

Vitamins and respiratory disease: antioxidant micronutrients in pulmonary health and disease

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The lungs are continually exposed to relatively-high O₂ tensions, and as such, in comparison with other organs, they represent a unique tissue for the damaging effects of oxidant attack. At particular times during a lifetime this every day challenge may increase exponentially. The first oxidative insult occurs at birth, when cells are exposed to a sudden 5-fold increase in O₂ concentration. Thereafter, the human lung, from infancy through to old age, can be subjected to deleterious oxidative events as a consequence of inhaling environmental pollutants or irritants, succumbing to several pulmonary diseases (including infant and adult respiratory distress syndromes, asthma, chronic obstructive pulmonary disease, cystic fibrosis and cancer) and receiving treatment for these diseases. The present paper will review the concept that consumption of a healthy diet and the consequent ability to establish and then maintain adequate micronutrient antioxidant concentrations in the lung throughout life, and following various oxidative insults, could prevent or reduce the incidence of oxidant-mediated respiratory diseases. Furthermore, the rationale, practicalities and complexities of boosting the antioxidant pool of the respiratory-tract lining fluid in diseases in which oxidative stress is actively involved, by direct application to the lung *v.* dietary modification, in order to achieve a therapeutic effect will be discussed.

Oxidative stress: Antioxidant vitamins: Lifespan: Pulmonary disease: Antioxidant therapy

The function of the lungs is to exchange gases between the tissues of the body and the outside environment. To do this job effectively, the adult human respiratory tract is composed of twenty-three generations of branching airways with approximately 300×10^6 alveoli, which produces an enormous surface area of about 140 m² that is in direct contact with the ambient air inhaled with each breath. Even under 'normal' circumstances (i.e. when an individual is in good health and resides in a relatively clean environment) the lung, in comparison with other organs, represents a unique tissue for oxidant stress, continually exposed to relatively-high O₂ tensions, inevitable pollutants and the products of metabolism derived from the pollutants. At particular times during a lifetime, however, this every day challenge may increase exponentially. For instance, *in utero* fetal lungs are exposed to relatively hypoxic tensions. These tensions rise abruptly at birth, having the potential to elicit oxidative injury in the neonate. Following the huge transition undertaken to begin life, the human

lung, from infancy through to old age, can then be subjected to many other insults that mediate damage, at least partly through the generation of reactive oxygen-derived free radicals. For example, inhalation of airborne irritants and pollutants, including cigarette smoke, O₃ and carcinogens (e.g. diesel exhaust), will generate excess reactive oxygen (ROS) and nitrogen species in the lung (Doelman & Bast, 1990; Rahman & MacNee, 1996). In addition, a typical component in most lung diseases and infections is the activation of inflammatory, immune and structural cells of the airways with consequent free radical generation (Barnes, 1990). Furthermore, several therapies lead to free radical-induced tissue damage, i.e. O₂ therapy for the treatment of prematurely born neonates and acute respiratory distress syndrome (ARDS), and chemotherapy and radiation for cancer patients. Lung tissue can also be destroyed during reperfusion after an ischaemic period such as that produced by surgery (Erzurum *et al.* 1993; Repine *et al.* 1997).

Abbreviations: ARDS, acute respiratory distress syndrome; CFTR, cystic fibrosis transmembrane conductance regulator; COPD, chronic obstructive pulmonary disease; FEV₁, forced expiratory volume in 1 s; GSH, reduced glutathione; NAC, N-acetylcysteine; ROS, reactive oxygen species; RTLFL, respiratory-tract lining fluid; SOD, superoxide dismutase.

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Thus, the lung is exposed not only directly to higher O₂ tensions and environmental oxidants, but also to oxidants that are produced by a variety of lung diseases and in the course of their therapies. If these oxidants were not scavenged, the delicate epithelial cells that line the respiratory tract would undergo free radical damage (i.e. oxidative stress), characterised by an increase in the oxidation of cellular lipids, proteins, carbohydrates and DNA and, as a consequence, impaired cellular functions and enhanced inflammatory reactions (Freeman & Crapo, 1982; Halliwell & Gutteridge, 1999; Droge, 2002).

To counteract this oxidative threat the lung is well endowed with antioxidant defences. The first and probably the most important line of defence against inhaled environmental oxidants and endogenous inflammatory-immune oxidant products is a thin, highly-complex and heterogeneous layer of lining fluid, the respiratory-tract lining fluid (RTLFL), that covers the respiratory epithelium. The RTLFL has a unique armamentarium of antioxidants, at a high concentration, including vitamin C (van der Vliet *et al.* 1999), urate (Peden *et al.* 1990), reduced glutathione (GSH) (Cantin *et al.* 1987), vitamin E (Mudway *et al.* 2001) and extracellular superoxide dismutase (SOD; Mudway *et al.* 2000), catalase and glutathione peroxidase (Avissar *et al.* 1996). Additional antioxidants include mucopolypeptide glycoproteins, caeruloplasmin, Fe-binding proteins (e.g. lactoferrin and transferrin) and small molecules such as bilirubin (Heffner & Repine, 1989). The composition and size of the RTLFL antioxidant pool differs markedly between individuals (Peden *et al.* 1990; Housley *et al.* 1995).

An understanding of the transport systems and turnover of RTLFL antioxidants, their relationships with the small-molecular-weight antioxidants and enzyme systems residing within the epithelial cells that line the respiratory tract, and the role that newly-described transport systems, e.g. vitamin C (Liang *et al.* 2001), may play in maintaining either extracellular or intracellular pools is still in its infancy. However, it is known that the antioxidant network of RTLFL is highly evolved and dynamic, in that deficiencies in certain aspects of the network can be offset by up-regulation of others (Mudway *et al.* 2001). This capability is critical, since the latest evidence suggests that the susceptibility of the lung to oxidative injury depends largely on its ability to up regulate protective ROS- and reactive nitrogen species-scavenging systems. Until recently, it has been speculated that the composition and quantity of antioxidants within the RTLFL might represent an important determinant of the oxidant challenge on the underlying respiratory epithelium, and therefore may determine an individual's susceptibility to respiratory disease throughout life. The latest evidence, however, lends more support to a subtler story, in which the speed at which lost antioxidant defences can be replaced is a more important determinant.

In the normal lung, therefore, complex and coordinated interactions of antioxidant compounds provide adequate protection of the distal lung structures from the damaging effects of oxidative attack. However, an imbalance between the production of oxidants and antioxidant capacity leads to the state of oxidative stress described earlier. As

many antioxidants are derived from the diet, attention has been paid to its quality, and in particular the intake of the micronutrient antioxidants, vitamins A, C and E, and how it may help to protect individuals from an oxidising environment and/or lung disease.

The present paper will discuss the concept that maintaining adequate pulmonary antioxidant vitamin concentrations at all stages throughout life could prevent or reduce the incidence of respiratory diseases. In addition, the rationale and practicalities of boosting RTLFL antioxidant concentrations in disease in order to achieve a therapeutic effect is discussed.

Oxidative challenge throughout life

Birth and the premature infant

The first known oxidative challenge of life occurs as early as birth, when lung cells are exposed to a sudden several-fold increase in O₂ concentration. A fully-developed lung armed with sufficient defence is therefore critical in ensuring that the newborn lung is resistant to high O₂ tensions. This prerequisite is clearly highlighted in the problems that can arise following a premature birth at approximately 32 weeks or earlier, when the structural and biochemical components of the human lung vital for normal respiration are not sufficiently developed. The extent of pulmonary immaturity in an infant born at this stage necessitates ventilation and the provision of supplementary O₂ that, in the presence of a severely-reduced antioxidant defence system, has the potential to increase the risk of toxicity to lung cells. Indeed, O₂-related lung injury of prematurely born neonates can in turn play a role in the progression to bronchopulmonary dysplasia, which is a common cause of morbidity and mortality in preterm infants (Northway *et al.* 1967; Jobe & Bancalari, 2001).

Evidence that premature infants are biochemically predisposed to oxidant injury comes from an association made between a delayed increase in RTLFL vitamin C concentration and increased risk of developing bronchopulmonary dysplasia (Vyas *et al.* 2001), and from studies (Boda *et al.* 1998) that cite a compromised glutathione system. Furthermore, there are studies (Buss *et al.* 2000) that suggest that premature infants that develop bronchopulmonary dysplasia have qualitative and quantitative differences in the oxidation of pulmonary lipids and proteins when compared with infants who do not develop bronchopulmonary dysplasia. These differences are most obvious in the first few days of life, suggesting that the process of lung injury that leads to the development of bronchopulmonary dysplasia occurs within hours to days of delivery and that oxidation is a major contributor to this pathological process.

While findings suggest that antioxidant supplementation to minimise oxidative stress in premature infants is as an appropriate therapeutic strategy, early attempts have not been successful (Rosenfeld *et al.* 1996; Davis *et al.* 2000; Table 1). Welty and co-workers (Welty, 2001) have suggested that this outcome may reflect an inability to deliver antioxidants in a timely manner to the areas in the lung in which deleterious oxidations are occurring. Further

Table 1. Antioxidant-based treatments for pulmonary diseases associated with oxidative stress

Disease	Antioxidant therapy	Reference
Premature infants with respiratory distress syndrome	Recombinant human Cu,Zn SOD	Rosenfeld <i>et al.</i> (1996), Davis <i>et al.</i> (2000)
Asthma	Se Mg and vitamin C Vitamin E	Hasselmark <i>et al.</i> (1993) Fogarty <i>et al.</i> (2003) Pearson <i>et al.</i> (2004)
Chronic obstructive pulmonary disease	Vitamins C and E, and β -carotene Vitamin C Vitamin C, α -lipoic acid and vitamin E NAC	Steinberg & Chait (1998) Dietrich <i>et al.</i> (2002) Dietrich <i>et al.</i> (2002) Boman <i>et al.</i> (1983), Rasmussen & Glennow (1888), British Thoracic Society Research Committee (1985), Bridgeman <i>et al.</i> (1991, 1994), Nagy <i>et al.</i> (1997)
Acute respiratory distress syndrome	NAL NAC NAC and procysteine EPA, γ -linolenic acid Vitamins C and E	Gillissen <i>et al.</i> (1997), Nagy <i>et al.</i> (1997) Suter <i>et al.</i> (1994), Walsh & Lee (1999), Jepsen <i>et al.</i> (1992) Bernard <i>et al.</i> (1997) Gadek <i>et al.</i> (1999) Nathens <i>et al.</i> (2002)
Cystic fibrosis	β -Carotene Vitamins A, C and E, and β -carotene	Winklhofer-Roob <i>et al.</i> (1995), Lepage <i>et al.</i> (1996) Wood <i>et al.</i> (2002)

NAC, N-acetylcysteine; NAL, nacystelyn; SOD, superoxide dismutase.

research is therefore necessary to determine both the nature and the location of the oxidative events that lead to the development of early lung injury, so that more appropriate and specific antioxidant interventions can be designed.

Childhood

If a healthy diet enriched with antioxidants can prevent or reduce the incidence of oxidant-mediated respiratory diseases, it is likely that such a response will take effect prenatally, or at least very early in life. This view is supported by the fact that lung growth continues throughout childhood into early adulthood (Burri, 1997) and the theory that the risk of developing chronic respiratory diseases is associated with maximum lung size, length of the plateau and the rate of decline in function (Dockery & Brunekreef, 1996). More specifically, vitamin C may have a role both in lung growth and development and in helping to reduce airway hyperactivity, and in doing so would influence childhood and adult lung function (Soutar *et al.* 1997; Romieu & Trenga, 2001). In addition, there is evidence that vitamin A is also involved in critical pathways for normal lung function in early life (Sempertegui, 2001).

Very little information exists on the effect of dietary intervention during pregnancy and/or infancy and pulmonary health. However, Martindale *et al.* (2005) have provided some evidence of nutritional programming *in vitro*, suggesting that maternal dietary vitamin E intake may lessen the risks of developing wheeze and eczema during the second year of life (Table 2). Surprisingly, they have found a positive association between maternal consumption of foods rich in vitamin C and wheezing symptoms and eczema in the second year of life. The most likely explanation given for this effect is residual confounding by factors associated with socio-economic status and/or a healthy lifestyle.

The most comprehensive investigation to date into the role of antioxidant vitamins in children's lung function (Gilliland *et al.* 2003), which examined cross-sectional data on fruit, vegetable, juice and vitamins A, C and E intakes from the Children's Health Study, has reported that low intakes of antioxidant vitamins A, C and E are associated with deficits in pulmonary function in both boys and girls. Whilst the effects of vitamin A are supported by a study conducted on rural Ethiopian children (Kassaye *et al.* 2001), the vitamin C findings are inconsistent with the only other reported investigation of the relationship between vitamin C and lung function during childhood (Cook *et al.* 1997; Table 2). This cross-sectional study of children in England and Wales has reported a positive association between forced expiratory volume in 1 s (FEV₁) and the frequency of fresh fruit consumption, and a weak association with green vegetable and salad consumption. No association between FEV₁ and serum vitamin C concentration was found, suggesting that other micronutrient antioxidants derived from the fruit and vegetables are more important than vitamin C.

Adulthood

Within the adult population there is increasing evidence to suggest that a diet rich in fruits and vegetables has a protective effect on lung function (Britton *et al.* 1995). In particular, a number of retrospective observational studies (Table 2) have reported that individuals with a high intake of antioxidants vitamins A, C and E tend to have increased lung function and decreased respiratory symptoms. For example, studies conducted by Cohen and colleagues (Tockman *et al.* 1986; Morabia *et al.* 1989) have shown that individuals with higher vitamin A intakes tend to have better lung function. Schwartz & Weiss (1994) have reported the findings of the National Health and Nutrition

Table 2. Epidemiological evidence for a relationship between antioxidant vitamins and lung function or symptoms

Study population and design	Antioxidant		Measured in	Measured outcome	Association			Reference
	Vitamin	Measured in			Beneficial	Adverse	None	
<i>In utero</i> Prospective, 2000 pregnant women	Vitamin E Vitamin C	Diet Diet		Wheeze during 2nd year	+			Martindale <i>et al.</i> (2005)
Childhood Cross-sectional, 2566 children, 11–19 years old	Vitamin C Vitamin E Vitamin A			FVC FEV ₁ and FEF _{25–75} FEF _{25–75} FEF _{25–75}	+	+	+	Gilliland <i>et al.</i> (2003)
Cross-sectional, 702 children, 6–9 years old	Vitamin A	MRDR		FEV ₁	+			Kassaye <i>et al.</i> (2001)
Cross-sectional, 2650 children, 8–11 years old	Vitamin C	Plasma		FEV ₁			+	Cook <i>et al.</i> (1997)
Adulthood Cross-sectional, 2633 adults	Vitamin C Vitamin E	Diet Diet		FEV ₁ , FVC FEV ₁ , FVC	+			Britton <i>et al.</i> (1995)
Cross-sectional, 1165 adults	Vitamin C Vitamin E	Diet Diet		FEV ₁ :FVC ≤ 0.65 FEV ₁ :FVC ≤ 0.65			+	Morabia <i>et al.</i> (1989)
Cross-sectional, 2526 adults	Vitamin C	Diet		FEV ₁	+			Schwartz & Weiss (1994)
Cross-sectional, 793 adults	β-Carotene, vitamin C and Se			Chronic non-specific lung disease			+	Miedema <i>et al.</i> (1993)
Cross-sectional, 6555 adults	Vitamin C Vitamin E	Diet		FEV ₁ and FVC FEV ₁ and FVC	+			Grievink <i>et al.</i> (1998)
Longitudinal, 1346 adults	Vitamin C Vitamin A, E and Mg	Diet		FEV ₁ FEV ₁	+		+	McKeever <i>et al.</i> (2002)
Old age Cross-sectional, 178 adults (≥65 years)	Vitamin C Vitamin E	Diet Diet		FEV ₁ and FVC FEV ₁ and FVC			+	Dow <i>et al.</i> (1996)

MRDR, modified relative dose response; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; FEF_{25–75}, 25 % and 75 % FVC; +, positive.

Examination Survey study, which indicate that the dietary intake of vitamin C is positively associated with the FEV₁. In the Zutphen study (Miedema *et al.* 1993) fruit intake was reported to be inversely related to the incidence of chronic non-specific lung disease, although no association was found with the estimated intake of several antioxidants, including β -carotene, vitamin C and Se. In the monitoring project on risk factors and health in The Netherlands (the MORGEN study) a high intake of vitamin C and β -carotene, but not vitamin E, was reported to be associated with a higher FEV₁ and forced vital capacity than a low intake of these antioxidants (Grievink *et al.* 1998). A more extensive investigation into the relationship, over time, between intake of vitamins C, E and A and Mg and a decline in lung function has been carried out by McKeever *et al.* (2002) in a study over a 9-year period, in a population in which the investigators had previously reported a cross-sectional association between FEV₁ and intake of both vitamin C and Mg (Britton *et al.* 1994, 1995). In this longitudinal analysis higher intakes of vitamin C, but not vitamins A, E and Mg were found to be associated with a lower rate of decline of FEV₁.

The mechanism behind these reported protective effects presumably involves provision, from the diet and via the blood, of adequate antioxidant reserves within the lung to deal with the production of ROS. However, the relationship between blood concentrations of dietary antioxidants and lung function is not clear; Ness *et al.* (1996) have reported that plasma vitamin C concentration correlate with both FEV₁ and forced vital capacity in men but not women. Furthermore, the extent to which RTLF antioxidant concentrations at all levels of the respiratory tract (especially the airways) can be influenced by dietary intake and/or plasma concentrations remains an important unanswered question (Kelly *et al.* 1999). The amount of antioxidant vitamins required from the diet to provide protection to a healthy individual in an uncompromised setting is likely to be finite, in that Mudway *et al.* (2000) have shown that FEV₁ is not influenced by vitamin supplementation in healthy vitamin C-replete subjects. However, in support of benefit from increased antioxidant intake in a compromised setting, field studies (Romieu *et al.* 1998; Grievink *et al.* 1999) have demonstrated that increased intakes of nutritional antioxidants can reduce the magnitude of lung function decrements in subjects exposed to high O₃ doses both occupationally (Romieu *et al.* 1998) and recreationally (Grievink *et al.* 1999).

Old age

Increasing age is accompanied by a progressive decline in lung function. While this decline is frequently accepted as the inevitable sign of the ageing process, it could relate to one or more environmental factors. For example, and pertinent to the present review, the quality of the diet of the elderly could influence the loss of lung function if it is paralleled by a low antioxidant intake (Kelly *et al.* 2003). In support of this hypothesis, Dow *et al.* (1996) have suggested that dietary intake of vitamin E in elderly subjects is related to lung function (Table 2). Furthermore, as the antioxidant status of the blood compartment is often

altered in old age, it is feasible that there is a similar change in RTLF antioxidants. Indeed, animal studies indicate that pulmonary GSH, the most abundant intracellular and extracellular non-protein thiol, decreases with age (Teramoto *et al.* 1994). This decrease may be the result of an age-related fall in synthetic capacity, or indeed through increased utilisation as a consequence of the low-grade inflammation identified in asymptomatic elderly subjects (Meyer *et al.* 1996), and in turn increased free radical fluxes in their airways. Thus, it is feasible that RTLF antioxidant status is compromised in the elderly, and this situation may be a consequence of decreased vitamin intakes, increased basal oxidative stress and/or decreased adaptive capacity.

Oxidative stress in respiratory disease

Asthma

Asthma is a chronic relapsing inflammatory disorder of the airways. As inflammatory cells generate and release ROS, asthmatic airways are liable to oxidative stress. The literature presents a convincing argument that acute asthma in adults is accompanied by oxidative and nitrosative stress (Henricks & Nijkamp, 2001). For example, studies have shown that inflammatory cells from peripheral blood and bronchoalveolar lavage fluid of asthmatic subjects generate more superoxide anion radicals than those from controls (Cluzel *et al.* 1987), exhaled air of patients with asthma contains elevated levels of various markers of oxidative stress (Crapo & Day, 1999; Montuschi *et al.* 1999; Corradi *et al.* 2001), whilst nitrotyrosine residues have been found to be distributed in both the airways and lung parenchyma of patients with asthma (Kaminsky *et al.* 1999).

The extent of oxidative stress will depend in part on the antioxidant defences available within the RTLF. Any deficiencies in this compartment will compound the ROS-mediated airway responses and tissue injury. Low levels of vitamin C and urate in the RTLF have been observed in adults with mild asthma, together with increased amounts of oxidised glutathione in their airways (Kelly *et al.* 1999). Interestingly, blood levels have been found to be normal or increased in these subjects, which may suggest increased oxidative stress in the asthmatic lung and/or defective transfer of these antioxidants from the blood compartment. This finding does, however, indicate that a plasma measurement alone is not a reliable indicator of airway antioxidant status.

Further evidence for an oxidant-antioxidant imbalance is provided by the finding of a decreased antioxidant capacity in plasma and bronchoalveolar lavage fluid of patients with asthma (De Raeve *et al.* 1997; Comhair *et al.* 2000). Bronchial epithelial cells isolated from patients with asthma not receiving corticosteroids were found to possess less Cu,Zn-SOD activity than epithelial cells obtained from control subjects (De Raeve *et al.* 1997), whilst there is a loss of SOD activity within minutes of an acute asthmatic response to segmental antigen instillation into the lung of individuals with atopic asthma (Comhair *et al.* 2000). However, when the antioxidant status in the RTLF of children with stable atopic asthma was compared with that

Table 3. Epidemiological evidence for a relationship between antioxidant vitamins and asthma

Study population and design	Antioxidant		Measured outcome	Association			Reference
	Vitamin	Measured in		Beneficial	Adverse	None	
Cross-sectional, 9074 adults	Vitamin C	Diet	Wheeze				Schwartz & Weiss (1990)
	Vitamin C	Serum		+			
Cross-sectional, 491 adult cases and 496 adult controls	Vitamin C	Plasma	Asthma symptoms			+	Grievink <i>et al.</i> (2000b)
	β -Carotene	Plasma	Asthma symptoms	+ bs			
Cross-sectional, ninety-four adult cases and 203 adult controls	Vitamin E	Diet and plasma	Current wheeze	+			Bodner <i>et al.</i> (1999)
	β -Carotene	Diet and plasma	Current wheeze	+ bs			
Cross-sectional, 114 child cases and 202 child controls	Vitamin E	Diet	Wheezy illness	+			Hijazi <i>et al.</i> (2000)
Longitudinal study, 77 866 adults	Vitamin C	Diet	Incidence of asthma			+	Troisi <i>et al.</i> (1995)
	Vitamin E	Diet	Incidence of asthma	+			
	Vitamin A and β -carotene	Diet	Incidence of asthma	+ bs			

bs, Borderline statistical significant; +, positive.

of children without asthma, it was found (Schock *et al.* 2001, 2003) that vitamin C, urate and vitamin E concentrations are similar in both groups, as are the markers for protein oxidation (carbonyls) and lipid peroxidation (thio-barbituric acid-reactive substances). These findings indicate that in asymptomatic children with minimal evidence of airway inflammation in the RTLF compartment, there are no measured indices that collectively suggest either deficiencies in antioxidant micronutrients or evidence of oxidative stress. Of interest, however, is a subgroup of children with asthma who were shown to exhibit increased bronchoalveolar lavage eosinophils associated with protein oxidation and possibly lipid peroxidation.

The prevalence of asthma has increased dramatically in many countries over recent decades, suggesting that environmental factors (one of which is dietary change) play a dominant role in the aetiology of the disease (McKeever & Britton, 2004). Seaton *et al.* (1994) have proposed that individuals in Westernised societies have progressively reduced their consumption of fruit and vegetables, and as a result have decreased pulmonary antioxidant defences, making them more susceptible to inhaled irritants and allergens. Furthermore the increased risk of asthmatic symptoms in association with low vegetable consumption in young Chinese subjects (Chen *et al.* 2004), who are more likely to have changed their dietary habits because of the rapid economic advances in China since 1978, provides further support for the concept of a protective effect of fruit, vegetables and antioxidant vitamins on asthma.

So, what is the evidence to support these hypotheses? The initial proposal of Seaton *et al.* (1994) has been examined in a case-control study (Soutar *et al.* 1997) in which it was found that those individuals with the lowest intakes of vitamin C and Mn are associated with a >5-fold increased risk of bronchial reactivity (Table 3). The various nutrients that have been shown in subsequent case-control or cross-sectional studies to be associated with a

reduced risk of asthma are vitamins A, C and E, flavones and flavonoids, Mg, Ca, Na, K, Se, Cu, Zn and fatty acids (Schwartz & Weiss, 1990; Greene, 1995; Bodner *et al.* 1999; Smit *et al.* 1999; Fogarty & Britton, 2000; Grievink *et al.* 2000a; Hijazi *et al.* 2000; Romieu & Trenga, 2001; Smit, 2001). However, longitudinal studies have shown that only vitamin E has a protective effect (Troisi *et al.* 1995). In a recent review of the effects of diet on asthma (McKeever & Britton, 2004) it was concluded that the hypothesis that individual nutrients alone or in combination have a major impact on clinical asthma severity or incidence is far from proved. These authors also suggest that if diet does play an important role in asthma, the most likely and efficient way of exploiting that effect is through dietary manipulation to increase intake of natural foods, and particularly fresh fruits and vegetables, in a balanced diet throughout life. The effect of fruit supplementation on the prevalence of asthma in young children is currently being assessed, capitalising on the phased introduction by the UK government of a scheme providing all 4–6-year-old children with a free portion of fruit every day at school (McKeever & Britton, 2004).

Evidence in support of the concept that antioxidants might influence asthmatic pathobiology is that: T-helper 1 and 2 cytokines are differentially regulated under conditions of oxidative stress (Malmberg *et al.* 2001); apoptotic death pathways, which are activated in the respiratory tract epithelia cells of subjects with asthma and are important in the clearance of airway phagocytes (Vignola *et al.* 2000), are known to be modulated by antioxidants (Droge, 2002); antioxidants may decrease phagocyte and non-phagocyte NADPH oxidase activities (Halliwell, 2000) and affect the production of NO (Mak *et al.* 2002). In clinical trials no effects have been demonstrated for vitamins C and E and Mg on asthma control (Fogarty *et al.* 2003; Pearson *et al.* 2004; Table 1), although these antioxidant vitamins have been shown to modulate the impact of O₃ exposure on the small airways of children with moderate to severe

asthma living in Mexico City (Romieu *et al.* 2002). Furthermore, evidence of a protective effect of Se is based on small numbers and/or weak outcomes (Hasselmark *et al.* 1993).

Alternative potential antioxidant therapies include the cysteine-donating compound N-acetylcysteine (NAC), which is a cellular precursor of glutathione and becomes deacetylated in the gut to cysteine after oral administration. NAC reduces disulfide bonds and has the potential to interact directly with oxidants and nullify their damaging effects (Aruoma *et al.* 1989). Other potential therapies include: ambroxyl, a mucolytic drug that can scavenge the hydroxyl radical and hypochlorous acid (Gillissen & Nowak, 1998); apocynin, an NADPH oxidase inhibitor that completely prevents production of ROS by granulocytes and macrophages (Stolk *et al.* 1994); SOD mimetics, to reduce the amount of superoxide anion in the airways (Salvemini *et al.* 1999, 2001). Since none of these agents has been tested in clinical trials, it is not known whether they have beneficial effects in asthma.

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is characterised by the progressive decline of lung function and the presence of airflow obstruction as a result of chronic bronchitis or emphysema. The single most important aetiological factor in the pathogenesis of this disease is cigarette smoke (Department of Health and Human Services, 1990; Doll *et al.* 1994). It is estimated that 90% of all patients with COPD are, or have been, smokers (Peto *et al.* 1992), but for unknown and probably complex reasons only about 20% of cigarette smokers develop the condition. One puff of cigarette smoke contains $\geq 10^{12}$ free radicals (Church & Pryor, 1985), and it is this rich source of oxidants, together with disease-related inflammation and infection, that are believed to contribute to the well-documented evidence of oxidative stress in patients with COPD (Rahman & MacNee, 1996; Repine *et al.* 1997).

Numerous observations have been made in studies in which different techniques have been used to measure a wide range of markers of oxidative stress in both the lung and systemic circulation of cigarette smokers and patients with COPD; they include: increased 4-hydroxy-2-nonenal-modified protein (a specific peroxidation product of linoleic acid) levels in airway and alveolar epithelial cells, endothelial cells and neutrophils in subjects with airway obstruction (Rahman *et al.* 2002); greater activation of alveolar macrophages obtained in lavage fluid from the lungs of smokers (Peto *et al.* 1992); increased levels of plasma thiobarbituric acid-reactive substances, serum malonyldialdehyde and superoxide anion production in circulating neutrophils of patients with acute exacerbations of COPD (Rahman *et al.* 1996, 1997; Calikoglu *et al.* 2002); elevated urinary levels of isoprostane $F_{2\alpha}$ (a biomarker of lipid peroxidation) in patients with COPD, with further elevation during exacerbations (Pratico *et al.* 1998). More recently, oxidative stress has been demonstrated by monitoring biomarkers of lipid peroxidation using the safe and simple technique of sampling the exhaled breath condensate from patients with chronic inflammatory lung

disease (Corradi *et al.* 2003a,b). Subsequent work (Corradi *et al.* 2004) has demonstrated that levels of malonyldialdehyde in exhaled breath condensate, but not induced sputum supernatant fraction, of subjects with COPD are negatively correlated with the severity of disease, which indicates that, in relation to lipid peroxidation products, exhaled breath condensate and induced sputum must be considered independent techniques.

As oxidants play such a pronounced role in cigarette smoke-induced lung damage, the status of pulmonary antioxidant defence mechanisms assumes paramount importance (Heffner & Repine, 1989; Bast *et al.* 1991; Halliwell, 1996). The question of whether antioxidant deficiency is related to COPD was raised by Taylor *et al.* (1986), who have reported a relationship between a deficiency in plasma antioxidant activity and an abnormal FEV₁:forced vital capacity in patients with COPD. Subsequently, a number of antioxidant disturbances have been observed in smokers and patients with COPD, but notable inconsistencies exist. Elevated levels of GSH have been reported in the bronchoalveolar lavage fluid of certain chronic smokers (Cantin *et al.* 1987; Linden *et al.* 1989), and a marginal increase in vitamin C has been observed in bronchoalveolar lavage fluid of smokers (Bui *et al.* 1992), but decreased levels of vitamin E have been demonstrated (Pacht *et al.* 1991). Plasma antioxidant capacity has also been shown to be decreased in smokers (Rahman *et al.* 1996) and in association with exacerbations of COPD (Rahman *et al.* 1997; Calikoglu *et al.* 2002), supporting earlier evidence of a depletion of vitamin C, vitamin E, β -carotene and Se in the serum of chronic smokers (Pelletier, 1970; Chow *et al.* 1986; Bridges *et al.* 1990; van Antwerpen *et al.* 1993; Mezzetti *et al.* 1995; Brown *et al.* 1997). However, a number of studies have revealed increased circulating antioxidant concentrations in cigarette smokers. For example, the levels of vitamins C and E have been shown to be increased in the plasma and internal mammary arteries of cigarette smokers compared with non-smokers (McGowan *et al.* 1984; Bui *et al.* 1992). Furthermore, erythrocytes from cigarette smokers contain increased levels of SOD and catalase, and are better able to protect endothelial cells from the effects of H₂O₂ (Toth *et al.* 1986).

These inconsistencies, together with the great individual variability of antioxidant levels in cigarette smokers, has led to suggestions that a protective response mechanism may exist (Repine *et al.* 1997), whereby low-grade oxidative stress can bring about a subsequent adaptive resistance to oxidative stress by increasing antioxidant defences. This system may explain why some cigarette smokers do not develop COPD while others do. The mechanisms for the induction of antioxidant enzymes, whether in erythrocytes, alveolar macrophages and/or lungs, by cigarette smoke exposure are currently unknown; however, it is likely to be a result of the induction of antioxidant genes and possibly other intrinsic factors, including dietary factors, that control the oxidant-antioxidant balance (Burney, 1995).

After cigarette smoking, α_1 -antitrypsin deficiency and environmental occupational exposures diet is considered an important additional risk factor for the development of COPD. This conclusion is based primarily on evidence

Table 4. Epidemiological evidence for a relationship between antioxidant vitamins and chronic obstructive pulmonary disease (COPD)

Study population and design	Antioxidant		Measured outcome	Association			Reference
	Vitamin	Measured in		Beneficial	Adverse	None	
Cross-sectional, 9074 adults	Vitamin C	Diet and serum	Bronchitis	+			Schwartz & Weiss (1990)
Cross-sectional, 6555 adults	Vitamin C	Diet	Cough, phlegm			+	Grievink <i>et al.</i> (1998)
			Productive cough			+	
	Vitamin E	Diet	Cough, phlegm			+	
Prospective, 793 adults			Productive cough		+		Miedema <i>et al.</i> (1993)
	β -Carotene	Diet	Cough, phlegm, Productive cough			+	
	Vitamin C	Diet	25-year incidence of CNSLD			+	
Prospective, ecological, sixteen cohorts of adults	β -Carotene	Diet	25-year COPD mortality			+	Tabak <i>et al.</i> (1998)
	Vitamin C	Diet				+	
	Vitamin E	Diet				+	
Cross-sectional, 491 adult cases and 496 adult controls	Vitamin E	Plasma	Chronic bronchitis			+	Grievink <i>et al.</i> (2000b)
			Dyspnea		+		
Cross-sectional, 29 133 adult male smokers	β -Carotene	Plasma	COPD symptoms	+ bs			Rautalahti <i>et al.</i> (1997)
	Vitamin E	Diet and serum	COPD symptoms	+			
	β -Carotene	Diet and serum		+			

bs, Borderline statistical significant; COPD, chronic obstructive pulmonary disease; CNSLD, chronic non-specific lung disease; +, positive.

of a relationship between antioxidants and lung function (Table 2); most cross-sectional studies have shown a beneficial effect of dietary or serum vitamin C or β -carotene (Britton *et al.* 1995; Chuwers *et al.* 1997; Grievink *et al.* 1998; Hu *et al.* 1998). However, most of the studies of the relationship between antioxidants and the prevalence or incidence of COPD symptoms have evaluated the effect of dietary intake only, and have showed no beneficial association with the intake of vitamin C, β -carotene or vitamin E (Miedema *et al.* 1993; Grievink *et al.* 1998, 2000b; Tabak *et al.* 1998; Table 4). On the other hand, The National Health and Nutrition Examination Survey II study did observe a beneficial association between both dietary and serum vitamin C and bronchitis symptoms (Schwartz & Weiss, 1990). Furthermore, the Alpha-Tocopherol Beta-Carotene Trial, which evaluated the potential benefit of vitamin E and β -carotene in smokers (Rautalahti *et al.* 1997), has reported a beneficial association between dietary vitamin E and β -carotene and presence of COPD symptoms at baseline but not after 6 years of supplementation. Several reasons have been proposed to explain this inconsistency: supplementation may have been for too short a period or too late in the disease process; incorrect doses may have been administered; dietary antioxidants may be more effective than supplementation (Grievink *et al.* 2000a). A possible explanation given for the lack of agreement between the results on lung function and COPD symptoms is that the lag-time for a beneficial effect on lung function differs from that for chronic respiratory symptoms (if, for example, the time to develop symptoms is longer than a decline in function); alternatively, lung function may be a more sensitive measure for the detection of small protective effects than respiratory symptoms (Grievink *et al.* 2000a).

If a protective effect of antioxidants on obstructive lung disease is observed, an interesting debate could ensue as to whether it is a direct effect. It is thought that asthma and COPD are partly caused by external triggers such as allergens in predisposed individuals, air pollution or cigarette smoke, all of which increase inflammation in the lung. Thus, subjects who have a low dietary intake or status of antioxidants and are exposed to these triggers are more likely to develop asthma or COPD than subjects who have a high antioxidant intake or status (Grievink *et al.* 2000a) and, as such, a lower basal level of inflammation.

Various approaches to redress the oxidant-antioxidant imbalance in COPD include supplementing smokers and/or COPD patients with vitamins C and E (Table 1). Whilst results in general have been disappointing, there is some evidence that these vitamins can reduce oxidative stress (Steinberg & Chait, 1998; Dietrich *et al.* 2002). The oral use of NAC in an attempt to enhance GSH and reduce the number of exacerbation days in patients with COPD has met with varying success (Boman *et al.* 1983; Rasmussen & Glennow, 1888; British Thoracic Society Research Committee, 1985; Bridgeman *et al.* 1991, 1994). Another therapeutic approach involves the use of n-acetylcysteine, which is a lysine salt of NAC with a neutral pH (NAC is acidic) that can consequently be administered as an aerosol into the lung without causing major side effects (Gillissen *et al.* 1997). Studies comparing the effects of n-acetylcysteine and NAC have found that both drugs enhance intracellular glutathione in alveolar epithelial cells and inhibit H₂O₂ and superoxide anion release from neutrophils harvested from the peripheral blood of smokers and patients with COPD (Nagy *et al.* 1997). Other approaches could involve: the molecular manipulation of antioxidant genes such as glutathione peroxidase; the development of molecules with

activity similar to antioxidant genes; the use of recombinant SOD. Gene-transfer techniques designed to increase cellular glutathione by targeting γ -glutamylcysteine synthetase activity also hold great promise in the management of oxidant-mediated injury in the lungs. Ultimately, it is hoped that the identification of the genes contributing to COPD and an increase in the knowledge of the mechanisms of the effects of oxidative stress could yield important insights into the pathogenesis of this disease, and provide new targets for therapeutic intervention.

Acute respiratory distress syndrome

ARDS is a complex disease with a high mortality rate and is characterised by severe respiratory failure, generalised lung inflammation, increased oxidative stress and diffuse lung oedema (Repine, 1992). It is associated with, and appears to be a consequence of, a large number of diverse disorders. The importance of ROS in the pathogenesis of ARDS was initially demonstrated by Cochrane *et al.* (1983), who showed that α_1 -proteinase inhibitor is inactivated in the RTLF of patients with ARDS. Subsequently, several clinical and experimental studies have provided additional observations showing that endothelial and epithelial injury in ARDS can be mediated through neutrophil-derived oxidants and macrophages or their products (Repine, 1992; Matthay *et al.* 1999; Lang *et al.* 2002). Further evidence for increased pulmonary oxidative stress has arisen from numerous studies of patients with ARDS showing, for example, higher levels of H_2O_2 in exhaled breath condensate (Baldwin *et al.* 1986; Sznajder *et al.* 1989), increases in plasma 4-hydroxy-2-nonenal-modified protein (Quinlan *et al.* 1996), increased levels of isoprostane in exhaled breath condensate (Carpenter *et al.* 1998) and protein damage, as indicated by elevated amino acid residues (Lamb *et al.* 1999) and increased carbonyl proteins (Lenz *et al.* 1999).

Enhanced oxidant generation in the presence of inadequate antioxidants may initiate and influence the progression of ARDS (Pacht *et al.* 1991; Repine, 1992; Metnitz *et al.* 1999; Lang *et al.* 2002). Decreased plasma concentrations of vitamin C, ubiquinol-10, vitamin E, β -carotene and Se have all been demonstrated in ARDS patients (Cross *et al.* 1990; Metnitz *et al.* 1999), suggesting that the antioxidant system is compromised. An investigation of the regulation of antioxidants in the epithelial lining fluid by Schmidt *et al.* (2004) has shown increased levels of the low-molecular-weight antioxidants vitamins A, C and E and uric acid but not GSH and plasmalogens in patients with ARDS. In contrast, a previous study (Pacht *et al.* 1991) has reported marked reductions in GSH levels in subjects with ARDS. A probable reason for this discrepancy is the use of a urea correction factor by Pacht *et al.* (1991) to try to account for bronchoalveolar lavage variability. Despite their report of an apparent up-regulation in alveolar antioxidants, Schmidt *et al.* (2004) observed oxidative stress (indicated by increased oxidised glutathione and F_2 -isoprostanes) in distal lung structures in patients with ARDS. Such findings highlight the fact that the precise modes of action of low-molecular-weight

antioxidants in biological fluids and tissues, the relative importance of individual antioxidants and their individual impact on total antioxidant capacity in the alveolar compartment remain poorly understood.

The numerous studies that give evidence of increased pulmonary stress in ARDS indicate that antioxidant therapy may be valuable in arresting the progress of the disease and decreasing morbidity and mortality (Table 1). Several studies have attempted to use NAC as a therapeutic agent in patients with ARDS or with acute lung injury with various predisposing factors for ARDS (Suter *et al.* 1994; Walsh & Lee, 1999). The intervention has failed to influence progression and improve mortality; in fact, increased pulmonary complaints in patients receiving NAC have been reported (Jepsen *et al.* 1992). In a clinical trial (Bernard *et al.* 1997) the use of both NAC and procysteine in patients with ARDS has been shown to correct the glutathione deficiency, but the progression of ARDS, morbidity and mortality is unchanged. Other attempts to test the antioxidant treatment hypothesis include an investigation into the effect of enteral feeding with EPA, γ -linolenic acid and antioxidants (Gadek *et al.* 1999), which reported improvements in neutrophil recruitment, gas exchange and ventilatory requirements, and a reduction in organ failures. In a more recent study (Nathens *et al.* 2002) early prophylactic antioxidant supplementation with vitamins C and E has been shown to reduce the rate of pulmonary morbidity and organ dysfunction in critically-ill surgical patients. ARDS, however, is a complex disease with a high mortality rate, and as such it is feasible that numerous therapeutic regimens, targeting the molecular pathways and consequences of oxidant-antioxidant imbalances, are needed to successfully arrest progression and improve mortality.

Cystic fibrosis

Patients with cystic fibrosis demonstrate the evidence linking an intake of dietary antioxidants with higher FEV_1 and forced vital capacity. As a result of a decreased production of pancreatic juices these individuals are unable to efficiently breakdown and absorb fat, including fat-soluble antioxidants such as vitamins A and E (Bye *et al.* 1985; Hommick *et al.* 1993). In addition, studies have reported low levels of vitamin C in patients with cystic fibrosis (Brown *et al.* 1997; Winklhofer-Roob *et al.* 1997). To further aggravate this problem, the recurrent airway infections and increased neutrophil activity experienced by patients with cystic fibrosis mean that they endure regular bouts of increased oxidative stress (Brown & Kelly, 1994). Interestingly, the decline with age of both lung function and antioxidant status in these patients (Brown *et al.* 1996) may be linked, although no long-term prospective studies have yet addressed this possibility.

Encouragingly, short-term intervention studies have shown that β -carotene supplementation reduces circulating markers of lipid peroxidation in patients with cystic fibrosis (Winklhofer-Roob *et al.* 1995; Lepage *et al.* 1996; Table 1). Wood *et al.* (2002) have also demonstrated that patients receiving a high-dose antioxidant supplement containing vitamins A, C and E, β -carotene and Se have an

improved antioxidant status, which appears to be linked to improved lung function.

Another potential mode of therapy relates to the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-dependent Cl⁻ channel that regulates epithelial surface fluid secretion, which in patients with cystic fibrosis is not functional because of mutations in the *CFTR* gene. The result is abnormal RTLF height, viscosity and composition, as well as decreased mucociliary clearance, that causes massive airway infections (McCray *et al.* 1999; Widdicombe, 1999; Jayaraman *et al.* 2001). Vitamin C (100 µM) has recently been reported to be a biological regulator of CFTR-mediated Cl⁻ secretion in epithelia (Fischer *et al.* 2004) that induces the opening of CFTR Cl⁻ channels *in vitro*, and when instilled into the nasal epithelium of human subjects effectively activates Cl⁻ transport *in vivo*. Since RTLF vitamin C is deficient in chronic inflammatory disorders of the airways, it may well represent a potential nutraceutical and pharmaceutical target for activating the secretion of Cl⁻, followed by fluid movement into the RTLF. This transport process may loosen sticky mucous secretions and increase mucociliary clearance of obstructed airways. Plasma vitamin C concentrations do not, however, usually exceed 90 µM, even at high oral doses of >1 g (Levine *et al.* 1996), suggesting the need for local delivery to the airway lumen via inhalation. In other pulmonary diseases, such as COPD and asthma, there is emerging evidence that post-translational damage to normal CFTR by ROS and reactive nitrogen species may contribute to the development of thickened airway secretions (Bebok *et al.* 2002). This finding would imply that functional CFTR-mediated Cl⁻ transport is a key mechanism for maintaining normal airway function and healthy lungs, and supports the pharmacological recovery of any dysfunction by vitamin C for a variety of clinical conditions.

Lung cancer

Lung cancer is the most common cancer in the world (World Cancer Research Fund and American Institute for Cancer Research, 1997). Despite therapeutic advances, the 5-year survival is only 10–15%. Thus, currently, the only efficient way to reduce the burden from lung cancer is prevention. Cigarette smoking is the major risk factor, accounting for about 90% of the cases. The high concentration of oxidants in cigarette smoke has been thought to contribute to its carcinogenic impact in the causation of lung cancer (Pryor, 1997; Hecht, 1999) and, in addition, its postulated role in cancer progression (Chandel & Schumacker, 2000). Amongst other risk factors, diet has been implicated (Ziegler *et al.* 1996), although despite the extensive research conducted in this area, the specific nutrients and mechanisms of the interaction remain to be elucidated.

Numerous prospective and retrospective studies have evaluated the role of fruit and vegetables in the aetiology of lung cancer (Steinmetz & Potter, 1991), and in the great majority of these studies lung cancer risk was shown to be reduced at high levels of consumption. The antioxidants vitamins have also received much attention. β-Carotene,

has been found to be inversely related to the risk of lung cancer in many prospective epidemiological studies, especially in studies measuring serum concentrations (Nomura *et al.* 1985; Menkes *et al.* 1986; Wald *et al.* 1988; Connett *et al.* 1989; Comstock *et al.* 1997). However, findings from two controlled trials, i.e. the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (Albanes *et al.* 1996) and the Beta-Carotene and Retinol Efficacy Trial (Omenn *et al.* 1996) contradict these earlier findings, but rather suggest increased risk of lung cancer among smokers with supplementary β-carotene. Data from prospective studies (Connett *et al.* 1989; Knekt *et al.* 1991; Yong *et al.* 1997), cohort studies (Nomura *et al.* 1985; Wald *et al.* 1987; Connett *et al.* 1989; Comstock *et al.* 1997) and one controlled trial (Albanes *et al.* 1996) suggest no role for vitamin E in lung carcinogenesis. Some prospective epidemiological studies suggest an inverse relationship between dietary vitamin C and the risk of lung cancer (Bandera *et al.* 1997; Ocké *et al.* 1997; Yong *et al.* 1997), but because of the high correlation between dietary vitamin C and vegetable and fruit intake the independent role of dietary vitamin C is difficult to estimate. Studies using prediagnostic plasma concentrations of ascorbic acid do not support the involvement of vitamin C in lung carcinogenesis (Stähelin *et al.* 1991; Comstock *et al.* 1997), and no controlled trials of vitamin C on lung cancer have been published.

Taken together these data suggest that supplementation with antioxidant vitamins cannot be recommended for the prevention of lung cancer. Whilst avoidance of cigarette smoking is the most effective method, causing an approximately 10-fold drop in the risk, the decreased risk associated with increased fruit and vegetable intake is 2-fold. A diet rich in fruit and vegetables, containing many potentially-beneficial nutrients and phytochemicals, can therefore only be recommended to reduce the risk of lung cancer, and certainly such dietary modification is unlikely to be harmful (Virtamo, 1999).

Other lung diseases and complications

The lung diseases summarised earlier in which oxidative stress is believed to play a part, and therefore in which dietary supplementation or antioxidant therapy may be useful, is not by any means exhaustive. Free radicals also participate in the pathogenesis of idiopathic interstitial pneumonias (Katzenstein & Myers, 1998; American Thoracic Society and European Respiratory Society, 2002), pulmonary fibrosis (Cantin *et al.* 1987; Strausz *et al.* 1990; Wallaert *et al.* 1990; Borok *et al.* 1991; Sherson *et al.* 1992; Schaberg *et al.* 1993; Nemery *et al.* 2001), sarcoidosis (Cassatella *et al.* 1989), allergic alveolitis (Behr *et al.* 2000) and asbestosis (Kamp *et al.* 1992; Kinnula, 1999). Furthermore, free radicals have a central role in pulmonary infections, pleural disorders, primary pulmonary hypertension and complications associated with lung transplantation (Kinnula *et al.* 1997; Kaneko *et al.* 1998; Archer & Rich, 2000; Balint *et al.* 2001). Several drugs are also associated with ROS generation and lung injury; the most widely investigated being the chemotherapeutic agent bleomycin (Tamagawa *et al.* 2000), which causes

oxidant-mediated lung injury and lung fibrosis (Kinnula *et al.* 1997). Numerous other chemotherapeutic agents, including carmustine, anthracyclines, antimetabolites and antibiotics (nitrofurantoin) can also lead to lung injury by ROS-mediated mechanisms (Halliwell & Gutteridge, 1996).

Concluding remarks

Research findings from a whole range of studies that have investigated nutritional programming *in vitro*, through to the role of a healthy diet in old age, support the hypothesis that during a lifespan a diet rich in the foods that provide a combination of antioxidant vitamins is likely to be beneficial for lung health. In this way a healthy antioxidant diet, along with other environmental and genetic factors, could make an important contribution to the population burden of preventable respiratory diseases. However, possible confounding influences of other dietary components should be considered when interpreting the various observational studies that have been undertaken and discussed in the present review. For example, high dietary intakes of a particular antioxidant may simply reflect intake of a good-quality diet, which may in turn contain other important dietary components.

Looking forward, longitudinal studies are needed to determine how early in life the benefits of a healthy antioxidant-rich diet operate, i.e. during childhood and adolescence, or whether it is the result of an accumulation of protective effects against oxidative damage throughout life.

A host of pulmonary diseases exist that are characterised by ongoing inflammation and accompanied by increased oxidative stress and subsequent lung injury. Modulation of these events by enhancing antioxidant levels offers unique opportunities for therapeutic prevention or inhibition of the progression of such diseases and, as such, the concept of antioxidant therapy administered in a targeted, timed and sustained way is of substantial current interest. Before this approach becomes reality, additional research is needed to better understand not only the molecular events involved in the pathogenesis of the specific disease, but also the complex actions and interplay between antioxidants and ROS and reactive nitrogen species in both the healthy lung and in disease states.

A comprehensive discussion of these complex issues deserves a review in itself; however, some of the main factors for consideration are:

- (a) ROS production may elicit a broad array of physiological responses. For example, they are important for the killing of invading micro-organisms and can influence the complicated network of signalling cascades responsible for gene regulation and protein metabolism (Droge, 2002; Lambeth, 2002). If therapeutic antioxidants are able to modulate the numerous redox-sensitive signalling pathways, they could elicit fundamental effects on almost all aspects of lung cell biology (Droge, 2002; Lambeth, 2002);
- (b) there is an increasing appreciation of the role of the antioxidant micronutrients, including vitamin C (Vissers *et al.* 2001; Carcamo *et al.* 2002) and vitamin E (Ricciarelli *et al.* 2001), as biological response modifiers. These micronutrients could therefore mediate functions seemingly unrelated to their actions on redox potential, including pathways related to immunomodulation and gene expression;
- (c) certain 'antioxidants', including polyphenolics and chemo-prevention agents, may function as mild pro-oxidants, and as such can influence cellular protective and adaptive antioxidant systems.

An improved understanding of these issues should prove useful in designing more appropriate antioxidant-based treatments and intervention trials in the future. These trials will then need to test the concept that antioxidant micronutrients can: (a) be effectively delivered to respiratory tract surfaces; (b) prevent harmful oxidations; (c) most importantly, be useful in the prevention and/or treatment of the clinical manifestations of pulmonary disease.

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