 62:503–8.
- 45 Tamay Z, Akcay A, Ones U, Guler N, Kilic G, Zencir M. Prevalence and risk factors for allergic rhinitis in primary school children. *Int J Pediatr Otorhinolaryngol* 2007; 71:463–71.
- 46 Nagel G, Linseisen J. Dietary intake of fatty acids, antioxidants and selected food groups and asthma in adults. *Eur J Clin Nutr* 2005; 59:8–15.
- 47 Grievink L, Smit HA, Ocke MC, van't Veer P, Kromhout D. Dietary intake of antioxidant (pro)-vitamins, respiratory symptoms and pulmonary function: the MORGEN study. *Thorax* 1998; 53:166–71.
- 48 Troisi RJ, Willett WC, Weiss ST, Trichopoulos D, Rosner B, Speizer FE. A prospective study of diet and adult-onset asthma. *Am J Respir Crit Care Med* 1995; 151:1401–8.
- 49 Wagner JG, Jiang Q, Harkema JR *et al.* γ -Tocopherol prevents airway eosinophilia and mucous cell hyperplasia in

- experimentally induced allergic rhinitis and asthma. *Clin Exp Allergy* 2007; **38**:501–11.
- 50 Okamoto N, Murata T, Tamai H, Tanaka H, Nagai H. Effects of alpha tocopherol and probucol supplements on allergen-induced airway inflammation and hyperresponsiveness in a mouse model of allergic asthma. *Int Arch Allergy Immunol* 2006; **141**:172–80.
 - 51 Zheng KC, Adjei AA, Shinjo M, Shinjo S, Todoriki H, Ariizumi M. Effect of dietary vitamin E supplementation on murine nasal allergy. *Am J Med Sci* 1999; **318**:49–54.
 - 52 Gu H, Itoh M, Matsuyama N *et al*. Toluene Diisocyanate exposure induces laryngo-tracheal eosinophilia, which can be ameliorated by supplementation with antioxidant vitamins in Guinea pigs. *Acta Otolaryngol* 2003; **123**:965–71.
 - 53 Jeong DW, Yoo MH, Kim TS, Kim JH, Kim IY. Protection of mice from allergen induced asthma by selenite. *J Biol Chemistry* 2002; **277**:17871–6.
 - 54 Hoffmann PR, Jourdan-Le Saux C, Hoffmann FW *et al*. A role for dietary selenium and selenoproteins in allergic airway inflammation. *J Immunol* 2007; **179**:3258–67.
 - 55 Lee CM, Chang JH, Moon DO *et al*. Lycopene suppresses ovalbumin-induced airway inflammation in a murine model of asthma. *Biochem Biophys Res Comm* 2008; **374**:248–52.
 - 56 Das M, Ram A, Ghosh B. Luteolin alleviates bronchoconstriction and airway hyperactivity in ovalbumin sensitised mice. *Inflamm Res* 2004; **58**:101–6.
 - 57 Moon H, Choi HH, Lee JY, Moon HJ, Sim SS, Kim CJ. Quercetin inhalation inhibits the asthmatic responses by exposure to aerosolized ovalbumin in conscious guinea pigs. *Arch Pharm Res* 2008; **31**:771–8.
 - 58 Jung CH, Lee JY, Cho CH, Kim CJ. Anti-asthmatic action of quercetin and rutin in conscious guinea pigs challenged with aerosolized ovalbumin. *Arch Pharmacol Res* 2007; **30**:1599–607.
 - 59 Rogerio AP, Kanashiro A, Fontanari C *et al*. Anti-inflammatory activity of quercetin and isoquercitrin in experimental murine allergic asthma. *Inflamm Res* 2007; **56**:402–8.
 - 60 Funaguchi N, Ohno Y, La BLB *et al*. Nairutin inhibits airway inflammation in and allergic mouse model. *Clin Exp Pharm Physiol* 2007; **34**:766–70.
 - 61 Kaur B, Rowe BH, Arnold E. Vitamin C supplementation for asthma. *Cochrane Database of Systematic reviews* 2009, Art. No.: CD000993, doi: 10.1002/14651858.CD000993.pub3.
 - 62 Shaheen SO, Newson RB, Rayman MP *et al*. Randomised, double blind, placebo-controlled trial of selenium supplementation in adult asthma. *Thorax* 2007; **62**:483–90.
 - 63 Fairris GM, Perkins PJ, Lloyd B, Hinks L, Clayton BE. The effects on atopic dermatitis of supplementation with selenium and vitamin E. *Acta Derm Venereol* 1989; **69**:359–62.
 - 64 Pearson PJK, Lewis SA, Britton J, Fogarty A. Vitamin E supplements in asthma: a parallel group randomised placebo controlled trial. *Thorax* 2004; **59**:652–6.
 - 65 Tsourelis-Nikita E, Hercogova J, Lotti T, Menchini G. Evaluation of dietary intake of vitamin E in the treatment of atopic dermatitis: a study of the clinical course and evaluation of the immunoglobulin E serum levels. *Int J Dermatol* 2002; **41**:146–50.
 - 66 Sahar E, Hassoun G, Pollack S. Effect of vitamin E supplementation on the regular treatment of seasonal allergic rhinitis. *Ann Allergy Asthma Immunol* 2004; **92**:654–8.
 - 67 Montano Velazquez BB, Jauregui-Renaud K, Banuelos Arias Adel C *et al*. Vitamin E effects on nasal symptoms and serum specific IgE levels in patients with perennial allergic rhinitis. *Ann Allergy Asthma Immunol* 2006; **96**:45–50.
 - 68 Yabuhara A, Macaubas C, Prescott SL *et al*. Th-2 polarised immunological memory to inhalant allergens in atopics is established during infancy and early childhood. *Clin Exp Allergy* 1997; **27**:1261–9.
 - 69 Dunstan JA, Breckler L, Hale J *et al*. Associations between antioxidant status, markers of oxidative stress and immune responses in allergic adults. *Clin Exp Allergy* 2006; **36**:993–1000.
 - 70 Devereux G, Barker RN, Seaton A. Ante-natal determinants of neonatal immune responses to allergens. *Clin Exp Allergy* 2002; **32**:43–50.
 - 71 Litonjua AA, Tantisira KG, Finn PW *et al*. Maternal antioxidant intake during pregnancy and cord blood lymphoproliferative responses. *Am J Resp Crit Care Med* 2004; **169**:A501.
 - 72 Warner JA, Jones CA, Jones AC, Warner JO. Prenatal origins of allergic disease. *J Allergy Clin Immunol* 2000; **105**:S493–8.
 - 73 Holt PG, Devereux G, Sly P. Early life origins of allergy and asthma. In: Holgate ST, Church MK, Lichenstein LM, eds. *Allergy*, 3rd Edn.. Philadelphia, USA: Mosby Elsevier, 2006; 223–32.
 - 74 Håland G, Carlsen KC, Sandvik L *et al*. Reduced lung function at birth and the risk of asthma at 10 years of age. *N Engl J Med* 2006; **355**:1682–9.
 - 75 Lucas JS, Inskip HM, Godfrey KM *et al*. Small size at birth and greater postnatal weight gain: relations to diminished infant lung function. *Am J Respir Crit Care Med* 2004; **170**:34–40.
 - 76 Prescott SL. Early origins of allergic disease: a review of processes and influences during early immune development. *Curr Opin Allergy Clin Immunol* 2003; **3**:125–32.
 - 77 Lynch JW, Kaplan GA, Salonen JT. Why do poor people behave poorly? Variation in adult health behaviours and psychosocial characteristics by stages of the socioeconomic lifecourse. *Soc Sci Med* 1997; **44**:809–19.
 - 78 Talvia S, Rasanen L, Lagstrom H *et al*. Longitudinal trends in consumption of vegetables and fruit in Finnish children in an atherosclerosis prevention study (STRIP). *Eur J Clin Nutr* 2006; **60**:172–80.
 - 79 Shaheen SO, Newson RB, Henderson AJ, Emmett PM, Sherriff A, Cooke M. Umbilical cord trace elements and minerals and risk of early childhood wheezing and eczema. *Eur Respir J* 2004; **24**:292–7.
 - 80 Devereux G, McNeill G, Newman G *et al*. Early childhood wheezing symptoms in relation to plasma selenium in pregnant mothers and neonates. *Clin Exp Allergy* 2007; **37**:1000–8.
 - 81 Litonjua AA, Rifas-Shiman S, Ly NP *et al*. Maternal antioxidant intake in pregnancy and wheezing illnesses in children at 2 y of age. *Am J Clinical Nutr* 2006; **84**:903–11.
 - 82 Devereux G, Turner SW, Craig LCA *et al*. Reduced 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.
 - 83 Willers SM, Devereux G, Craig LCA *et al*. Maternal food consumption during pregnancy and asthma, respiratory and atopic symptoms in 5-year-old children. *Thorax* 2007; **62**:772–8.
 - 84 Fitzsimon N, Fallon UB, O'Mahony D *et al*. Mothers' dietary patterns during pregnancy and the risk of asthma symptoms in children at 3 years. *Irish Med J* 2007; **100**:27–32.
 - 85 Chatzi L, Torrent M, Romieu I *et al*. Mediterranean diet in pregnancy is protective for wheeze and atopy in childhood. *Thorax* 2008; **63**:507–13.

- 86 Azzi A, Gysin R, Kempna P *et al.* The role of α -tocopherol in preventing disease: from epidemiology to molecular events. *Molec Aspects Dis* 2003; **24**:325–36.
- 87 Reiter E, Jiang Q, Christen S. Anti-inflammatory properties of α - and γ -tocopherol. *Molec Aspects Med* 2007; **28**:668–91.
- 88 Li-Weber M, Giasisi M, Trieber MK, Krammer PH. Vitamin E inhibits IL-4 gene expression in peripheral blood T-cells. *Eur J Immunol* 2002; **32**:2401–8.
- 89 Han SN, Ha WK, Beharka A, Smith DE, Bender BS, Meydani SN. Vitamin E supplementation increases T helper 1 cytokine production in old mice infected with influenza virus. *Immunology* 2000; **100**:487–93.
- 90 Malmberg KJ, Lenkei R, Petersson M *et al.* A short-term dietary supplementation of high doses of vitamin E increases T helper 1 cytokine production in patients with advanced colorectal cancer. *Clin Cancer Res* 2002; **8**:1772–8.
- 91 Wang Y, Huang DS, Eskelson CD, Watson RR. Long term dietary vitamin E retards development of retrovirus induced dysregulation in cytokine production. *Clin Immunol* 1994; **72**:70–5.
- 92 Meydani SN, Han SN, Wu D. Vitamin E and immune response in the aged: molecular mechanisms and clinical implications. *Immunol Rev* 2005; **205**:269–81.
- 93 Roper RL, Brown DM, Phipps RP. Prostaglandin E2 promotes B lymphocyte Ig isotype switching to IgE. *J Immunol* 1995; **154**:162–70.
- 94 Kalinski P, Hilkens CM, Snijders A, Snijdwint FG, Kapsenberg ML. IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naive T helper cells. *J Immunol* 1997; **159**: 28–35.
- 95 Hilkins CM, Snijders A, Snijdwint FG, Wierenga EA, Kapsenberg ML. Modulation of T-cell cytokine secretion by accessory cell-derived products. *Eur Respir J* 1996; **22**:90S–4S.
- 96 Tan PH, Sagoo P, Chan C *et al.* Inhibition of NF- κ B and oxidative pathways in human dendritic cells by antioxidative vitamins generates regulatory T cells. *J Immunol* 2005; **174**:7633–44.
- 97 Wakikawa A, Utsuyama M, Wakabayashi A, Kitagawa M, Hirokawa K. Vitamin E enhances the immune functions of young but not old mice under restraint stress. *Exp Gerontol* 1999; **34**: 853–62.
- 98 Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA* 2007; **297**:842–57.