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Dietary antioxidants and environmental stress

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Air is one of our most important natural resources; however, it is also in the front line for receiving environmental pollution. Air quality decreased markedly following the industrial revolution, but it was not until the great London Smog in 1952 that air quality made it onto the political agenda. The introduction of the Clean Air Act in 1956 led to dramatic decreases in black smoke and SO_2 concentrations over the next two decades, as domestic and industrial coal-burning activities ceased. However, as these improvements progressed, a new threat to public health was being released into the air in ever-increasing quantities. Rapid motorisation of society from the 1960s onwards has led to the increased release of atmospheric pollutants such as tiny particles (particulate matter of $<10\,\mu m$ in aerodynamic diameter) and oxides of N, and the generation of the secondary pollutant O_3. These primary and secondary traffic-related pollutants have all proved to be major risks factors to public health. Recently, oxidative stress has been identified as a unifying feature underlying the toxic actions of these pollutants. Fortunately, the surface of the lung is covered with a thin layer of fluid containing a range of antioxidants that appear to provide the first line of defence against oxidant pollutants. As diet is the only source of antioxidant micronutrients, a plausible link now exists between the sensitivity to air pollution and the quality of the food eaten. However, many questions remain unanswered in relation to inter-individual sensitivity to ambient air pollution, and extent to which this sensitivity is modified by airway antioxidant defences.

Antioxidants: Oxidative stress: Air pollution: Lung function: Vitamins

Air is one of the most important natural resources on which all life depends. The atmosphere is, however, also in the front line for receiving environmental pollution. Increased industrialisation of the major cities in the UK gave rise to a marked increase in air pollution. For example, smoke plumes from factories and power stations, which combined with fog to produce wintertime smogs, were characteristic of cities such as London. Indeed, during the Victorian era such smogs were an integral component of the tourist attraction of London, as typified by Monet's frequent visits to paint the charismatic London smog. About 50 years later, on 4 December 1952, this view changed dramatically when an anticyclone settled over London. As a result, the wind dropped and the smog that built up over London did not dispel as usual but lasted for 5 d. During this time, many thousands of the population died and many more became sick. The dramatic increase in air pollution during the first week in December was subsequently agreed to be responsible for this public health disaster. Examination of the data revealed that SO_2 concentrations had increased 7-fold, peaking at $2000\,\mu g/m^3$ and black smoke levels had increased 3-fold to approximately $1600\,\mu g/m^3$ during the smog episode. Moreover, peaks in both factors coincided with the time of the greatest number of deaths. In response to sustained public and political pressure the government of the day introduced the Clean Air Act in 1956 (UK Parliament, 1956) to reduce the emission of atmospheric pollutants. As a consequence, during the 1960s air quality in many cities in the UK went through a marked transition and deaths directly attributable to 'smog type' air pollution largely disappeared. However, as industrial and domestic use of coal was decreasing there was an increase in the number of vehicles on the roads. Furthermore, the introduction of the motorway network and improved road infrastructure led to vastly increased annual mileage averages. For example, vehicle traffic increased 7-fold between 1960 and 2000 from 50×10^9 vehicle km per year to 350×10^9 vehicle km per year. As a consequence, attention

Abbreviations: FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; PM, particulate matter; RTLF, respiratory-tract lining fluid.
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Table 1. Types and sources of airborne pollutants

Pollutant	Source
NO_2	Combination of fossil fuels from stationary sources: heating; power generation; motor vehicles
O_3	Strong oxidising agent formed in the air through a complex series of reactions involving sunlight (UV radiation) on NO_2 and hydrocarbons
Particulate matter (PM)	Mixture of solid and liquid particles of various sizes suspended in air: coarse fraction (2·5–10·0 μm), mechanically produced and natural PM; fine fraction (0·1–2·5 μm), small PM, may be formed from gaseous reactions; ultrafine fraction (<0·1 μm), formed by nucleation resulting from condensation reactions

has shifted from the traditional sources of pollution in the energy and industrial sectors to traffic-related pollution. These new pollutants broadly fall into primary source pollutants such as oxides of N and particulate matter (PM) and secondary photochemical pollutants such as O_3 (see Table 1). Although all these pollutants are bad for public health, the effects of PM particularly have caught the attention of researchers in the last decade. Two USA-based studies have been primarily responsible for this increased profile, as they have both provided convincing evidence that the concentration of PM in air is related to life expectancy (Dockery *et al*. 1993; Pope *et al*. 1995). Importantly, the decrease in life span is estimated to be 1–2 years, which is large when compared with other environmental risk factors related to mortality. As a consequence of this work and a number of follow-up studies (Abbey *et al*. 1999; Dockery, 2001; Pope *et al*. 2002), air pollution is attracting increasing attention from both the political and scientific communities. As a result of increased awareness of the problem, weather forecasts in newspapers and on television now warn the public when poor air quality threatens, whilst free government telephone numbers can be used to obtain more detailed information. When air quality is poor, sensitive individuals such as asthmatics are advised to avoid strenuous exercise, particularly outdoor exercise. Clearly, any form of exercise increases the breathing rate and hence the amount of (contaminated) air that is entering the lung.

Although legislation has been introduced in the UK, across Europe and in the USA to control traffic emissions, pollution still exists and its eventual resolution will require substantial change in the attitudes of many individuals and all governments. In the meantime, the impact of traffic-generated air pollutants will probably be present for several decades. For those in the public health arena who have to deal with the problem it is clear that the current science base does not provide an adequate understanding of the impact of traffic-related pollutants on health. Considerably more work is therefore required before a firm understanding of the mechanisms underlying pollution-induced effects on human health is obtained.

The effects of air pollution

Inhalation of pollutants at concentrations encountered during typical episodes induces a range of symptoms in patients with established respiratory conditions and even in some healthy subjects (Koren *et al*. 1989; Devlin *et al*. 1991; Schelegle *et al*. 1991; Aris *et al*. 1993). For example, breathing only slightly elevated concentrations of O_3 (120–240 $\mu g/m^3$) results in a range of respiratory symptoms in a small percentage (10–20) of the healthy population (Mudway & Kelly, 2000). Symptoms include decreased lung function, increased airway hyper-reactivity and pulmonary inflammation. Those individuals with pre-existing conditions such as asthma and chronic obstructive pulmonary disease generally experience an exacerbation of their symptoms. Similarly, NO_2 arising from traffic outdoors and gas appliances indoors results in a time-dependent inflammatory response in the lung (Sandstrom *et al*. 1991).

Epidemiological studies have consistently reported associations between particulate air pollution, especially PM <10 μm in aerodynamic diameter, and adverse health effects, increasing morbidity and mortality (Samet *et al*. 2000). However, it is by no means clear how exposure to PM, typically as low as 30 $\mu g/m^3$, can produce these health effects and which components of PM mediate them. Although epidemiological evidence suggests that it is the fine (PM of 0·1–2·5 μm in aerodynamic diameter) or ultrafine (PM of <0·1 μm in aerodynamic diameter) fraction that contains the toxic components, there is no general agreement. Moreover, the wide spectrum of disease end points (from cardiovascular death to an asthma attack) suggests that more than one component may be driving the health effects.

Air pollution and oxidative stress

The three traffic-related pollutants causing most concern include the primary pollutants NO_2 and PM of <10 μm, and the secondary pollutant O_3. All these pollutants are toxic to the lung, and in ambient air they usually coexist in varying combinations. Although the properties of these pollutants vary markedly, they all have one common feature, which is that they can cause oxidative stress. Oxidative stress is a relatively new term in biology that was introduced by Sies (1991) to describe non-lung phenomena. However, the concept is a global one and can relate to events occurring in any tissue. Oxidative stress is a situation in which the prooxidant–antioxidant balance is disturbed. This imbalance can occur when the generation of oxidant molecules, or free radicals, exceeds the available antioxidant defences (for an overview of the distribution of intracellular and extracellular antioxidant defences in the lung, see Fig. 1). The damage arising from aberrant free radical activity is often loosely referred to as oxidative stress, and it is characterised by the presence of increased

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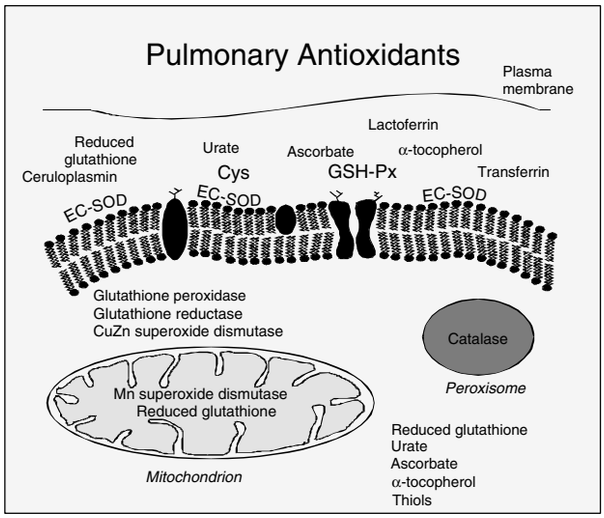

Fig. 1. Distribution of antioxidants in the extracellular and intracellular compartments of the lung. Cys, cysteine; GSH-Px, glutathione peroxidase; EC-SOD, extracellular superoxide dismutase.

cellular concentrations of oxidised lipids, proteins and nucleic acids. Many of these oxidation products become ineffective; for example, many enzymes become inactivated following oxidation. Of the three ambient air pollutants of primary concern: NO_2 is a free radical, as it contains unpaired electrons in its outer electron orbital; O_3, although not a free radical, is a powerful oxidant, second only to Fl in redox potential; much of the PM carries on its surface metal and organic contaminants that are capable of generating free radicals (Kelly, 2003).

On entering the lung, ambient pollutants do not come into direct contact with respiratory epithelial cells, but rather their first contact is with the fluid layer that covers the respiratory epithelium, the respiratory-tract lining fluid (RTLF; Fig. 2). Thus, the responses observed following exposure to ambient pollutants are thought to be mediated through oxidation reactions occurring within this fluid air–lung interface (Pryor, 1994). In the case of O_3, and to a lesser extent NO_2, because of its low solubility and high reactivity (Medinsky & Bond, 2001), these reactions appear to be confined to the RTLF. As a result, little O_3 is thought to react directly with the underlying cells. Instead, its toxicity is transmitted to the respiratory epithelium by secondary oxidation products formed by the direct ozonisation of RTLF lipids (Pryor *et al*. 1995). These species trigger the underlying cells to elaborate inflammatory signals (Kafoury *et al*. 1999), resulting in airway neutrophilia with ensuing tissue injury. Particulate pollution can, in contrast, cross the RTLF layer; however, it is likely that in making the transition the oxidative nature of the particle surface will be changed as a result of reactions with components of the RTLF (Kelly, 2003).

Antioxidants and normal lung function

Opposing the formation of cytotoxic species in the RTLF is a network of antioxidants: ascorbate (van der Vliet *et al*. 1999), urate (Peden *et al*. 1990), reduced glutathione (Cantin *et al*. 1987), α-tocopherol (Mudway *et al*. 2001), extracellular superoxide dismutase (Mudway *et al*. 2001) and glutathione peroxidase (Avissar *et al*. 1996). All these antioxidants are free-radical scavengers, but many (ascorbate and urate) also function as sacrificial targets for O_3, reacting rapidly with this oxidant to limit its interaction with RTLF lipids and proteins (Pryor *et al*. 1995). It has been proposed that the composition and quantity of antioxidants within the RTLF might therefore represent an important determinant of an individual's responsiveness to O_3 (Kelly *et al*. 1995). Indeed, there is evidence (discussed later, see p. 582) that increased antioxidant intake may protect against the effects of air pollution.

Both the extracellular compartment (RTLF) and the intracellular components of the lung are well endowed with antioxidant defences (Fig. 1). Whilst there is evidence to suggest that good lung function is associated with diets rich in fruits and vegetables (Britton *et al*. 1995), there is little data linking basal pulmonary or blood antioxidant levels to an individual's responsiveness to oxidant gases under controlled conditions (Blomberg *et al*. 1999; Mudway *et al*. 2001). It is, however, conceivable that boosting the plasma concentrations of these antioxidants above the normal range could have a protective effect, if this strategy results in augmented RTLF antioxidant concentrations.

Interest in dietary antioxidants first arose in the area of lung disease in the 1980s, with the suggestion from retrospective observational studies that individuals with low intakes of dietary antioxidants have decreased lung function. Studies by Cohen and colleagues (Tockman *et al*. 1986; Morabia *et al*. 1989) indicated that dietary retinol levels are an important predictor of lung function, in that individuals with higher retinol intakes tend to have better lung function. Schwartz & Weiss (1990), reporting the findings of the National Health and Nutrition Examination Survey study, observed that the dietary intake of vitamin C is positively associated with a measure of lung function (forced expiratory volume in 1 s; FEV_1). Strachan *et al*. (1991) found that smokers with high intakes of fresh fruit and fruit juice have a higher FEV_1 than those with a low intake of these foods. In the Zutphen study (Miedema *et al*. 1993) it was shown that fruit intake is inversely related to the incidence of chronic non-specific lung disease, but there is no association with the estimated intakes of several antioxidants, including β-carotene, vitamin C and Se. In the MORGEN study (the monitoring project on risk factors and health in The Netherlands) it was found that a high intake of vitamin C and β-carotene, but not vitamin E, is associated with a higher FEV_1 and forced vital capacity (total volume expired; FVC) than a low intake of these antioxidants, suggesting that these nutrients do have a protective effect on lung function (Grievink *et al*. 1998).

In addition to these epidemiological studies, there are a number of cross-sectional studies in which associations have been made between blood concentrations of dietary antioxidants and lung function and/or respiratory symptoms. Taylor *et al*. (1986) have reported that patients with chronic obstructive pulmonary disease who have an abnormal FEV_1:FVC have decreased plasma antioxidant levels. The findings of the National Health and Nutrition

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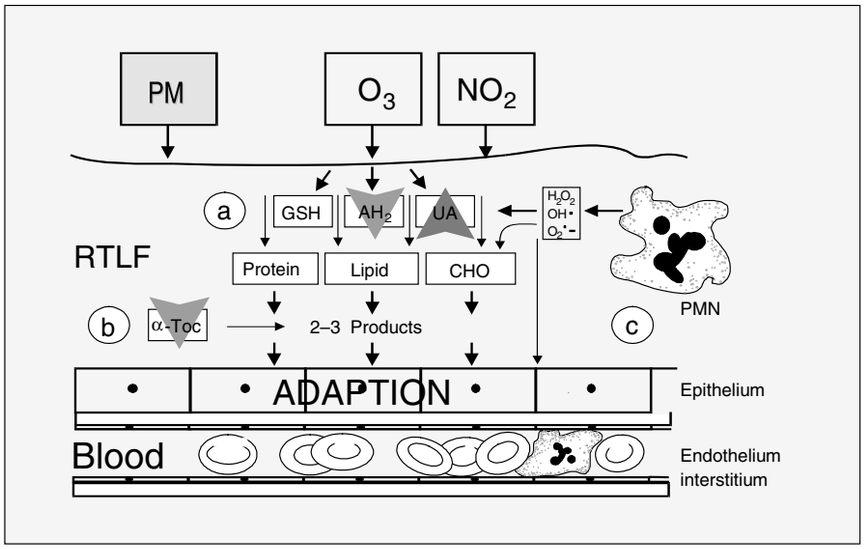

Fig. 2. Schematic model of how oxidant air pollutants interact with the lung. RTLF, respiratory tract lining fluid; GSH, reduced glutathione; AH_2, ascorbic acid; UA, uric acid; α-Toc, α-tocopherol; PMN, polymorphonucleoctyes; CHO, carbohydrate. (a), First interactions are between the pollutants and the protecting hyrdrophilic antioxidants; (b), subsequent reactions probably occur with antioxidants on the respiratory epithelium; (c), oxidative reactions lead to the generation of chemotactic signals, which lead to the influx of activated polymorphonucleoctyes onto the lung surface.

Examination Survey II study (Schwartz & Weiss, 1994) suggest that both bronchitis and wheeze are negatively associated with serum vitamin C levels. Ness *et al*. (1996) have reported that plasma vitamin C concentration correlate with both FEV_1 and FVC in men but not women. More recently, Mudway *et al*. (2000) have shown that FEV_1 is not influenced by vitamin C supplementation in healthy vitamin C-replete subjects.

As outlined earlier, a growing body of evidence links the intake of vitamins and provitamin β-carotene with lung function; i.e. a high intake of these dietary components is associated with higher FEV_1 and FVC than low intakes, presumably as a result of improved antioxidant reserves to deal with the inevitable increases in reactive oxygen species. This scenario has been demonstrated in patients with cystic fibrosis who, because of decreased production of pancreatic juices, are unable to efficiently breakdown and absorb fat-soluble vitamin antioxidants such as vitamins E and A (Bye *et al*. 1985; Homnick *et al*. 1993). This problem, along with recurrent airway infections and increased neutrophil activity, means that patients with cystic fibrosis endure regular bouts of increased oxidative stress (Brown & Kelly, 1994). Moreover, as both lung function and antioxidant status decline with age in patients with cystic fibrosis (Brown *et al*. 1996), it is possible that these two events are linked, although no long-term prospective studies have yet addressed this interesting 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). More recently, Wood *et al*. (2002) have demonstrated that high-dose antioxidant supplementation (/d; 200 mg vitamin E, 300 mg vitamin C, 25 mg β-carotene, 90 μg Se, 500 μg vitamin A) improves the antioxidant status of patients with cystic fibrosis, which appears to be linked with an improvement in lung function.

Air pollution and antioxidant intake

In support of the proposed benefit from increased antioxidant intake, recent field studies have demonstrated that increased intakes of nutritional antioxidants can reduce the magnitude of lung function decrements in subjects exposed to high O_3 doses both occupationally (shoe cleaners in Mexico City; Romieu *et al*. 1998) and recreationally (Dutch cyclists; Grievink *et al*. 1999). In these studies it was observed that prolonged periods of supplementation (75 mg vitamin E, 650 mg vitamin C, 15 mg β-carotene daily for 1·5 months in the Mexico City study; 100 mg vitamin E, 500 mg vitamin C daily for 15 weeks in the Dutch cyclist study) are associated with protection against average O_3 backgrounds of 67·3 μg/m^3 and 38·5 μg/m^3 respectively. Notably, major O_3 episodes were only recorded in the Mexico City study, with numerous hourly episodes >110 μg/m^3.

Corroboration for these findings has been provided in a number of controlled chamber studies. Chatham *et al*. (1987) have reported protection against O_3-induced (600 μg/m^3 for 2 h, with intermittent exercise) decrements in FVC in subjects receiving a daily supplement of 528 mg vitamin E for 14 d with a single 1 g vitamin C dose taken immediately before the O_3 challenge. Notably, in this study no protection was observed with vitamin C alone, and no protection against FEV_1, forced expiratory flow (25th–75th percentile), or specific airway conductance

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decrements was observed. A recent (double-blinded) study has examined a fuller range of response end points in two groups of sixteen subjects exposed to O_3 at 800 μg/m^3 for 2 h while exercising at a ventilation rate of 20 l/min per m^2 following placebo and vitamin supplementation (250 mg vitamin C, 33 mg vitamin E and a 370 g (12 oz) vegetable cocktail rich in carotenoids daily for 14 d; Samet *et al*. 2001). In this study a small yet significant blunting of the O_3-induced decrement in FEV_1 ($P = 0{\cdot}05$) was observed in the supplemented group with a similar trend ($P = 0{\cdot}06$) observed in the FVC response. No protection against O_3-induced airway inflammation was reported. In this study subjects were initially placed on a restricted-antioxidant diet for 7 d before entering the placebo or vitamin predosing period, such that their initial vitamin C, vitamin E, and β-carotenoid concentrations were low (group means of 34·2, 13·3 and 1·4 μmol/l respectively). In field studies in which protection has been observed basal blood antioxidant levels, when measured, have been at the upper end of the normal range (Grievink *et al*. 1998, 1999; Romieu *et al*. 1998). Whilst these studies have examined responses in healthy subjects, a recent study by Trenga *et al*. (2001) has addressed whether vitamin supplementation (500 mg vitamin C and 264 mg vitamin E daily for 4 weeks) would protect asthmatics against O_3-induced (120 μg/m^3 for 45 min, with mild exercise) lung function decrements and following subsequent provocation with SO_2 (100 and 250 μg/m^3). No difference between the pre- and post-O_3 lung function decrements (FVC, FEV_1, forced expiratory flow (25th–75th percentile) and positive expiratory flow) was observed between the supplement and placebo groups. Similarly, no difference was observed in the lung function decrement after SO_2. Indeed, overall responses actually appeared to be larger in the vitamin-receiving group. In each of these studies statistical power is limited both by small group sizes and the fact that subjects do not act as their own controls. Further, although chamber studies have reported protection against O_3 with vitamin supplementation the end points in which protection was seen are not consistent, and the magnitude of the protective effect is small.

Although it has recently been hypothesised that low antioxidant intake may result in accelerated loss of lung function with ageing (Kelly, 2003), few studies have explored whether there is a relationship between antioxidant intake and lung function in childhood. Cook *et al*. (1997) have reported a positive association between FEV_1 and the frequency of fresh fruit consumption and a weak association with green vegetable and salad consumption. As they found no association between FEV_1 and serum vitamin C concentration, they concluded that other micronutrient antioxidants derived from these foods are more important than ascorbic acid. More recently, Gilliland *et al*. (2003) have reported that low intakes of antioxidant vitamins are associated with deficits in pulmonary function in both boys and girls participating in the Children's Health Study in California. Importantly, the deficits amongst boys with asthma are large enough to be clinically significant. Gilliland *et al*. (2003) have suggested that low antioxidant intake during childhood may contribute to the risk of developing obstructive lung disease during adulthood, as well as increased morbidity and mortality associated with low FEV_1.

In conclusion, the air breathed contains many products of human activity that pose important challenges to the health of the population. Atmospheric oxidative pollutants in particular pose an important challenge to the maintenance of normal lung function. Gaseous air pollutants, such as O_3, are thought to play a role in the causation and aggravation of asthma and allergic responses that are increasingly prevalent in children. Although still under active discussion, many investigators now believe that oxidative stress plays a central role in the impact of air pollutants such as O_3 and PM. Oxidative stress, by definition, is an imbalance between oxidants and antioxidants. As many antioxidants are derived from the food eaten, increased attention is being paid to the quality of the diet, and how this strategy may help protect the population from an oxidising environment.

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