Effects of dietary antioxidants on the immune function of middle-aged adults

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The immune system is highly reliant on accurate cell–cell communication for optimal function, and any damage to the signalling systems involved will result in an impaired immune responsiveness. Oxidant-mediated tissue injury is a particular hazard to the immune system, since phagocytic cells produce reactive oxygen species as part of the body's defence against infection. Adequate amounts of neutralizing antioxidants are required, therefore, to prevent damage to the immune cells themselves. Many antioxidants can be obtained directly from the diet (e.g. ascorbic acid, α-tocopherol, carotenoids and polyphenolic flavonoids) or require micronutrients as integral components (e.g. Se in the metalloenzyme glutathione peroxidase (*EC* 1.11.1.9)). Numerous epidemiological studies have found strong associations between diets rich in antioxidant nutrients and a reduced incidence of cancer, and it has been suggested that a boost to the body's immune system by antioxidants might, at least in part, account for this. Although more striking effects have been observed in the elderly, there is also evidence that antioxidant nutrients can modify cell-mediated immune responses in younger individuals. Indeed, it might be essential to have an adequate intake of antioxidant nutrients from an early age in order to help prevent the development of, or at least delay the onset of, several degenerative disorders. The present paper will review the effects of specific nutrients on immune function in young to middle-aged human subjects, focusing on the antioxidant vitamins C and E, and on Se. A further review, dealing more specifically with the effects of carotenoids on human immune function, will be presented at a forthcoming meeting of the Nutrition Society.

Antioxidants: Immune function: Vitamin C: Vitamin E

Free radicals and reactive oxygen species

Free radicals are highly-reactive molecules containing one or more unpaired electrons. Examples of free radicals are superoxide ($O_2^{\bullet -}$) and hydroxyl (OH•). The term 'reactive oxygen species' (ROS) is a collective one that includes not only oxygen-centred radicals but also some non-radical derivatives of oxygen, such as H_2O_2, singlet oxygen and HOCl. H_2O_2 can very easily break down, particularly in the presence of transition metal ions (e.g. Fe^{2+}), to produce the hydroxyl radical, the most reactive and damaging of the oxygen free radicals:

$$H_2O_2 + Fe^{2+} \rightarrow OH\bullet + OH^- + Fe^{3+}.$$

Free radicals are generated during normal cellular metabolism and are also made deliberately. For example, $O_2^{\bullet -}$ plays an essential role in the intracellular killing of micro-organisms by activated phagocytes. Free radical production in cells can be significantly increased by certain toxic 'redox-cycling' compounds such as various drugs and CCl_4. Exogenous sources of free radicals include ozone, exposure to u.v. radiation in sunlight, and cigarette smoke.

All the major classes of biomolecules are vulnerable to free radical damage. Free radicals cause strand breaks in DNA (Halliwell & Aruoma, 1991), which potentially can lead to subsequent misrepair and tumour cell formation. An example of free radical-mediated damage to proteins is the formation of cataracts, resulting from the damage to the crystallins in the lens of the eye. However, lipids are probably most susceptible to free radical attack, particularly long-chain polyunsaturated fatty acids which contain several double bonds. The oxidative destruction of poly-unsaturated fatty acids, known as lipid peroxidation, can be extremely damaging, since it proceeds as a self-perpetuating chain reaction.

Abbreviation: ROS, reactive oxygen species.

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Antioxidant defences

Since ROS are produced *in vivo*, organisms have evolved antioxidant defence systems either to prevent the generation of ROS or to intercept any that are produced. They exist in both the aqueous and membrane compartments of cells and can be enzymes or non-enzymes. Catalase (*EC* 1.11.1.6) and glutathione peroxidase (*EC* 1.11.1.9) are enzymes which can safely decompose peroxides, particularly H_2O_2 produced during the respiratory burst involved in microbial killing in phagocytic cells, whilst superoxide dismutase (*EC* 1.15.1.1) intercepts or 'scavenges' free radicals. Most free-radical scavengers are not enzymes, and many are obtained through the diet. In cell membranes the most important is α-tocopherol, the major member of the vitamin E family. This molecule acts as a 'chain-breaking antioxidant', intercepting lipid peroxyl radicals and so terminating lipid peroxidation chain reactions. Another group of lipid-soluble compounds that can act as antioxidants are the carotenoids, such as β-carotene, lycopene and lutein, found in highly-pigmented fruits and vegetables (Mangels *et al.* 1993). The polyene structure of these compounds allows the molecules to quench, or inactivate, singlet oxygen and free radicals. The major water-soluble free-radical scavenger is ascorbic acid (vitamin C), which also plays a role in 'sparing' vitamin E, by regenerating α-tocopherol from the oxidized tocopheroxyl radical (Bendich *et al.* 1986). More recently, attention has also focused on the antioxidant properties of plant polyphenols, found in tea and red wines (Rice-Evans, 1995), but considerably more information on the absorption, metabolism and excretion of these compounds in human subjects is required before their relative contribution to preventing oxidative damage can be assessed.

Oxidative stress and the immune system

Viewed from the perspective of a 'two-pan balance' with ROS in one pan and antioxidants in the other pan, it is thought that tipping the balance in favour of the ROS is a major contributor to several degenerative disorders such as cancer and cardiovascular diseases (Table 1) and to the ageing process in general. Striking improvements in immune function have been observed in elderly individuals following supplementation with antioxidant nutrients (see Lesourd, 1999), but there is growing evidence that effects can also be observed in younger healthy individuals. Strong associations between diets rich in antioxidant nutrients and a reduced incidence of cancer have been observed in numerous epidemiological studies, and it has been suggested that a boost to the body's immune system by antioxidants might, at least in part, account for this finding. Indeed, it is probably crucial to attempt to keep the balance of ROS to antioxidants as level as possible, ideally by dietary means rather than by taking supplements, from as early an age as possible, in order to prolong, if not prevent, the onset of many age-related disorders.

Table 1. Degenerative disorders associated with oxidative damage

Cancer
Cardiovascular disease
Stroke
Cataract
Degeneration of the macular area of the retina
Immunosenescence
Ageing

The immune system is particularly sensitive to oxidative stress. Immune cells rely heavily on cell–cell communication, particularly via membrane-bound receptors, to work effectively. Cell membranes are rich in polyunsaturated fatty acids which, if peroxidized, can lead to a loss of membrane integrity, altered membrane fluidity (Baker & Meydani, 1994), and result in alterations in intracellular signalling and cell function. It has been shown that exposure to ROS can lead to a reduction in cell-membrane receptor expression (Gruner *et al.* 1986). In addition, the production of ROS by phagocytic immune cells can damage the cells themselves if they are not sufficiently protected by antioxidants.

Selenium

Se is concentrated in tissues involved in the immune response, such as lymph nodes, spleen and liver (Spallholz *et al.* 1990), and various components of the immune system have been shown to be impaired if dietary intake of Se is inadequate (for review, see McKenzie *et al.* 1998). A major deficiency is seen in the microbicidal activity of phagocytes (Serfass & Ganther, 1975) and this is thought to be due to the fact that Se is an integral component of glutathione peroxidase. Once a micro-organism has been engulfed into a phagocytic cell, the phagocytic vesicle fuses with lysosomes and the pathogen is destroyed by the respiratory burst, which generates ROS from molecular oxygen. It has been suggested that a lack of glutathione peroxidase can lead to damage of lysosomal membranes by lipid hydroperoxides, resulting in the release of various hydrolytic enzymes into the cytoplasm, with a subsequent reduction in cell function. Supplementation with Se appears to boost cell-mediated immune responses and, as well as protecting against oxidative damage, Se can also upregulate the expression of the high-affinity interleukin-2 receptor on T lymphocytes (Roy *et al.* 1994). This may, at least in part, explain the stimulatory effect of Se on B-cell antibody production, since this is regulated by T-cells.

In passing, it is worth mentioning the recent work of Beck and colleagues (Beck, 1998) who have demonstrated that Se-deficient mice infected with the coxsackievirus B3, develop an increased myocarditis compared with adequately-fed mice. The deficiency in Se was associated with a change to the viral genotype, converting the virus from a benign to a virulent strain (which involved six nucleotide changes). It is thought that this resulted from an enhanced ability of the virus to replicate in deficient hosts, thereby increasing the chances of a mutation occurring. This finding has obvious implications for populations containing individuals with poor nutritional status, since once the mutations have occurred even nutritionally-adequate hosts will be susceptible to viral-induced disease.

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Ascorbic acid

Vitamin C appears to affect most aspects of the immune system. It is found in high concentrations in leucocytes, it is rapidly utilized during infection, and reduced plasma levels are often associated with reduced immune function. Animal and human studies have suggested that the dietary requirements for vitamin C are increased in cancer, surgical trauma and infectious diseases. However, the belief that high intakes of vitamin C will prevent the onset of the common cold has not been scientifically substantiated, although it is generally agreed that the associated symptoms following infection can be reduced by a moderate intake (Coulehan *et al.* 1974). Pauling's (1970) claims regarding the effects of vitamin C on the common cold certainly inspired a great deal of interest in the effect of this vitamin on immune function in the 1970s and early 1980s (for reviews, see Thomas & Holt, 1978; Siegel, 1993), but research in this area has subsequently declined, and there have been very few recent studies examining the effects of vitamin C on the immune system in healthy younger individuals. In one study, where a group of healthy young males was given 1–3 g vitamin C/d for 3 weeks, there was an increase in neutrophil motility to lipopolysaccharide-activated autologous serum and in mitogen-stimulated lymphocyte proliferation following supplementation (Anderson *et al.* 1980). At the other end of the spectrum, to determine the effect of moderate vitamin C deficiency, Jacob *et al.* (1991) studied a group of young healthy non-smokers who were asked to consume an ascorbic acid-deficient diet which became gradually supplemented with vitamin C, starting at 5 mg/d, and progressing up to 250 mg/d. Ascorbic acid concentrations in plasma and leucocytes dropped initially to < 50 % of baseline, and there was no change in lymphocyte proliferation. However, blood levels of glutathione and NAD(P) decreased during ascorbic acid depletion, as did the delayed hypersensitivity responsiveness to intradermal injection of seven recall antigens. On repletion, there was a recovery in delayed hypersensitivity response at intakes of 60 or 250 mg vitamin C/d, but not in lymphocyte proliferation. Jacob *et al.* (1991) suggest that the inconsistency between these two tests regarding the influence of vitamin C on cell-mediated immunity may result from higher sensitivity of the delayed hypersensitivity test, involvement of cells other than those isolated for *in vitro* cultures in the *in vivo* delayed hypersensitivity response, or other unknown factors. The lack of an effect on lymphocyte proliferation at an intake of 250 mg/d suggests that, at least in young individuals, only levels of vitamin C that approach pharmacological doses can produce a quantifiable effect on this index of immune function. However, at present, there is no marker of immune function that is known to be indicative of a long-term beneficial effect in terms of reducing the incidence of degenerative disorders in later life. Although not an immunological study, one recent report provides an excellent example of the potential need to maintain adequate intakes of antioxidant nutrients in the middle years of life to prevent the accumulative damage caused by ROS being made manifest in later years. Jacques *et al.* (1997) examined the cross-sectional relationship between age-related lens opacities and vitamin C supplement use over a 10–12-year period in women without diagnosed cataract or diabetes. Use of vitamin C supplements for 10 years or more was associated with a 77 % lower prevalence of early lens opacities and an 83 % lower prevalence of moderate lens opacities compared with women who did not use supplements. Women who consumed vitamin C supplements for less than 10 years showed no evidence of a reduced prevalence of early opacities, suggesting that long-term consumption of vitamin C supplements may substantially reduce the development of age-related lens opacities. While the use of supplements might be required to obtain sufficient intakes of vitamin C to prevent this form of oxidative damage, it is hoped that the intake required to maintain optimal immune function can be obtained from a healthy diet containing fruit and vegetables rich in antioxidants. This should be the case, since epidemiological studies of populations having a lower incidence of cancer suggest that the benefits are associated with the intake of increased amounts of these foodstuffs rather than the taking of supplements.

Vitamin C has been used to treat some clinical phagocytic cell dysfunctions. In Chediak-Higashi syndrome, which is characterized in part by defective neutrophil functions, vitamin C supplementation has been shown to increase neutrophil chemotaxis, improve bactericidal activity and reduce the length of clinical illness (Boxer *et al.* 1976). Vitamin C also appears to be beneficial in the treatment of chronic granulomatous disease (Anderson, 1982) and in recurrent pyogenic infections (Rebora *et al.* 1980).

Ascorbate provides important antioxidant protection to plasma lipids and lipid membranes (Frei *et al.* 1989) and can also neutralize phagocyte-derived oxidants released extracellularly (Anderson & Lukey, 1987), thereby preventing oxidant-mediated tissue damage, particularly at sites of inflammatory activity. Other mechanisms which have been proposed for the immunostimulatory effects of vitamin C include: modulation of intracellular cyclic nucleotide levels, modulation of prostaglandin synthesis, enhancement of cytokine production, antagonism of the immunosuppressive interaction between histamines and leucocytes, the protection of 5-lipoxygenase (*EC* 1.13.1.34) (Anderson *et al.* 1990). There is a need for further research, not only into the mechanisms by which vitamin C can enhance immune cell function, but also to define the optimal levels of intake required to boost the immune system in younger individuals.

Vitamin E

Studies of human subjects and animals, in either states of deficiency or at supradietary levels, suggest strongly that α-tocopherol is involved in maintaining immune cell function. Since vitamin E is the most effective chain-breaking lipid-soluble antioxidant present in cell membranes, it is considered likely that it plays a major role in maintaining cell membrane integrity by limiting lipid peroxidation by ROS. Vitamin E deficiency states are associated with depressed B-cell antibody production and T-cell proliferation to mitogenic stimulation, and an increased rate of infection. As with other dietary antioxidants, a marked improvement in immune indices can be seen in the elderly following supplementation with vitamin E (Meydani & Beharka, 1996), but there is also evidence that increased

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intakes can modulate the function of immune cells in younger individuals. For example, supplementation with 300 mg vitamin E/d depressed the bactericidal activity of leucocytes from a group of healthy young men (Prasad, 1980). This finding, together with the results of a study suggesting that prolonged high-dose intakes of α-tocopherol (1600 mg/d) can lead to inhibition of neutrophil phagocytosis (Boxer, 1986), has led to the suggestion that perhaps vitamin E supplements should only be taken in moderation to prevent an increased susceptibility to infection. However, paradoxically, a community-based survey reported a negative correlation between plasma vitamin E levels and infectious disease episodes (Chavance *et al.* 1985), although this was in an elderly population.

Recently, Deveraj *et al.* (1996) examined the effect of high dose α-tocopherol supplementation (1200 mg/d) on *ex vivo* monocyte function. After 8 weeks of supplementation the *in vitro* release of ROS and lipid oxidation was decreased, both in the resting state and in lipopolysaccharide stimulated cells compared with both baseline and a 6-week washout period (when levels had returned to baseline). A similar effect was observed after culturing the cells in the presence of the protein kinase C inhibitor, Calphostin C, suggesting that inhibition of the activity of this enzyme might be a mechanism by which α-tocopherol can inhibit ROS release and lipid oxidation. Also, Deveraj & Jialal (1997) reported a decrease in interleukin-1β release from resting and activated monocytes following vitamin E supplementation, and have suggested that this inhibition is mediated in part by inhibition of 5-lipoxygenase, thereby decreasing leukotriene B4 levels, an agonist for interleukin-1β. It is also possible that vitamin E and, indeed, other antioxidant nutrients can influence a variety of inflammatory processes by inhibiting the activity of the transcription factor nuclear factor-κB. This protein transcription factor is required for maximal transcription of many cytokines, including interleukin-1β, and it is thought that the generation of ROS is a vital link in mediating nuclear factor-κB activation by a variety of stimuli (Sen & Packer, 1996; Jackson *et al.* 1998). Vitamin E might also down-regulate the positive feedback mechanism by which interleukin-1β can, in turn, activate nuclear factor-κB (Blackwell & Christman, 1997).

Several studies have examined the effect of vitamin E in cigarette smokers. Cigarette smoke contains millions of free radicals per puff, and other compounds present can stimulate the formation of other highly-reactive molecules (Pryor & Stone, 1993). Serum levels of vitamin E (as well as vitamin C and β-carotene) and lung vitamin E concentrations are significantly lower in smokers compared with non-smokers (Bendich, 1994) and even supplementation with 2400 mg/d for 3 weeks failed to restore lung vitamin E levels to that found in non-smokers (Pacht *et al.* 1986). Circulating phagocytes from smokers produce high levels of free radicals, which probably in part accounts for the depressed immune function observed in smokers (Hughes *et al.* 1985; Johnson *et al.* 1990), and there is some evidence that vitamin E supplementation can reduce the overproduction of ROS by phagocytic cells from current smokers (Richards *et al.* 1990).

Reduced levels of vitamin E have been reported in human immunodeficiency virus-infected individuals. Passi *et al.* (1993) found that plasma vitamin E levels were significantly lower in a group of 200 human immunodeficiency virus-positive individuals compared with controls, but whether this is related to an inadequate intake of this vitamin is unclear. Dietary diaries from a group of 100 human immunodeficiency virus-infected asymptomatic men did not indicate an inadequate intake of vitamin E, but plasma levels were low or marginally low in 74 % of the men (Beach *et al.* 1992). In a study of patients who had developed acquired immune deficiency syndrome an inverse relationship was observed between serum vitamin E levels and severity of disease (Favier *et al.* 1994).

Carotenoids

The close association observed in epidemiological studies between high intakes of carotenoid-rich foodstuffs and a reduced incidence of cancer stimulated several studies of the effects of these compounds on immune function. Once again, emphasis has been given to studying effects in the elderly (for review, see Bendich, 1996), but there is also evidence that carotenoids can enhance immune function in healthy middle-aged individuals (Hughes *et al.* 1997). An associated review, dealing specifically with the effects of carotenoids on human immune function, will be presented at a forthcoming meeting of the Nutrition Society.

Conclusions

The present review has selected examples of studies demonstrating an effect of dietary antioxidants on the immune system of adult humans during mid-life. It is evident that there is a scarcity of studies performed in this age-group, particularly in healthy subjects. A major challenge in the area of nutritional immunology is to identify specific effects of dietary antioxidants on the immune system at this stage in life (when it is assumed to be functioning at an 'optimal' level) which are indicative of a beneficial effect that might only be seen in later years, in terms of a reduced incidence of age-related diseases.

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