Chapter 17

Dietary antioxidants: a consideration of factors influencing requirements

Nutrients with an antioxidant role

The potential beneficial effects from antioxidants in protecting against disease have been used as an argument for recommending increasing intakes of several nutrients above those derived by conventional methods. If it is possible to quantify such claims, antioxidant properties should be considered in decisions concerning the daily requirements of these nutrients. This section examines metabolic aspects of the most important dietary antioxidants – vitamins C and E, the carotenoids, and several minerals – and tries to define the populations which may be at risk of inadequacy to determine whether antioxidant properties *per se* should be and can be considered in setting a requirement. In addition, pro-oxidant metabolism and the importance of iron are also considered.

Members of the Food and Nutrition Board of the National Research Council in the United States, recently defined a dietary antioxidant as a substance in foods which significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiologic function in humans (*1*). It is recognised that this definition is somewhat narrow because maintenance of membrane stability is also a feature of antioxidant function (*2*) and an important antioxidant function of both vitamin A (*3*) and zinc (*4*). However, it was decided to restrict consideration of antioxidant function in this document to nutrients which were likely to interact more directly with reactive species.

The need for biologic antioxidants

It is now well established that free radicals, especially superoxide ($O_2^{\cdot -}$), nitric oxide ($NO^{\cdot}$), and other reactive species such as H_2O_2, are continuously produced *in vivo* (*5-7*). Superoxide in particular is produced by leakage from the electron transport chains within the mitochondria and microsomal P450 systems (*8*) or formed more deliberately, for example, by activated phagocytes as part of the primary immune defence in response to foreign substances or to combat infection by micro-organisms (*9*). Nitric oxide is produced from L-arginine by nitric oxide synthases, and these enzymes are found in virtually every tissue of the mammalian body, albeit at widely different levels (*7*). Nitric oxide is a free radical but is believed to be essentially a beneficial metabolite and indeed it may react with lipid peroxides and function as an antioxidant (*10*). Nitric oxide also serves as a mediator whereby macrophages express cytotoxic activity against micro-organisms and neoplastic cells (*11*). If nitric oxide is at a sufficiently high concentration, it can react rapidly with superoxide in the absence of a catalyst to form peroxynitrite. Peroxynitrite is a potentially damaging nitrogen species which can react through several different mechanisms, including the formation of an intermediate with the reactivity of the hydroxyl radical (*12*).

To cope with potentially damaging reactive oxidant species (ROS), aerobic tissues contain endogenously produced antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase and several exogenously acquired radical-scavenging substances such as vitamins E and C and the carotenoids (*13*). Under normal conditions the high concentrations of SOD maintains superoxide concentrations too low to

allow the formation of peroxynitrite. It is also important to mention the antioxidant reduced glutathione (GSH). GSH is ubiquitous in aerobic tissues, and although it is not a nutrient, it is synthesised from sulfhydryl-containing amino acids and is highly important in intermediary antioxidant metabolism (*14*).

Integrated antioxidant defences protect tissues and are presumably in equilibrium with continuously generated ROS to maintain tissues metabolically intact most of the time. Disturbances to the system occur when production of ROS is rapidly increased, for example, by excessive exercise, high exposure to a xenobiotic compounds (such as an anaesthetic, pollutants, or unusual food), infection, or trauma. Superoxide production is increased by activation of NADPH oxidases in inflammatory cells or after the production of xanthine oxidase, which follows ischaemia. The degree of damage resulting from the temporary imbalance depends on the ability of the antioxidant systems to respond to the oxidant or pro-oxidant load. Fruits and vegetables are good sources of many antioxidants, and it is reported that diets rich in these foods are associated with a lower risk of the chronic diseases of cancer (*15*) and heart disease (*16*). Hence, it is believed that a healthful diet maintains the exogenous antioxidants at or near optimal levels thus reducing the risk of tissue damage. The most prominent representatives of dietary antioxidants are vitamin C, tocopherols, carotenoids, and flavonoids (*17-19*). Requirements for flavonoids are not being considered at this time and work on this subject is still very much in its infancy. In contrast, several intervention studies have been carried out to determine whether supplements of the other nutrients can provide additional benefits against such diseases.

The components in biologic tissues make an ideal mixture of substrates for oxidation. Polyunsaturated fatty acids (PUFAs), oxygen, and transition metals are present in abundance but are prevented from reaction by cellular organisation and structure. PUFAs are present in membranes but are always found with vitamin E. Transition metals, particularly iron, are bound to both transport and storage proteins; abundant binding sites on such proteins prevent overloading the protein molecule with metal ions. Tissue structures, however, break down during inflammation and disease, and free iron and other transition metals have been detected (*20, 21*). Potentially damaging metabolites can arise from interactions between transition metals and the ROS described above. In particular the highly reactive hydroxyl radical can be formed by the Fenton (*reaction 1*) and Haber-Weiss reactions (*reaction 2*; with an iron-salt catalyst) (*22*). Pathologic conditions greatly increase the concentrations of both superoxide and nitric oxide, and the formation of peroxynitrite has been demonstrated in macrophages, neutrophils, and cultured endothelium (*reaction 3*) (*12, 23*). Peroxynitrite can react through several different mechanisms, including the formation of an intermediate with the reactivity of the hydroxyl radical (12).

$$Fe^{2+} + H_2O_2 = Fe^{3+} + OH^{\cdot} + OH^{-} \qquad 1$$

$$O_2^{-\cdot} + H_2O_2 = O_2 + OH^{\cdot} + OH^{-} \qquad 2$$

$$NO + O_2^{-\cdot} = ONOO^{\cdot} \qquad 3$$

During inflammation or other forms of stress and disease, new measures are adopted by the body to counter potential pro-oxidant damage. The body alters the transport and distribution of iron by blocking iron mobilisation and absorption and stimulating iron uptake from plasma by liver, spleen, and macrophages (*3, 24, 25*). Nitric oxide has been shown to play a role in the coordination of iron traffic by mimicking the consequences of iron starvation and leading to the cellular uptake of iron (*26*). The changes accompanying disease are generally termed the acute-phase response and are, generally, protective (*27*). Some of the changes in plasma acute-phase reactants which affect iron at the onset of disease or trauma are shown in ***Table 57***.

Table 57

Systems altered in disease which reduce risk of autoxidation

System	Changes in plasma	Physiologic objectives
Mobilisation and metabolism of iron	Decrease in transferrin Increase in ferritin Increase in lactoferrin Increase in haptoglobin Decrease in iron absorption Movement of plasma iron from blood to storage sites.	Reduce levels of circulating and tissue iron to reduce risk of free radical production and pro-oxidant damage. Reduce level of circulating iron available for microbial growth.
Positive acute phase proteins	Increase in antiproteinases Increase in fibrinogen	Restriction of inflammatory damage to diseased area.
White blood cells	Variable increase in white blood cells of which 70% are granulocytes.	Production of reactive oxygen species to combat infection. Scavenge vitamin C to prevent interaction of vitamin C with free iron.
Vitamin C metabolism	Uptake of vitamin C from plasma by stimulated granulocytes. Reduction of plasma vitamin C in acute and chronic illness or stress-associated conditions. Temporary fall in leukocyte vitamin C associated with acute stress.	Reduce levels of vitamin C in the circulation – because it is a potential pro-oxidant in inflamed tissue – or where free iron may be present. Facilitate movement of vitamin C to tissues affected by disease (e.g., lungs in smokers). Protect granulocytes and macrophages from oxidative damage.

Source: Modified from Koj (*28*) and Thurnham (*3, 29, 30*).

Pro-oxidant activity of biologic antioxidants

Most biologic antioxidants are antioxidants because when they accept an unpaired electron, the free radical intermediate formed has a relatively long half-life in the normal biologic environment. The long half-life means that these intermediates remain stable for long enough to interact in a controlled fashion with intermediates which prevent autoxidation, and the excess energy of the surplus electron is dissipated without damage to the tissues. Thus it is believed that the tocopheroxyl radical formed by oxidation of α-tocopherol is sufficiently stable to enable its reduction by vitamin C or GSH to regenerate the quinol (*31, 32*) rather than oxidizing surrounding PUFAs. Likewise the oxidized forms of vitamin C, the ascorbyl free radical and dehydroascorbate, may be recycled back to ascorbate by GSH or the enzyme dehydroascorbate reductase (*13*). The ability to recycle these dietary antioxidants may be an indication of their physiologic essentiality to function as antioxidants.

Carotenoids are also biologic antioxidants but their antioxidant properties very much depend on oxygen tension and concentration (*33, 34*). At low oxygen tension β-carotene acts as a chain-breaking antioxidant whereas at high oxygen tension it readily autoxidizes and exhibits pro-oxidant behaviour (*33*). Palozza (*34*) reviewed much of the evidence and suggests that β-carotene has antioxidant activity between 2 and 20 mmHg of oxygen tension, but at the oxygen tension in air or above (>150 mmHg) it is much less effective as an antioxidant and can show pro-oxidant activity as the oxygen tension increases. Palozza (*34*) also suggests that autoxidation reactions of β-carotene may be controlled by the presence of other antioxidants (e.g., vitamins E and C) or other carotenoids. There is some evidence that large supplements of fat-soluble nutrients such as β-carotene and other carotenoids may compete with each other during absorption and lower plasma concentrations of other nutrients derived from the diet. However, a lack of other antioxidants is unlikely to explain the increased incidence of lung cancer in the α-tocopherol β-carotene intervention study, because there was no difference in cancer incidence between the group which received both β-carotene and α-tocopherol and the groups which received one treatment only (*35*).

The free radical formed from a dietary antioxidant is potentially a pro-oxidant as is any other free radical. In biologic conditions which might deviate from the norm, there is always the potential for an antioxidant free radical to become a pro-oxidant if a suitable receptor molecule is present to accept the electron and promote the autoxidation (*36*). Mineral ions are particularly important pro-oxidants. For example, vitamin C will interact with both copper and iron to generate cuprous or ferrous ions, respectively, both of which are potent pro-oxidants (*29, 37*). Fortunately, mineral ions are tightly bound to proteins and are usually unable to react with tissue components unless there is a breakdown in tissue integrity. Such circumstances can occur in association with disease and excessive phagocyte activation, but even under these circumstances there is rapid metabolic accommodation in the form of the acute-phase response to minimise the potentially damaging effects of an increase in free mineral ions in extra-cellular fluids (***Table 57***).

Nutrients associated with endogenous antioxidant mechanisms

Both zinc and selenium are intimately involved in protecting the body against oxidant stress. Zinc combined with copper is found in the cytoplasmic form of SOD whereas zinc and magnesium occur in the mitochondrial enzyme. SOD occurs in all aerobic cells and is responsible for the dismutation of superoxide (*reaction 4*):

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ = H_2O_2 + O_2 \qquad 4$$

Hydrogen peroxide produced as a product of dismutation reaction is removed by GPx of which selenium is an integral component (*reaction 5*). To function effectively, this enzyme also needs a supply of hydrogen, which it obtains from GSH. Cellular concentrations of GSH are maintained by the riboflavin-dependent enzyme glutathione reductase.

$$H_2O_2 + 2GSH = GSSG + 2H_2O \qquad 5$$

Four forms of selenium-dependent GPx have been described which have different activities in different parts of the cell (*38*). In addition, a selenium-dependent thyrodoxin reductase was recently characterised in human thyrocytes. Thyrodoxin reductase may be particularly important to the thyroid gland because it can cope with higher concentrations of peroxide and hydroperoxides generated in the course of thyroid hormone synthesis better than can GPx (*39*). It is suggested that in combination with iodine deficiency, the inability to remove high concentrations of hydrogen peroxide may cause atrophy in the thyroid gland, resulting in myxedematous cretinism (*39*).

SOD and GPx are widely distributed in aerobic tissues and, if no catalytic metal ions are available, endogenously produced superoxide and hydrogen peroxide at physiologic concentrations may have limited, if any, damaging effects (*36*). SOD and GPx are of fundamental importance to the life of the cell, and their activity is not readily reduced by deficiencies in dietary intake of these nutrients. In contrast, enzyme activity can be stimulated by increased oxidant stress (e.g., ozone) (*40*). Activities of zinc-dependent enzymes have been shown to be particularly resistant to the influence of dietary zinc (*41*), and although erythrocyte GPx activity correlates with selenium when the intake is below 60–80 μg/day (*42*), there is no evidence of impaired clinical function at low GPx activities found in humans. Nevertheless, one selenium intervention study reported remarkably lower risks of several cancers after 4.5 years of selenium at 200 μg/day (*43*). The effects were so strong on total cancer mortality that the study was stopped prematurely. However, the subjects were patients with a history of basal or squamous cell carcinomas and were not typical of the general population. In addition, a prospective analysis of serum selenium in cancer patients (*44*) (1.72 μmol/L) found very little difference from concentrations in matched controls (1.63 μmol/L) although the difference was significant (*45*). Furthermore, areas with high selenium intakes have a lower cancer incidence than do those with low intakes, but the high selenium areas were the least industrialized (*45*).

Nutrients with radical-quenching properties

Vitamins C and E are the principal nutrients which possess radical-quenching properties. Both are powerful antioxidants, and the most important difference between these two compounds stems from their different solubility in biologic fluids. Vitamin C is water soluble and is therefore especially found in the aqueous fractions of the cell and in body fluids whereas vitamin E is highly lipophilic and is found in membranes and lipoproteins.

Vitamin E

Vitamin E falls into the class of conventional antioxidants which are generally phenols or aromatic amines (see ***Chapter 9***). In the case of the four tocopherols that together constitute vitamin E, the initial step involves a very rapid transfer of phenolic hydrogen to the recipient free radical with the formation of a phenoxyl radical from vitamin E. The phenoxyl radical is resonance stabilised and is relatively unreactive towards lipid or oxygen. It does not therefore continue the chain (*33, 46*). However, the phenoxyl radical is no longer an antioxidant and to maintain the antioxidant properties of membranes, it must be recycled or repaired – that is,

reconverted to vitamin E – because the amount of vitamin E present in membranes can be several thousand-fold less than the amount of potentially oxidizable substrate (*47*). Water-soluble vitamin C is the popular candidate for this role (*31*), but thiols and particularly GSH can also function *in vitro* (*32, 48-50*).

There are eight possible isomers of vitamin E, but α-tocopherol (5,7,8 trimethyl tocol) is the most biologically important antioxidant *in vivo* (*46*). In plasma samples, more than 90 percent is present as α-tocopherol but there may be approximately 10 percent of γ-tocopherol. In foods such as margarine and soy products the γ form may be predominant and palm oil products are rich in the tocotrienols.

Vitamin E is found throughout the body in both cell and sub-cellular membranes. It is believed to be orientated with the quinol ring structure on the outer surface (i.e., in contact with the aqueous phase) to enable it to be maintained in its active reduced form by circulating reductants such as vitamin C (*31*). Within biologic membranes, vitamin E is believed to intercalate with phospholipids and provide protection to PUFAs. PUFAs are particularly susceptible to free radical–mediated oxidation because of their methylene-interrupted double-bond structure. The amount of PUFAs in the membrane far exceeds the amount of vitamin E, and the tocopherol-PUFAs ratios are highest in tissues where oxygen exposure is greatest and not necessarily where the PUFAs content is highest (*47*).

Oxidation of PUFAs leads to disturbances in membrane structure and function and is damaging to cell function. Vitamin E is highly efficient at preventing the autoxidation of lipid and it appears as if the primary, and possibly only, role in biologic tissues is to provide this function (*46*). Autoxidation of lipid is initiated by a free radical abstracting hydrogen from PUFA to form a lipid radical (*reaction 6*) which is followed by a rearrangement of the double-bond structure to form a conjugated diene. *In vitro* the presence of minute amounts of peroxides and transition metals will stimulate the formation of the initial radical. Oxygen adds to the lipid radical to form a lipid peroxide (*reaction 7*) which then reacts with another lipid molecule to form a hydroperoxide and a new lipid radical (*reaction 8*). This process is shown in general terms below for the autoxidation of any organic molecule (RH), where the initial abstraction is caused by a hydroxyl radical ($OH^{\cdot}$).

$$RH + OH^{\cdot} = R^{\cdot} + H_2O \qquad 6$$

$$R^{\cdot} + O_2 = ROO^{\cdot} \qquad 7$$

$$ROO^{\cdot} + RH = ROOH + R^{\cdot} \qquad 8$$

Autoxidation or lipid peroxidation is represented by reactions 6 and 7. The process stops naturally when reaction between two radicals (*reaction 9*) occurs more frequently than does reaction 8.

$$ROO^{\cdot} + ROO^{\cdot} = \text{non-radical products} \qquad 9$$

The presence of the chain-breaking antioxidant, vitamin E (ArOH), reacts in place of RH shown in reaction 8 and donates the hydrogen from the chromanol ring to form the hydroperoxide (*reaction 10*). The vitamin E radical ($ArO^{\cdot}$, tocopheroxyl radical) which is formed is fairly stable and therefore stops autoxidation. Hydroperoxides formed by lipid peroxidation can be released from membrane phospholipids by phospholipase A2 and then degraded by GPx in the cell cytoplasm (*see* ***Chapter 15***).

$$ROO^{\cdot} + ArOH = ArO^{\cdot} + ROOH \qquad 10$$

Vitamin C

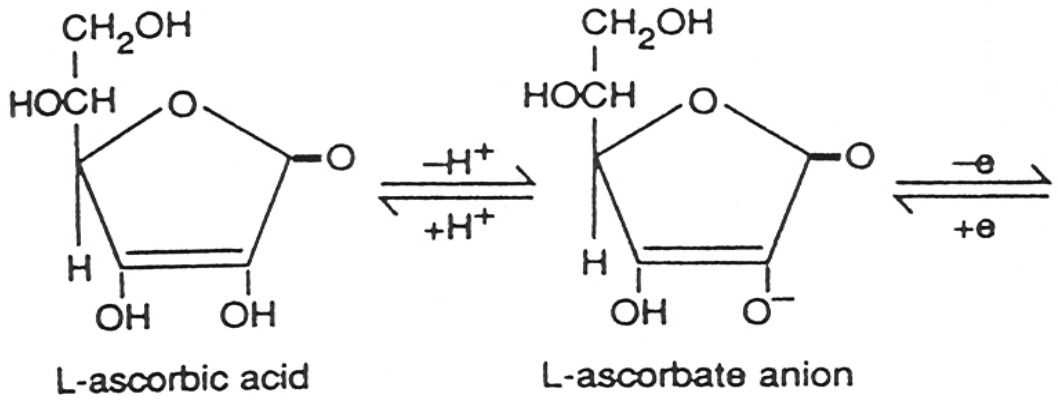
Many, if not all of the biologic properties of vitamin C are linked to its redox properties (see ***Chapter 6***). For example, essential defects in scurvy such as the breakdown of connective tissue fibres (*51*) and muscular weakness (*52*) are both linked to hydroxylation reactions in which ascorbate maintains loosely bound iron in the ferrous form to prevent its oxidation to the ferric form, which makes the hydroxylase enzymes inactive (*53*). Ascorbate exhibits similar redox functions in catecholamine biosynthesis (*53*) and in microsomal cytochrome P450 enzyme activity, although the latter may only be important in young animals (*54*). In the eye, vitamin C concentrations may be 50 times higher than in the plasma and may protect against the oxidative damage of light (*55*). Vitamin C is also present in the gonads, where it may play a critical role in sperm maturation (*56*). Spermatogenesis involves many more cell divisions than does oogenesis, resulting in an increased risk of mutation. Fraga *et al.* (57) reported that levels of sperm oxidized nucleoside 8-OH-2'-deoxyguanosine (an indicator of oxidative damage to DNA) varied inversely with the intake of vitamin C (5-250 mg/day). No apparent effects on sperm quality were noted. Frei (*58*) also showed that vitamin C was superior to all other biologic antioxidants in plasma in protecting lipids exposed *ex vivo* to a variety of sources of oxidative stress. The importance of vitamin C in stabilising various plasma components such as folate, homo-cysteine, proteins, other micronutrients, *etc*. has not been properly evaluated. When blood plasma is separated from erythrocytes, vitamin C is the first antioxidant to disappear.

Vitamin C is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical (***Figure 27***). As a scavenger of ROS, ascorbate has been shown to be effective against the superoxide radical anion, hydrogen peroxide, the hydroxyl radical, and singlet oxygen (*59, 60*). Vitamin C also scavenges reactive nitrogen oxide species to prevent nitrosation of target molecules (*61*). The ascorbyl free radical can be converted back to reduced ascorbate by accepting another hydrogen atom or it can undergo further oxidation to dehydroascorbate. Dehydroascorbate is unstable but is more fat soluble than ascorbate and is taken up 10–20 times more rapidly by erythrocytes, where it will be reduced back to ascorbate by GSH or NADPH from the hexose monophosphate shunt (*56*).

Thus, mechanisms exist to recycle vitamin C similarly to those for vitamin E. The existence of a mechanism to maintain plasma ascorbate in the reduced state means that the level of vitamin C necessary for optimal antioxidant activity is not absolute because the turnover will change in response to oxidant pressure. Recycling of vitamin C will depend on the reducing environment which exists in metabolically active cells. In atrophic tissues or tissues exposed to inflammation, cell viability may fail and with it the ability to recycle vitamin C. In such an environment, the ability of newly released granulocytes (*62*) or macrophages (*63*) to scavenge vitamin C from the surrounding fluid may be invaluable for conservation of an essential nutrient as well as reducing the risk of ascorbate becoming a pro-oxidant through its ability to reduce iron (*37*).

Figure 27

Vitamin C can donate a hydrogen atom and form a relatively stable ascorbyl free radical



β-*Carotene and other carotenoids*

Many hundreds of carotenoids are found in nature but relatively few are found in human tissues, the five main ones being β-carotene, lutein, lycopene, β-cryptoxanthin, and α-carotene (*17, 18, 64*). β-Carotene is the main source of pro-vitamin A in the diet. There are approximately 50 carotenoids with pro-vitamin A activity, but β-carotene is the most important and is one of the most widely distributed carotenoids in plant species (*64*). Approximately 2–6 mg β-carotene is consumed by adults daily in developed countries (*65, 66*) and similar amounts of lutein (*67*) and lycopene (*66*) are probably also consumed. Smaller amounts may be consumed in the developing world (*68, 69*). β-Cryptoxanthin is a pro-vitamin A carotenoid which is found mainly in fruits (*66*). Consumption is small but bio-availability of carotenoids may be greater from fruit than vegetable, so its contribution to dietary intake and vitamin A status may be higher than the amount in the diet would predict.

β-Carotene has the general structure of this group of compounds and has two 6-membered carbon rings (β-ionone rings) separated by 18 carbon atoms in the form of a conjugated chain of double bonds. The latter is responsible for the antioxidant properties of the molecule (*33, 70, 71*). β-Carotene is unique in possessing two β-ionone rings in its molecule which are essential for vitamin A activity. The chemical properties of the carotenoids closely relate to the extended system of conjugated double bonds, which occupies the central part of carotenoid molecules, and various functional groups on the terminal ring structures. The ROS scavenged by carotenoids are singlet oxygen and peroxyl radicals (*33, 72-74*). Carotenoids in general and lycopene specifically are very efficient at quenching singlet oxygen (*72, 73*). In this process the carotene absorbs the excess energy from singlet oxygen and then releases it as heat. Singlet oxygen is generated during photosynthesis; therefore, carotenoids are important for protecting plant tissues, but there is limited evidence for this role in humans. However, ß-carotene has been used in the treatment of erythropoietic protoporphyria (*75*), which is a light-sensitive condition which in some persons respond to treatment with amounts of β-carotene (in excess of 180 mg/day) (*76*). It has been suggested that large amounts of dietary carotenes may provide some protection against solar radiation but results are equivocal. No benefit was reported when large amounts of β-carotene were

used to treat persons with a high risk of non-melanomatous skin cancer (*77*). However, two carotenoids – lutein (3,3'-dihydroxy α-carotene) and zeaxanthin (the 3,3'-dihydroxylated form of β-carotene) – are found specifically associated with the rods and cones in the eye (*78*) and may protect the retinal pigment epithelium against the oxidative effects of blue light (*79, 80*).

Burton and Ingold (*33*) were the first to draw attention to the radical-trapping properties of β-carotene. Using *in vitro* studies, they showed that β-carotene was effective in reducing the rate of lipid peroxidation at the low oxygen concentrations found in tissues. Because all carotenoids have the same basic structure, they should all have similar properties. Indeed, several authors suggest that the hydroxy-carotenoids are better radical-trapping antioxidants than is β-carotene (*81, 82*). It has also been suggested that because the carotenoid molecule is long enough to span the bilayer lipid membrane (*83*), the presence of oxy functional groups on the ring structures may facilitate similar reactivation of the carotenoid radical in a manner similar to that of the phenoxyl radical of vitamin E (*33*).

There is some evidence for an antioxidant role for β-carotene in immune cells. Bendich (84) suggested that β-carotene protects phagocytes from auto-oxidative damage; enhances T and B lymphocyte proliferative responses; stimulates effector T cell function; and enhances macrophage, cytotoxic T cell, and natural killer cell tumoricidal capacity. Some data are in conflict with evidence of protective effects on the immune system (*85, 86*) and other data have found no effect (*87*). An explanation for the discrepancy may reside in the type of subjects chosen. Defences may be boosted in those at risk but it may not be possible to demonstrate any benefit in healthy subjects (*88*).

A requirement for antioxidant nutrients

Free radicals are a product of tissue metabolism, and the potential damage which they can cause is minimised by the antioxidant capacity and repair mechanisms within the cell. Thus in a metabolically active tissue cell in a healthy subject with an adequate dietary intake, damage to tissue will be minimal and most of the damage occurring will be repaired (*36*). An important dietary source of antioxidant nutrients is the intake of fruit and vegetables, and it is now well established that persons consuming generous amounts of these foods have a lower risk of chronic disease than do those whose intake is small (*15, 16, 89*). These observations suggest that the antioxidant nutrient requirements of the general population can be met by a generous consumption of fruit and vegetables and the slogan "5 portions a day" has been promoted to publicize this idea (*90*).

Occasionally, damage may occur which is not repaired and the risk of this happening may increase in the presence of infection or physical trauma. Such effects may exacerbate an established infection or may initiate irreversible changes leading to a state of chronic disease (e.g., a neoplasm or atherosclerotic lesions). Can such effects also be minimised by a generous intake of dietary antioxidants in the form of fruit and vegetables or are supplements needed?

It is generally recognised that certain groups of people have an increased risk of free radical - initiated damage. Premature infants, for example, are at increased risk of oxidative damage because they are born with immature antioxidant status (*91-93*) and this may be inadequate for coping with high levels of oxygen and light radiation. People who smoke are exposed to free radicals inhaled in the tobacco smoke and have an increased risk of many diseases. People abusing alcohol need to develop increased metabolic capacity to handle the extra alcohol load. Similar risks may be faced by people working in environments where there are elevated levels of volatile solvents (e.g., petrol and cleaning fluids, in distilleries, chemical plants, *etc*.). Car drivers and other people working in dense traffic may be exposed to elevated

levels of exhaust fumes. Human metabolism can adapt to a wide range of xenobiotic substances, but metabolic activity may be raised with the consequent production of more ROS which are potentially toxic to cell metabolism.

Of the above groups, smokers are the most widely accessible people and this has made them a target for several large antioxidant-nutrient intervention studies. In addition, smokers often display low plasma concentrations of carotenoids and vitamin C. However, no obvious benefits to the health of smokers have emerged from these studies and, in fact, β-carotene supplements were associated with an increased risk of lung cancer in two separate studies (*35, 94*) and with more fatal cardiac events in one of them (*95*). Other risk groups identified by their already having had some non-malignant form of cancer, such as non-melanomatous skin cancer (*77*) or a colorectal adenoma (*96*), showed no effect on subsequent recurrences after several years of elevated intakes of antioxidant nutrients. The use of β-carotene (77) or vitamin E alone or in combination with vitamin C (96) showed no benefits. Thus, the results of these clinical trials do not support the use of supplementation with antioxidant micronutrients as a means of reducing cancer or even cardiovascular rates although in the general population, toxicity from such supplements is very unlikely.

Some intervention trials however have been more successful in demonstrating a health benefit. Stitch and colleagues (*97, 98*) gave large quantities of β-carotene and sometimes vitamin A to chewers of betel quids in Kerala, India, and to Canadian Inuits with pre-malignant lesions of the oral tract and showed reductions in leukoplakia and micronuclei from the buccal mucosa. Blot and colleagues (*99*) reported a reduction (13 percent) in gastric cancer mortality in people living in Linxian Province, People's Republic of China, after a cocktail of β-carotene, vitamin E, and selenium. These studies are difficult to interpret because the subjects may have been marginally malnourished at the start and the supplements may have merely restored nutritional adequacy. However, correcting malnutrition is unlikely to be the explanation for the highly successful selenium supplementation study of US patients with a history of basal or squamous cell cancers of the skin (*43*). Interestingly, the intervention with 200 μg/day of selenium for an average of 4.5 years had no effect on the recurrence of the skin neoplasms (relative risk [RR]1.10, confidence interval 0.95–1.28). However, analysis of secondary endpoints showed significant reductions in total cancer mortality (RR 0.5) and incidence (RR 0.63) and in the incidences of lung, colorectal, and prostate cancers. The mean age of this group was 63 years and obviously they were not a normal adult population, but results of further studies are awaited with keen interest. Lastly, results of the Cambridge Heart Antioxidant Study should be mentioned because they provide some support for a beneficial effect of vitamin E in persons who have had a myocardial infarction (*100*). Recruits to the study were randomly assigned to receive vitamin E (800 or 400 mg/day) or placebo. Initial results of the trial suggested a significant reduction in non-fatal myocardial infarctions but a non-significant excess of cardiovascular deaths (*100*).The trial officially ended in 1996, but mortality has continued to be monitored and the authors now report significantly fewer deaths in those who received vitamin E for the full trial (*101*) (*see* ***Chapter 9***).

In conclusion, some studies have shown that health benefits can be obtained by some people with increased risk of disease from supplements of antioxidant nutrients. The amounts of supplements used have, however, been large and the effect possibly has been pharmacologic. Further work is needed to show whether more modest increases in nutrient intakes in healthy adult populations will delay or prevent the onset of chronic disease. The evidence available regarding health benefits to be achieved by increasing intakes of antioxidant nutrients does not assist in setting nutrient requirements.

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