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REVIEW

Air pollution, oxidative stress and dietary supplementation: a review

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ABSTRACT: The aim of the present review was to provide an up-to-date overview of the biological and epidemiological evidence of the role of oxidative stress as a major underlying feature of the toxic effect of air pollutants, and the potential role of dietary supplementation in enhancing antioxidant defences.

A bibliographic search was conducted through PubMed. The keywords used in the search were ''air pollutant'', ''oxidative stress'', ''inflammation'', ''antioxidant polyunsaturated fatty acids'' and ''genetics''. In addition, the authors also searched for biomarkers of oxidative stress and nutrients.

The review presents the most recent data on: the biological and epidemiological evidence of the oxidative stress response to air pollutants; the role of dietary supplementation as a modulator of these effects; and factors of inter-individual variation in human response. The methodology for further epidemiological studies will be discussed in order to improve the current understanding on how nutritional factors may act.

There is substantial evidence that air pollution exposure results in increased oxidative stress and that dietary supplementation may play a modulating role on the acute effect of air pollutants. Further epidemiological studies should address the impact of supplementation strategies in the prevention of air-pollution-related long-term effects in areas where people are destined to be exposed for the distant future.

KEYWORDS: Air pollution, antioxidants, nutrition, oxidative stress

Epidemiological studies have clearly shown that air pollution exposure is associated with a range of respiratory and cardiovascular health effects and increased mortality [1]. Recent research has identified oxidative stress as one potential feature underlying the toxic effect of air pollutants, which trigger a number of redox sensitive signalling pathways, such as those of inflammatory response and cytokine production [2–5]. Toxicity may arise from an imbalance of biological pro-oxidant and antioxidant processes [6] linked to increased exposure to oxidants or the presence of impaired antioxidant defences [7, 8]. This imbalance has long been recognised in investigations of ozone (O_3) [9], one of the most potent oxidants, and more recent studies have focused on this particular mechanistic hypothesis [10]. Since diet is a major source of antioxidants, it is important to examine whether antioxidant defence mechanisms could be increased by dietary means to protect against air pollutants as this could have major public health consequences [11]. To provide an up-to-date overview on the biological and epidemiological evidence of the role of oxidative stress as a major underlying feature of the toxic effect of air pollutants and the potential role of dietary supplementation as an enhancer [11] of antioxidant defences, a bibliogaphic search was conducted through PubMed. The keywords used in the search were ''air pollutant'', ''oxidative stress'', ''inflammation'', ''antioxidant'' (vitamin C, vitamin E, carotenoids), ''polyunsaturated fatty acids'' (PUFA) and ''genetics''. In addition, the current authors searched for biomarkers of oxidative stress, biomarkers of antioxidant intake (selenium, flavonoids, carotenoids, vitamin C, vitamin E), and n-3 PUFA. Various recent reviews have been published on these issues [1–5, 7–10, 12–34], therefore, the present authors refer to these and mostly focus on the latest findings. Thus, the purpose of this up-to-date overview is five-fold. First, the relevance of oxidative stress as a common mechanism for

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effects of ambient air pollutants will be summarised. Secondly, the role of antioxidants in oxidative stress will be briefly discussed. Thirdly, the evidence for dietary supplements as modulating the adverse effects due to air pollution will be reviewed. Fourthly, the relevance of factors that may interact with a subjects' response to exogenous oxidative stress will be discussed. Finally, the need to further investigate the relevance of dietary supplementation as an approach to protect from adverse effects of air pollution will be discussed.

BIOLOGICAL AND EPIDEMIOLOGICAL EVIDENCE

Oxidative stress and air pollutants

Several air pollution components have been related to particulate toxicity. An important determinant of the acute inflammatory response appears to be the dose of bio-available transition metals (such as copper, vanadium, chromium, nickel, cobalt and iron), organic compounds (such as polycyclic aromatic hydrocarbons) and biological fractions (such as endotoxins) [35, 36]. The oxidative stress mediated by particulate matter (PM) may arise from: direct generation of reactive oxygen species (ROS) from the surface of soluble compounds; altered function of mitochondria or reduced nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase; and activation of inflammatory cells capable of generating ROS and reactive nitrogen species (RNS), as well as oxidative DNA damage [37, 38]. The particle provides a template for electron transfer to molecular oxygen in these reduction and oxidation (redox) cycling events [39]. In addition, target cells, such as airway epithelial cells and macrophages, generate ROS in response to particle uptake by biologically catalysed oxidation reactions that occur in the cell membrane and mitochondria [4, 40–42]. *In vitro* studies have shown that inhaled PM causes expression of nuclear factor (NF)-κB-related genes and oxidant-dependent NF-κB activation [43, 44]. The dose of bio-available transition metal, rather than particulate mass, may be the primary determinant of acute inflammatory response [35, 37, 44]. However, other studies suggest that the hydrosoluble fraction is responsible for the oxidative damage to DNA [45]. The biological component of particles also seems to be related to oxidative stress [46], as well as bacterial endotoxin that induce the liberation of tumour necrosis factor (TNF)-α and interleukin (IL)-6 by macrophages [36].

Strong oxidative activity and the effective depletion of lung lining fluid antioxidants have been reported in large studies of ambient PM <2.5 μm ($PM_{2.5}$) [17]. To defend against the oxidative damage, cells use up their stores of a key antioxidant, glutathione. The glutathione depletion can induce a state of cellular stress, which triggers an increase in the production of antioxidant enzymes through activation of a transcription factor nuclear factor-erythroid 2-related factor 2 [17]. Failure to overcome oxidative stress leads to the activation of additional intracellular signalling cascades that regulate the expression of cytokine and chemokine genes [15]. These products are produced locally in target tissues as well as systemically, and lead to widespread pro-inflammatory effects remote from the site of damage. In addition, PM appears to inhibit protective enzymes involved in oxidative stress responses depending on their toxicity (copper/zinc superoxide dismutase, manganese superoxide dismutase, glutathione peroxidase and glutathione reductase) [47].

Diesel exhaust particles (DEPs) have a high content of elemental and organic carbon and are thought to be particularly toxic [15]. These particles consist of a carbon core with adsorbed organic compounds, such as polyaromatic hydrocarbons, quinones and redox-active metals, and the capacity of DEPs to induce oxidative stress is largely related to these adsorbed components. Animal experimental models, cell culture experiments and cell free systems involving DEPs have shown oxidative stress response and oxidative DNA damage. Human studies have shown increased neutrophils, B cells and alveolar macrophages in bronchoalveolar lavage fluid and an increased amount of pro-inflammatory cytokines, chemokines and adhesion molecules [48]. Exposure to DEPs has been shown to increase airway resistance, increase IL-6 and IL-8 in lavage fluid, increase IL-8 mRNA expression in bronchial mucosa and upregulate endothelial adhesion molecules P-selectin and vascular cell adhesion molecule-1 [49]. ROS formed at the epithelial level after DEP exposure upregulate IL-10, promoting antigen-presenting cells and allergy to pollen [15]. However, controlled exposure to DEP in human subjects has been shown to respond with an increase in low molecular antioxidants in the alveolar compartment [50]. The role of oxidative stress in response to DEPs and other particles is further supported by *in vitro* studies in which ROS are generated by macrophages, neutrophils, eosiniphils and epithelial cells after stimulation by DEPs or particles [15]. Interestingly, low sulphur diesel combined with engine filters blocked a range of responses to DEPs including the oxidative stress responses in mice [51].

Alteration of autonomic functions also appears to be partly associated with oxidative stress [14]. Long-term exposure to low concentrations of $PM_{2.5}$ has been shown to alter vasomotor tone, lead to vascular inflammation and potentiate atherosclerosis induced by high-fat chow in susceptible mice [52]. Although epidemiological evidence suggests that it is the fine ($PM_{2.5}$) or ultrafine (PM <0.1 μm) fraction that contains the toxic components; the large spectrum of disease end-points (from cardiovascular to asthma attack) suggest that more than one component may be driving the health effects [2].

O_3 is a very reactive gas whose uptake depends on the availability of antioxidants in the lining fluids, and its toxicity appears to be transmitted to the respiratory epithelium by secondary ROS formed by direct ozonisation of respiratory tract lining fluid lipids [16]. Alteration of the cell membrane translating an induction of lipid peroxidation and a significant modification of the redox status has been observed [53], as well as the activation of transcription factors such as NF-κB and increased expression of a range of pro-inflammatory cytokines and adhesion genes [2, 6]. O_3 has been shown to react readily with ascorbic acid, uric acid and thiols, and exposure of these molecular species to O_3 results in their rapid depletion [6]. When these defence mechanisms are overwhelmed, O_3 may injure the underlying cells by inducing lipid peroxidation and activating inflammatory gene expression [6, 53]. Like O_3, nitrogen dioxide (NO_2) reacts with substrates present in the lung lining fluid compartment. The oxidised species arising from the reaction between NO_2 and lining fluid are responsible

for the signalling cascade of inflammatory cells into the lung [54–56].

A hierarchical oxidative stress model has been proposed to explain the dose–dependent response to air pollutant exposure [57]. Low exposure would lead to the formation of ROS activating an antioxidant response, followed by the transcription of enzymes important in detoxification, cytoprotective and antioxidant responses. These include phase II enzymes, whose induction serves as a detoxification mechanism (*e.g.* NAD(P)H:quinone oxidoreductase 1 (Nqo1) and glutathione *S*-transferase). At higher exposure, the transcription NF-κB and activator protein-1 responses would be activated. This would lead to NF-κB and mitogen-activated protein kinase signalling, altering the function of mitochondria or NADPH, and to increased expression of pro-inflammatory cytokines (such as TNF-α and IL-8 and IL-6) and genes coding adhesion molecules [2, 6, 15, 43, 44]. Any enhanced inflammatory response would lead to additional generation of ROS and RNS, together with oxidative DNA damage (fig. 1) [15, 37, 38].

Antioxidants and oxidative stress

Antioxidants in the lung are the first line of defence against oxygen free radicals. The respiratory tract lining fluids (RTLF) contain a range of low molecular weight antioxidants similar to those found in blood plasma, including reduced glutathione, ascorbic acid (vitamin C), uric acid and α-tocopherol (vitamin E). They also contain antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase, thioredoxin reductase, catalase and the metal binding proteins ceruloplasmin and transferrin [2, 7]. All these antioxidants are free radical scavengers but also function as sacrificial targets for O_3 (ascorbate and urate) and react rapidly with this oxidant to limit its interaction with RTLF lipids and proteins [58]. The composition and quantity of antioxidants in the RTLF may represent an important determinant of individual responsiveness to air pollutants but should be thought of as a dynamic equilibrium with the antioxidant defences within the epithelium and the more remote plasma pool [59]. Controlled studies suggest that exposure to O_3 results in a depletion of RTLF antioxidants followed by an enhancement of the movement of antioxidants to the RTLF [60] or increased synthesis [3, 59]. Similarly, low-dose diesel exposure challenge in healthy volunteers was followed by an increase of inflammatory markers in bronchial lavage. No inflammatory response was seen in the alveolar compartment, but both reduced glutathione and urate concentrations were increased following diesel exposure suggesting differential antioxidant responses in the conducting airway and alveolar regions [50].

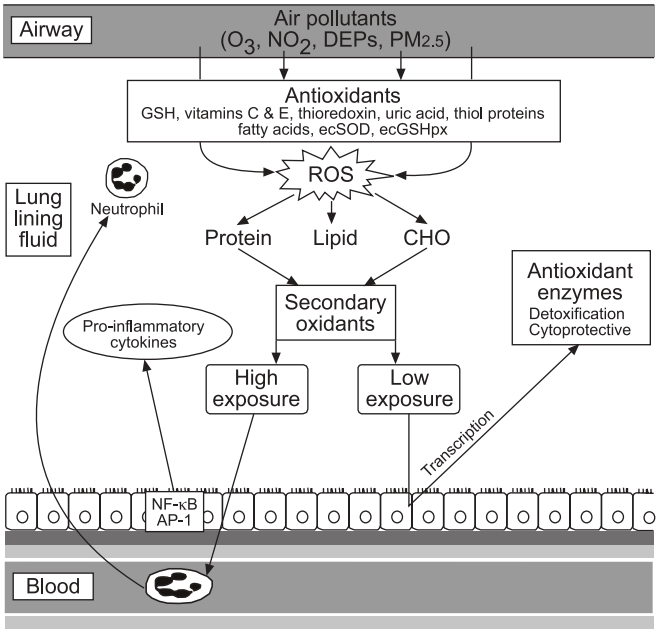

FIGURE 1. A model of the reaction of oxidants in the airway. Inhaled pollutants, such as ozone (O_3), nitrogen dioxide (NO_2), particulate matter <2.5 μm ($PM_{2.5}$) or diesel exhaust particulates (DEPs), react with nonenzymatic antioxidant constituents of the respiratory tract lining fluid including: reduced glutathione (GSH); vitamin C; uric acid; and enzymatic antioxidants, such as extracellular superoxide dismutase (ecSOD), extracellular glutathione peroxidise (ecGSHpx) and thioredoxin. These molecules provide a protective screen against these pollutants. If defences are exceeded, the production of reactive oxygen species (ROS) is increased and oxidants may react with organic molecules, such as proteins or lipids, and alter the epithelium resulting in: cell activation and initiation of the inflammatory process; activation of neutrophils; and liberation of cytokines, chemokines and adhesion molecules. CHO: carbohydrate; NF-κB: nuclear factor-κB; AP-1: activator protein-1. Modified from [3].

As many antioxidants are derived from the diet, several dietary factors have been implicated; mainly because of their potential role in inflammatory reactions. The following section will focus mostly on nutrients that have been used in supplementation studies to modulate the impact of air pollutants or might interact with the immune response. These factors include antioxidant vitamins, omega-3 fatty acids and other micronutrients that might affect the immune response.

Antioxidant nutrients

Vitamin C

Vitamin C, a water-soluble vitamin, is an abundant antioxidant substance and is widely distributed throughout the body including the extracellular lining fluid of the lung [17]. Ascorbate is an excellent reducing agent and scavenges free radical and oxidants. *In vitro* evidence suggests that vitamin C has a role as a chemical reducing agent both intracellularly and extracellularly. Intracellular vitamin C might prevent protein oxidation and regulate gene expression and mRNA translation. This is particularly relevant for the lung which is exposed to oxidative substances. Extracellular vitamin C protects against oxidants and oxidant-mediated damage [61]. It contributes to antioxidant activity through scavenging a variety of free radicals and oxidants, *in vitro*, including superoxide radical (O^{2-}), peroxyl radicals, hydrogen peroxide, hypochlorous acid, singlet oxygen, oxidant air pollutants and oxidants that leak from activated neutrophils and macrophages [59, 61]. While the terminating product dehydroascorbate can be regenerated to ascorbate by intracellular enzymes, in particular thioredoxin

reductase, which catalyses its regeneration [62], this regeneration is unlikely in the RTLF because of the lack of enzymes. Therefore, the maintenance of ascorbate level in the RTLF requires transportation from cellular sources or from the plasma pool [59]. Ascorbate also acts indirectly to prevent lipid peroxidation [59] and contributes to the regeneration of membrane-bound oxidised vitamin E [63]. Ascorbate plays a role in immune function and is transported into neutrophils and lymphocytes [18]. Whilst ascorbate has many antioxidant actions, it also has the capacity to act as a pro-oxidant in the presence of transition metals [64].

Vitamin E

Vitamin E, a lipid-soluble vitamin, represents the principal defence against oxidant-induced membrane injury in human tissue because of its role in breaking the lipid peroxidation chain reaction [64]. It is a potent peroxyl radical scavenger and especially protects PUFAs within the phosphsolipid biological membrane and in plasma liproteins [65]. It also decreases production of prostaglandin E_2, a metabolite of arachidonic acid produced by lipid peroxidation of lung cells after O_3 exposure [19]. Vitamin E appears to play a major role as an integral constituent of alveolar surfactant, whose quantity and composition conditions normal lung function [66].

β-Carotene

β-Carotene, a precursor to vitamin A and other carotenoids, accumulates in tissue membranes, scavenges O^{2-} and reacts directly with peroxyl free radicals generated by O_3 [67]. It could, therefore, play a role in the control of inflammation and immune response through its antioxidant properties. However, recent research has shown that high-dose carotenoid supplementation may lead to both antioxidant and pro-oxidant reactions [68], depending on the redox potential of the biological environment in which it acts [69].

Other antioxidants, such as flavonoids, are scavengers of superoxide anions and peroxyl radicals [70]. In addition to antioxidant activities, flavonoids can modulate cell signalling pathways [20]. Selenium, an essential trace element that plays a role in the detoxification of peroxides and free radicals [67], could also play an important role in the prevention of lung injury [21]. As an integral part of the glutathione peroxidases and thioredoxin reductase, selenium probably interacts with every nutrient that affects the pro-oxidant/antioxidant balance of the cell. It also appears to support the activity of vitamin E in limiting lipid oxidation [71].

Omega-3 PUFA

Increased intake of omega-3 PUFA (n-3 PUFA) can decrease the inflammatory reaction by changing the contents of lipid membranes and other substrates, which are in turn the substrates for eicosanoid production [72]. The substitution of n-3 PUFA (α-linoleic acid; 18:3n-3 and eicosapentaenoic acid (EPA); 20:5n-3) for n-6 fatty acids (linoleic acid; 18:2n-6) in the membrane leads to the production of less potent inflammatory mediators (prostaglandin E_3 instead of prostaglandin E_2, and leukotriene 5 instead of leukotriene 4) [72]. Prostaglandin E_2 has been shown to act on T-lymphocytes to reduce the formation of interferon (IFN)-γ without affecting the formation of IL-4. This may lead to the development of allergic sensitisation, since IL-4 promotes the synthesis of immunoglobulin E whereas IFN-γ has the opposite effect [73]. Leukotriene 4, a potent stimulator of airway smooth muscle cells, increases post-capillary vascular permeability and mediates asthma by vasoconstriction and mucus secretion. The competitive interactions between n-6 PUFA and n-3 PUFA determine the cellular contents of arachidonic acid and EPA.

Increased intake of n-3 PUFA appears to decrease the risk of sudden and nonsudden death from myocardial infarction and nonfatal myocardial infarction [74–76]. The protective effect of n-3 PUFA may be linked, in part, to its cardiac and arrhythmic effects, including increasing heart rate variability (HRV) [22, 74, 77]. There is a positive correlation between the baseline cell membrane concentrations of n-3 PUFA and the degree of HRV, both in healthy subjects and in patients with coronary artery disease [23, 78]. Along with increasing HRV, other anti-arrhythmic mechanisms of n-3 PUFA have also been described, including the capacity to stabilise the electrical activity of cardiac myocytes by modulating sarcolemmal ion channels and voltage-dependent sodium channels [24], and the capacity to reduce myocardial infarct size in animal models of ischaemia and reperfusion [24]. N-3 PUFA also appear to: decrease the risk of thrombosis; decrease serum triglyceride levels, slowing the growth of atherosclerotic plaque; improve vascular endothelial function; lower blood pressure; and decrease inflammation [79].

Other micronutrients and immune functions

Micronutrients such as zinc, vitamin A and folic acid can also influence several components of immunity, altering the function of macrophages and thus their role in innate immunity and inflammation. Studies have shown that deficiencies in these micronutrients can significantly alter macrophage phagocytosis and their production of cytokines (IL-1 and IL-6, TNF-α and IFN-γ). These deficiencies also alter natural killer cell function, neutrophil motility and antimicrobial activity [25].

Nutrient supplementation and effects of air pollution

The effects on air pollutant toxicity of nutrient supplementation at levels higher than is physiologically required have been studied in both animals and humans and summarised previously [2, 11, 17, 80].

Experimental animal studies

Results of animal studies suggest that supplementation with vitamin C and vitamin E modulates the pulmonary response to exposure to photo-oxidants, such as O_3 or NO_2 [17, 81], and that vitamin C, uric acid and glutathione located in the respiratory tract lining fluid are consumed on exposure to O_3 and NO_2 [16, 82, 83]. Dietary deficiency of vitamin C appears to quickly translate to decreased levels of vitamin C in blood and RTLF [84]. Temporary vitamin E deficiency may induce reversible changes in the expression of pro-inflammatory markers, reduce surfactant lipid synthesis in alveolar type II cells and favour the development of injury in response to air pollution insults [66]. Further experimental studies using antioxidants, iron chelators or other substances support the role of ROS as mediators of the effects of particulates [37, 54]. Oxidative stress appears to play a critical role in the activation

of NF-κB, and cytokine-induced NF-κB activation is prevented after treatment with antioxidants or metal chelators [54]. *N*-acetylcysteine, a powerful antioxidant, had a protective effect on inflammatory response and oxidative stress damage in rats exposed to coal dust [85] and on changes in heart rate and decrease in HRV in rats exposed to urban air particles [86].

Human studies

There is little information on the impact of antioxidant supplementation on the acute effects of air pollution exposure in humans. Most existing studies have focused on the changes of acute lung function. Other outcomes included bronchial airway reactivity, inflammatory response and changes in HRV but are less numerous and consistent. All these studies were experimental studies using supplements.

Antioxidant supplementation

Lung function and airway reactivity

Early studies used experimental protocols with single pollutants and a small number of healthy adults. Levels of O_3 and NO_2 were very high (usually close to 1,000 $\mu g \cdot m^{-3}$ and >3,000 $\mu g \cdot m^{-3}$, respectively) and subjects were supplemented for a relatively short period of time with high doses of vitamin C or vitamin E (eight to 16 times the USA recommended daily allowance of vitamin C (60 $mg \cdot day^{-1}$) and vitamin E (8 $mg \cdot day^{-1}$)) [2, 87–89]. A modulating effect of antioxidant supplementation was observed in some studies of acute lung function changes [89] and airway reactivity [87] but not in others.

More recent experimental studies have addressed conditions in which the O_3 level and supplement doses were lower. In a study of asthmatic adults, a cocktail of vitamin C (500 mg) and vitamin E (400 UI) protected against a decrease in peak expiratory flow from SO_2 challenge after O_3 exposure [90]. In another study [91], subjects were first deprived of vitamin C and then supplemented with a relatively low dose of vitamin C (250 mg), vitamin E (100 mg) and vegetable cocktail. Supplementation protected against acute change in lung function (forced expiratory volume in one second and forced vital capacity) after O_3 challenge. However, in well nourished individuals sensible to O_3, supplementation with vitamin C (500 mg) and vitamin E (100 mg) provide no protective effect on inflammatory response or lung function decrease after O_3 challenge. This lack of protection was observed despite elevated plasma vitamin C (+60.1%) and vitamin E (+51.4%) concentrations following supplementation, and increased vitamin C concentrations in the airways after supplementation following O_3 exposure [92].

Supplementation studies conducted in free-living populations of healthy exercising adults (the Netherlands) or adults exposed to high levels of air pollutants (Mexico) support the hypothesis that antioxidant supplementation protects against the acute effects of O_3 on lung function. In these studies, healthy adults were randomised to receive vitamin C (650 mg), vitamin E (75 mg) and β-carotene (15 mg) for several weeks [80, 93–95]. More recently, a study of asthmatic children exposed to high levels of air pollutants in Mexico City also suggested that supplementation with vitamin C (250 $mg \cdot day^{-1}$) and vitamin E (50 $mg \cdot day^{-1}$) had a modulating effect on acute lung function changes [96]. The positive effect of antioxidant supplementation was mostly found in children genetically susceptible to the effects of oxidants (glutathione *S*-transferases (GST)M1 null genotypes) [97].

Inflammatory response

Only three studies have evaluated the impact of antioxidant supplementation on airway inflammatory response to air pollutant exposure. SAMET *et al.* [91] observed no difference in the bronchoalveolar lavage content of polynuclear cells and other inflammatory markers between supplement and placebo groups after O_3 challenge. Similarly, Mudway *et al.* [92] reported no effect of supplementation with vitamin C and vitamin E on O_3-induced neutrophilia in healthy individuals responsive to O_3. In contrast, asthmatic children heavily exposed to air pollutants and supplemented with vitamin C and vitamin E had significantly lower levels of IL-6 and IL-8 in nasal lavage than children receiving placebo [98].

n-3 PUFA supplementation

Lung function and inflammatory response

The impact of n-3 PUFA supplementation on asthmatic symptoms and exercise-induced bronchoconstriction has been examined among asthmatic subjects in various recently reviewed studies [12, 34, 99]. Most of these studies enrolled a small number of asthmatic patients randomly assigned to receive a high dose of n-3 PUFA (3–4 g of EPA) for a short time-period (6–10 weeks); results were inconsistent. Studies with longer intervention periods, from 6 months to 1 yr, also led to inconsistent results with some studies showing improvement in lung function [100, 101] or inflammatory markers [101–103], or no effect [104]. The dosage and duration of n-3 PUFA supplementation, and the type of asthmatic patients differed between studies and may explain the discrepancy between these studies [12, 34]. The Cochrane database of systematic reviews identified 22 studies but included only nine that fulfilled the inclusion criteria and concluded that data were insufficient to determine the effect of n-3 PUFA in asthma. None of these studies include information on air pollution.

Cardiovascular effect

Increased intake of n-3 PUFA either from dietary sources or as a pharmacological supplementation has been shown to decrease the risk of mortality from coronary heart disease [105]. In a randomised trial conducted in nursing home residents, supplementation with 2 $g \cdot day^{-1}$ of fish oil (each 1 g capsule contained 83.2 % of omega-3 fatty acids) significantly decreased the effect of $PM_{2.5}$ on time and frequency domain parameters of HRV [106] This is one of two studies providing evidence that oxidant stress is one of the mechanisms explaining the effect of particle air pollution on the cardiovascular system [107]. The other study reported that statins had a mitigating effect on the HRV effects of particulate air pollution in subjects genetically susceptible to oxidative stress (lacking the GSTM1 allele) [108].

Modifiers of an individual's response to oxidative stress

Under the biological model of oxidative stress one would expect factors that modify the response to oxidative stress to also alter the effects of air pollution. Thus, nutritional status, chronic diseases and genetic factors are candidates to

determine susceptibility to oxidative stress-related effects of air pollution [26] as all these conditions are related to poor antioxidant defence.

Nutritional status

Antioxidant vitamin supplementation provides some protection against the adverse effect of O_3 on lung function in asthmatic children with slight deficiencies in these nutrients [96], and to adults depleted in vitamin C [91]. In contrast, vitamin supplementation did not protect against O_3-induced lung function decrement in well nourished subjects [109].

Chronic diseases

Most chronic diseases are associated with chronic inflammation [13, 27, 28, 110–112], which might increase susceptibility to the additional oxidative stress caused by air pollution exposure. In particular, subjects with asthma [29], chronic obstructive lung diseases [113], diabetes [114] and cardiovascular diseases [115] have all been shown to have antioxidant deficiency [13] and be more susceptible to the effects of air pollution [108, 115]. As observed in the case of cigarette smoke, a significant source of oxidative stress, air pollutants would lower antioxidant defences, with deleterious health consequences [116, 117]. Evidence of the potential beneficial effect of antioxidants can be found in studies of elderly subjects in which treatment with statins [108] and n-3 PUFA supplementation [106] had a beneficial effect on response to particulate exposure.

Genetic susceptibility

As oxidative stress is an important pathway activated/involved in the adverse effects of air pollution, the genes involved are of primary interest. Most studies have focused on single gene polymorphisms; however, it is likely that there will be a hierarchy of genes determining susceptibility, rather than one individual gene driving this process [15].

GST enzymes: GSTM1, GSTP1

GST are phase II xenobiotic metabolising enzymes that participate in the detoxification of ROS by catalysing their conjugation with glutathione [118, 119]. The common null allele of *GSTM1* results in a complete lack of the enzyme and reduced or no conjugation activity [120]. It has been associated with an increase in asthma and wheezing among children exposed to environmental tobacco smoke *in utero*, with a decrease in lung function growth [121, 122], and also with a rapid decline in lung function in smokers [123]. In addition, polymorphic *GSTM1* has been shown to act as a modifier of the lung response to fire smoke [124] and O_3 [125]. Antioxidant supplementation with vitamin C and E appears to modulate the effect of O_3 in asthmatic children homozygous for the *GSTM1* null allele [97]. Allergen sensitive subjects with low responsive genotypes show enhanced susceptibility to the adjuvant effects of DEP [126]. A *GSTM1* polymorphism has also been shown to increase sensitivity to PM, as evidenced by greater changes in HRV [108]. Moreover, glutaryl coenzyme A inhibitors, *i.e.* statins, with known antioxidant and anti-inflammatory properties mitigate against the effects of ambient particles on HRV in subjects lacking the *GSTM1* allele [107, 108].

Other genes

The Toll-like receptor 4 (*TLR4*; xr 4) gene has been implicated in innate immunity and endotoxin susceptibility [127] and has been hypothesised to play a role in O_3-induced hyperpermeability [26]. TNF-α (Xr17) has been related to lung function changes after O_3 exposure [128] and to an increased risk of asthma and wheezing that can be modified by O_3 exposure [129]. TNF has been identified as a candidate gene for O_3-induced airway inflammation and hyperresponsiveness [130]. Polymorphisms in TNF and lipoteichoic acid have been associated with respiratory effects of O_3 in humans [128]. *Arginase II* has been associated with an increased risk of asthma in children, and the association appeared stronger among children with a smoking parent [131] suggesting that air pollutants could also play a role.

Gene–gene interactions

O_3-induced acute effects on respiratory function have been shown to be smaller in subjects with *GSTM1* null and *NOQ1* Pro/Pro genotypes [132]. Similarly, a study examining asthma risk in a population highly exposed to O_3 showed that the risk of asthma was significantly associated with the *NOQ1* genotype in subjects with the null genotype for *GSTM1* [133]. Both genes have a specific function in antioxidative activities.

FURTHER EPIDEMIOLOGICAL RESEARCH

There is now substantial evidence that air pollution exposure results in increased oxidative stress, alterations in immune regulation and repeated inflammatory responses that overcome lung defences to disrupt the normal regulatory and repair processes [10, 15]. As summarised previously, despite a plausible mechanistic model linking air pollution, oxidative stress and dietary supplementation, evidence is not sufficient. Further randomised controlled trials (RCTs) are needed in order to better understand the potential protective effect of nutrient supplementation on the effect of air pollution on respiratory and cardiovascular functions and inflammatory responses.

RCTs provide a good alternative to maximise contrast in nutrient intake for evaluating the interaction of dietary factors and air pollutants and should be conducted in both the controlled setting and in free-living populations. A controlled setting will allow assignment of air pollutant exposure and, therefore, provide an accurate representation of the health effects and potential modulating effects of supplementation, while RCT conducted in free-living populations will have the advantage of representing real-life conditions.

Susceptible subjects, such as those with pre-existing respiratory or cardiac disease, micronutrient deficiency or genetic susceptibility, are the most likely to benefit from nutritional intervention (see Modifier of response section); therefore, RCTs should focus on these population subgroups. Short- and long-term effects can be studied; however, the major challenge in long-term effect studies is to assess the appropriate time-frame of exposure for the induction of the disease and, therefore, the relevant period and duration of the supplementation. There is accumulating evidence that exposure during lung development in foetal life and early childhood plays a major role, as in the case of maternal smoking [134–136]. Therefore, RCTs of pregnant females with specific risks (such as asthmatic or

TABLE 1 Biomarkers of oxidative stress most commonly used in clinical and epidemiological studies

Type of measurement	Biomarker	Biological sample	Laboratory technique	Sensitivity and specificity	Comments	[Ref.]
TAC	TRAP TRAP + R-PE	Plasma Serum	Fluorescence	Good Possible artefactual confounding	Measures the cumulative action of all antioxidants present in plasma and body fluids TRAP: indirect measure TRAP+R-PE: direct measure of peroxyl radical attack on R-PE. Affected by protein concentration Plasma better than serum	[140–142]
Lipid peroxidation	TBARS	Tissue Plasma Serum	Spectrophotometry Colourimetry Fluorometry	Low specificity	Easy to use Indirect measure	[143, 144]
	MDA-TBA derivatisation	Plasma Serum EBC Urine	TBARS HPLC/MS HPLC-UV/Vis HPLC with fluorescence detection	Low specificity Good	Measures MDA, end product of lipoperoxidation. MDA is generated mainly by arachidonic acid and docosahexaenoic acid With HPLC detection, MDA is not a specific product of lipid peroxidation	[143–145]
	Free MDA	Plasma Serum	HPLC HPCE	Good Good	Low amount of plasma needed Fast and practical for clinical measurements Low detection limit	[145, 146]
	4-hydroxynonenal 4-hydroxy	Tissue Blood Urine	ELISA GC/MS	Good	HNE is a toxic product of lipid peroxidation and second toxic messenger of free radicals	[147, 148]
	Hydrocarbons: ethane and pentane	EBC	GC	Penthane: low specificity Ethane: good	Hydrocarbons are produced through peroxidation of fatty acids in cellular biomembranes, by ROS Ethane: faster chromatographic measurement compared with other hydrocarbons; better marker for lipid peroxidation Background level of pentane and isoprene in human breath difficult to separate pentane from isoprene by chromatography Possible contamination with ambient air ethane and pentane	[149–152]
	Conjugated dienes	Plasma Serum	Spectrophotometry HPLC	Validity still questionable	Other biological substances, even polyunsaturated fatty acids, absorb in the same UV region CD generation continues *ex vivo* after sampling Plasma CD is >90% derived from 9, 11 diene-conjugated linoleic acid from dietary dairy products	[150, 153]
	LDL oxidation	Plasma	*Ex vivo* LDL by CD assay with spectrophotometric determination	Good	Measures the rate of CD formation Cannot be known for certain whether the *in vitro* situation accurately reflects *in vivo* events Should reflect the antioxidant defence system. Vitamin E has shown reasonably consistent effects in increasing the resistance of LDL to oxidation	[143, 153–155]
		Plasma Serum	*In vivo* LDL-BDC with spectrophotometric determination	Good	Faster and simpler to perform than the *ex vivo* procedure Measures amount baseline diene conjugation	[156]
	Oxidised LDL	Plasma	ELISA	Poor	These modifications may occur independently of lipid peroxidation Still unclear whether it can serve as a peripheral marker High variability	[144, 152, 157]
	Lipid hydroperoxides: CEOOH	Plasma	HPLC assay with chemiluminescence detection	Not confirmed	Not detectable in young healthy controls Direct indicator of lipid peroxidation	[144, 158]

TABLE 1 Continued.

Type of measurement	Biomarker	Biological sample	Laboratory technique	Sensitivity and specificity	Comments	[Ref.]
Eicosanoids	F2-isoprostane	Plasma Serum Urine EBC	HPLC GC/MS ELISA	Good	These markers reflect respiratory tract integrity between reactive nitrogen species and ROS Interaction with other prostanoids Potent biological activity 8-iso-$PGF_{2\alpha}$ is a major component of total F_2 isoprostanes In plasma, possibility of artefactual generation due to arachidonic acid autoxidation Better in urine - less interaction	[143, 144, 152, 159, 160]
	PGE_2	EBC Plasma Sputum	HPLC/MS/MS ELISA GC/MS	 Good	Not flow dependent in healthy subjects	[159–162]
	LTB_4	EBC Plasma Serum Urine Sputum BAL	GC/MS HPLC ELISA	Good	LTB_4 is a potent neutrophil chemoattractant and may contribute to airway narrowing by producing local oedema and increasing mucus secretion	[159–161]
Nitrogen reactive species	Nitrite: NO_2^- Nitrate: NO_3^-	EBC Plasma	Colourimetry Fluorometry Ionic chromatography GC/MS HPLC	Good	In healthy children, nitrite values are not related to levels of exhaled NO Both nitrite and nitrate quantification	[159, 163–166]
	S-nitrosothiols 3-nitrotyrosine	Plasma BAL	Fluorometry GC/MS	Good	Formed by glutathione peroxidise; a selenium-dependent enzyme	[159, 167–169]
DNA oxidation	8-OHdG	Urine DNA	ELISA CG/MS HPLC/ECD	Poor	May be influenced by the metabolic rate and also by excision repair GC/MS: level of 8-OHdG overestimated ELISA values higher than HPLC values	[143, 170–173]
	8-oxoGua	DNA	CG-MS HPLC-ECD HPLC-MS Comet assay ELISA	Good	HPLC-ECD generally yields lower values Enzymatic approach: FPG may detect lesions other than 8-oxo-7, 8-dihydroguanine; the method relies on indirect calibration Reported strong correlation between overnight and 24 h urinary 8-oxodGuo#	[174, 175]
	8-oxodGuo	24 h urine	CG-MS HPLC-ECD HPLC-MS Comet assay ELISA	Good	HPLC-ECD generally yields lower values Enzymatic approach: FPG may detect lesions other than 8-oxo-7, 8-dihydroguanine; the method relies on indirect calibration Reported strong correlation between overnight and 24 h urinary 8-oxodGuo#	[174, 175]
	Modified comet assay	DNA	SCGE	Good	Measures DNA strand breaks Proportion of DNA in the tail indicates the frequency of breaks Particularly sensitive to oxidative attack by H_2O_2	[143, 176]
	HmdU	Plasma Serum	ELISA	Good	Autoantibody to oxidised DNA Product of thymine oxidation	[143, 177, 178]
Protein oxidation	Protein carbonyl	Plasma Lung aspirate	Colourimetric method ELISA HPLC	Good	Measures generic oxidation; does not differentiate between those protein carbonyl arising directly from protein oxidation and those formed by adduction of other oxidised products	[143, 153, 179]

TABLE 1 Continued.

Type of measurement	Biomarker	Biological sample	Laboratory technique	Sensitivity and specificity	Comments	[Ref.]
Other	GSH	Sputum Plasma Saliva	Spectrophotometry	Good	GSH is a protective antioxidant against oxidative stress Level of GSH depends on biological sample	[159, 180–184]
		BAL	Reverse phase HPLC	Good		
		EBC	HPLC /with fluorescence detection	Good		
	GSH/GSSG ratio	Plasma Serum	Colourimetry HPLC NL	Good	Decrease in GSH/GSSG indicates chronic oxidative stress	[153, 185]
	H_2O_2	EBC	Spectrophotometry Fluorometry Chemiluminescence	Poor: high variation	Concentration appears to be expiratory flow rate dependent Wide variability in mean exhaled H_2O_2 concentration in healthy nonsmoking adults Other factors: exercise, food, beverage intake	[159, 186–188]
	CC16	Serum BALF	Latex immunoassay ELISA	Good	These tests evaluate the integrity of respiratory tract Peripheral marker CC16 protects the respiratory tract against oxidative stress and inflammation	[189–192]
	Thioredoxin	Serum	ELISA	Good	Thioredoxin is induced by oxidative stress and secreted by cells	[193–195]

TAC: total antioxidant capacity; TRAP: total radical trapping antioxidant parameter; R-PE: R-phycoerythrin; TBARS: thiobarbituric acid-reactive substances; MDA-TBA: malondialdehyde-thiobarbituric acid; HPLC: high performance liquid chromatography; MS: mass spectometry; EBC: exhaled breath condensate; UV/Vis: UV/visible detection; HPCE: high performance capillary electrophoresis; HNE: 4-hydroxynonenal; GC/MS: gas chromatography/MS tandem; ROS: reactive oxygen species; CD: conjugated dienes; LDL: low-density lipoprotein; BDC: baseline diene conjugation; CEOOH: cholesteryl ester hydroperoxides; PG: prostaglandin; LTB_4: leukotriene B_4; BAL: bronchoalveolar lavage; NO: nitric oxide; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; ECD: electrochemical detection; 8-oxoGua: 8-oxo-7,8-dihydroguanine; FPG: fasting plasma glucose; SCGE: single cell microgel electrophoresis; 8-oxodGuo: 8-oxo-7,8-dihydro-2′-deoxyguanosine; HmdU: 5-hydroxymethyl-2′-deoxyuridine; GSH: reduced glutathione; GSSG: oxidised glutathione (disulfide form); NL: nasal lavage; BALF: BAL fluid(s). #: $r=0.93$, $p<0.01$.

atopic mothers) might provide some insight into the role of antioxidants and n-3 PUFA as modulators of the air pollution effect. In these studies, a major challenge is the accurate assessment of air pollution exposure, oxidative stress, biomarkers of nutritional status and health outcomes. Standardisation of these factors within and between studies is crucial to allow comparability of results. In the following section some issues to be considered in future studies will be discussed.

Air pollution exposure

Contrasts in exposure need to be maximised to be able to distinguish between effects in the placebo group and smaller or no effects in the supplemented groups. Depending on the study design and hypotheses tested, either temporal or spatial contrast should be large. Multicentre studies including areas with contrasting air pollution levels and the enrolment of random samples of participants within each centre might be an option. Moreover, the design of the exposure assessment must take into account the relationship between measured or measurable markers of oxidant pollution and personal exposure to the pollutant relevant to the hypothesis. For example, there are often large indoor/outdoor ratios in O_3 concentrations and these can be very heterogeneous across homes. Personal O_3 concentration may be very poorly correlated with ambient levels in certain areas. It might be useful to measure the redox activity of ambient pollutants or the antioxidant depletion rates, as these may be the most relevant characteristics in the hypothesised pathways of redox imbalance. Various assays have been developed to measure the redox activity of particles, such as OH radical formation or antioxidant depletion rates [137]. However, the measurement methods may need further development to be applicable in epidemiological studies, in particular, for personal exposure assessment.

Biomarkers of oxidative stress

The advantage of using biomarkers is that they integrate both the effects of oxidant exposure and the full range of antioxidant protective mechanisms *in vivo* [30]. However, samples can be oxidised during handling, processing and analysis, so there is potential for artefacts in estimates of baseline levels of oxidation markers. The magnitude of this problem varies between biomarkers [31, 138]. Most of these biomarkers include measures of lipid, DNA and protein oxidation. Recent review articles provide broad coverage of this topic [30, 139]. Table 1 presents a summary of oxidative stress biomarkers useful for clinical and epidemiological studies including: the type of marker; the biological media for measurement; the laboratory techniques most frequently used; an appreciation of its

TABLE 2 Biomarkers of nutrient intake most commonly used in clinical and epidemiological studies

Type of measurement	Biological sample	Laboratory technique	Comments	Characteristics and sources	[Ref.]
Carotenoids β-Carotene α-Carotene Lycopene Lutein Xanthine β-Cryptoxanthin	Serum Plasma Induced sputum Adipose tissue	HPLC	Poor bioavailability in raw food, improved by mild cooking or heating (*e.g.* lycopene in tomato juice) Reflect short-term intake Need to control for cholesterol level Adipose tissue reflects long-term exposure May not reflect concentration in target tissue	Liposoluble Red, orange and yellow fruits and vegetables (sweet potato, carrots, winter squash) Green vegetables	[143, 196–198]
Tocopherols α-Tocopherol γ-Tocopherol	Serum Plasma Adipose tissue	HPLC	Serum and plasma reflect short-term intake Need to control for cholesterol level Adipose tissue reflects long-term exposure	Liposoluble Vegetable and seed oils (corn, safflower, soy) Beans, eggs, green vegetables	[143, 199]
Vitamin C	Serum Plasma	HPLC	Vitamin C in food can be destroyed by exposure to high temperature, oxidation or cooking in large amount of water Response to intake up to 50–90 mg·day^{-1}, then eliminated by renal clearance Reflects short-term intake Predicts intake at low level of vitamin intake	Hydrosoluble Fruits: papaya, canteloupe, citrus fruits, strawberries Vegetables: cauliflower, broccoli, brussel sprouts, kale, sweet peppers	[143, 200]
Selenium	Plasma Toenail	Atomic absorption spectrophotometry HPLC	At higher levels of intake, the correlation between plasma selenium concentration and dietary intake depends on the chemical form of selenium in the diet Selenium content of cereals and grains depends on the soil content Plasma reflects short-term intake Nail and whole blood reflect long-term exposure (>26–56 weeks)	Cereals and grains Animal products, especially organ meats and seafood	[143, 201–204]
Flavonoids	Serum Urine	HPLC	Measures the usual dietary intake over 1 week	Apples, lemons, oranges Potatoes, cauliflower Tea Skin of tubers and roots Red wine	[205, 206]
Isoflavonoids	Serum Urine	GC/MS HPLC	Sex differences in metabolism and excretion	Legumes: soybeans, beans, lentils, chickpeas.	[207–209]

TABLE 2 Continued.

Type of measurement	Biological sample	Laboratory technique	Comments	Characteristics and sources	[Ref.]
Lignans	Serum -24–72 h urine	HPLC	Sex differences in metabolism and excretion	Oil seeds (flax seed, soybean, rapeseed) Whole-grain cereals (wheat, oats, rye), legumes, vegetables; fruits	[207–209]
PUFA **n-3 PUFA** **n-6 PUFA**	Free fatty acids in serum or plasma Components of circulating triglycerides Phospholipids Cholesterol esters Red blood cell membranes EBC Adipose tissue	HPLC GC/MS GLC	Samples are temperature and oxygen sensitive Potential for oxidation and degeneration over time Free fatty acids, phospholipids and cholesterol esters represent the intake over the last few days or meals Serum fatty acids appear to be sensitive to changes in diet; high fluctuation (10–12%) and lab error <5% Components of triglycerides represent intake over the past few hours RBC reflect longer term intake (half-life of RBC: 120 days) RBC sample: collected whole blood is suspended in phosphate buffer and centrifuged; packed red cells are stored at -80°C RBC: may contain lower levels of n-3 and n-6 PUFA Adipose tissue reflects long-term intake if no severe weight loss has occurred	Fish oils Fish and shellfish Soy and canola oil	[210–212]
Folate	Serum RBC	ELISA	Serum: short-term folate RBC: dietary intake over last 120 days	Leafy vegetables Dry beans and peas, fortified cereal Some fruits	[213, 214]
Zinc	Plasma Cells Erythrocyte, monocyte, neutrophil, platelet Hair Nails Urine	Atomic absorption spectrometry	Plasma: most frequently used Possibility of no association between zinc intake and plasma zinc Cells: complex sample preparation Poor sensitivity, imperfect specificity	Oysters Animal proteins Beans Nuts Pumpkin and sunflower seeds	[202, 215–218]

HPLC: high performance liquid chromatography; GC: gas chromatography; MS: mass spectrometry; PUFA: polyunsaturated fatty acids; GLC: gas liquid chromatography; EBC: exhaled breath condensate; RBC: red blood cells.

sensitivity and specificity based on the literature review; and some additional comments [140–195].

Biomarkers of exposure to antioxidant nutrients and n-3PUFA

These biochemical indicators have the advantage of integrating different food sources and providing a better estimation of the internal dose, *i.e.* a closer indication of the amount of nutrient available after absorption and metabolism [33]. They can also be used in intervention studies to monitor compliance with the supplement. However, they are subject to measurement errors and sampling, storage, handling and laboratory analysis and temporality issues need to be carefully considered [30]. Table 2 presents a summary of biomarkers of antioxidant and n-3 PUFA intake used in clinical and epidemiological studies including: the type of marker; the biological media for measurements; the laboratory techniques most frequently used; the characteristics and food sources of these nutrient biomarkers; and some additional comments [196–218].

Health end-points

The limited validity of symptoms of respiratory or cardiac diseases has been extensively discussed [219, 220]. Objective outcomes, such as lung function, nitric oxide in exhaled breath, carotid intimae-media thickness, electrocardiographic abnormalities or HRV, are less prone to bias and may be a good alternative but their long-term predictive value is uncertain. Biological indicators, such as pro-inflammatory markers (*e.g.* IL-6, IL-4, TNF-α, IFN-γ) in sera, exhaled breath and nasal lavage, and peripheral inflammatory markers (*e.g.* cell counts, fibrinogen, C-reactive protein, von-Willebrand factor, prostaglandin E2, plasminogen activator inhibitor, cell adhesion molecules) might provide useful information about potential mechanisms of air pollutant exposure. However, they are subject to large within-person variability and limited specificity as they are common to different end-points; therefore, serial measurements over the study period are required. In addition, intra-individual variability and the temporal frame need to be considered for any of the transient end-points. A mechanistic approach that includes evaluation of several end-points at the clinical and biological levels seems most appropriate. Further understanding of the crucial role of transcription factors, DNA methylation and RNA control of gene expression will provide new perspectives on the complex interaction of air pollutants and nutritional factors.

CONCLUSION

Oxidative stress is one of the main mechanisms by which air pollutants affect respiratory and cardiovascular health. Short-term randomised supplementation trials suggest that antioxidant vitamins and n-3 polyunsaturated fatty acids might protect against the acute effect of these pollutants, particularly in vulnerable subgroups [80, 96, 106]. However, the evidence is still limited because of the small sample size in most studies and the lack of comprehensive assessment of baseline nutritional status and oxidative stress response. Future studies should include randomised control trials of antioxidant or n-3 polyunsaturated fatty acid supplementation in susceptible populations and measure clinical, as well as intermediate, outcomes and biomarkers of oxidative stress and nutrient intake considering factors, such as reproducibility, inter- *versus* intra-person variability, detection limits and specificity and sensitivity of these markers. Doses and duration are still under debate but harmonisation between studies is desirable for comparison purposes.

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