

8. Dietary antioxidants

8.1 Nutrients with an antioxidant role

The potential beneficial effects of 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 physiological function in humans (1). It is recognized 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.

8.2 The need for biological antioxidants

It is now well established that free radicals, especially superoxide ($O_2^{\cdot-}$), nitric oxide (NO^{\cdot}), and other reactive species such as hydrogen peroxide (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 microorganisms (9). Nitric oxide is produced from L-arginine by nitric oxide synthases, and these enzymes are

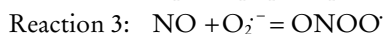
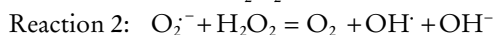
found in virtually every tissue of the mammalian body, albeit at widely different levels (7). Nitric oxide is a free radical but is believed to be essentially a beneficial metabolite and indeed it may react with lipid peroxides and function as an antioxidant (10). Nitric oxide also serves as a mediator whereby macrophages express cytotoxic activity against microorganisms 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 through a reaction with a 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 as well as several exogenously acquired radical-scavenging substances such as vitamins E and C and the carotenoids (13). Under normal conditions, the high concentrations of SOD maintain superoxide concentrations at a level 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 synthesized 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 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, as 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 diseases such as those mentioned above.

The components of biological tissues are an ideal mixture of substrates for oxidation. Polyunsaturated fatty acids (PUFAs), transition metals, and oxygen are present in abundance but are prevented from reaction by cellular organization 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 during these periods (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).



During inflammation or other forms of stress and disease, the body adopts new measures to counter potential pro-oxidant damage. The body alters the transport and distribution of iron by blocking iron mobilization 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 8.1.

8.3 Pro-oxidant activity of biological antioxidants

Most biological antioxidants are antioxidants because when they accept an unpaired electron, the free radical intermediate formed has a relatively long half-life in the normal biological environment. The long half-life means that these intermediates remain stable for long enough to interact in a controlled fashion with other 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

TABLE 8.1
Systems altered during disease which reduce risk of autoxidation

System	Changes in plasma	Physiologic objectives
Mobilization 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 antiproteinasases 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

Sources: modified from Koj (28) and Thurnham (3, 29, 30).

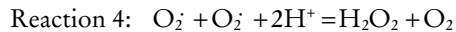
GSH to regenerate the quinol (31, 32) rather than oxidizing surrounding PUFAs. Similarly, the oxidized forms of vitamin C, the ascorbyl free radical and dehydroascorbate, may be recycled back to ascorbate by GSH or the enzyme dehydroascorbate reductase (13). The ability to recycle these dietary antioxidants may be an indication of their physiological essentiality to function as antioxidants.

The biological antioxidant properties of the carotenoids depend very much 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) has reviewed much of the evidence and has suggested 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 suggested 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 intake of large quantities of fat-soluble nutrients such as β -carotene and other carotenoids may cause them to 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 that was observed in a 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).

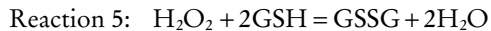
The free radical formed from a dietary antioxidant is potentially a pro-oxidant as is any other free radical. In biological conditions that deviate from the norm, there is always the potential for an antioxidant free radical to become a pro-oxidant if a suitable receptor molecule is present to accept 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 minimize the potentially damaging effects of an increase in free mineral ions in extracellular fluids (Table 8.1).

8.4 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. Superoxide dismutase is present in all aerobic cells and is responsible for the dismutation of superoxide (reaction 4).



Hydrogen peroxide is a product of this dismutation reaction and 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 reduced glutathione (GSH). Cellular concentrations of GSH are maintained by the riboflavin-dependent enzyme glutathione reductase.



Four forms of selenium-dependent GPx have been described, each with different activities in different parts of the cell (38). In addition, a selenium-dependent enzyme, thioredoxin reductase, was recently characterized in human erythrocytes. Thioredoxin 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 has been 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 physiological concentrations probably 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 zinc and selenium. 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 in subjects taking supplements for 4.5 years at doses of 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 (43). In addition, a prospective analysis of serum selenium in cancer patients (44) ($1.72 \mu\text{mol/l}$) found very little difference from concentrations in matched controls ($1.63 \mu\text{mol/l}$) 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).

8.5 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 biological 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.

8.5.1 Vitamin E

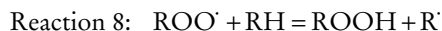
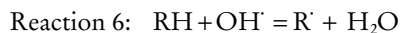
Vitamin E falls into the class of conventional antioxidants which generally consist of phenols or aromatic amines (see Chapter 5). In the case of the four tocopherols that, together with the four tocotrienols constitute vitamin E, the initial step involves a very rapid transfer of phenolic hydrogen to the recipient free radical with the formation of a phenoxyl radical from vitamin E. The phenoxyl radical is resonance stabilized 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 (i.e. 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 this role in vitro (32, 48–50).

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

Vitamin E is found throughout the body in both cell and subcellular 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 biological 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-PUFA ratios are highest in tissues where oxygen exposure is greatest and not necessarily where the PUFA 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 that its primary, and possibly only, role in biological tissues is to perform 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).



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



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 10 on selenium).



8.5.2 Vitamin C

Many, if not all of the biological properties of vitamin C are linked to its redox properties (see Chapter 7). For example, the consequences of 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 by 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 biological antioxidants in plasma in protecting lipids exposed *ex vivo* to a variety of sources of oxidative stress. The importance of vitamin C in stabilizing various plasma components such as folate, homocysteine, proteins and other micronutrients 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 (i.e. L-ascorbate anion, see Figure 8.1). 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, which are similar 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

of which are essential for vitamin A activity. The antioxidant properties of the carotenoids closely relate to the extended system of conjugated double bonds, which occupies the central part of carotenoid molecules, and to the various functional groups on the terminal ring structures (33, 70, 71). The reactive oxidant species scavenged by carotenoids are singlet oxygen and peroxy radicals (33, 72–74). Carotenoids in general and lycopene specifically are very efficient at quenching singlet oxygen (72, 73). In this process the carotenoid 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 some evidence for an antioxidant role in humans. β -Carotene has been used in the treatment of erythropoietic protoporphyria (75) (a light-sensitive condition) with amounts 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 individuals 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 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. However, there are data which conflict with the evidence of the protective effects of β -carotene on the immune system (85, 86) and other data which 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).

8.6 A requirement for antioxidant nutrients

Free radicals are a product of tissue metabolism, and the potential damage which they can cause is minimized 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, if it does occur, will be repaired (36). Fruit and vegetables are an important dietary source of antioxidant nutrients, and it is now well established that individuals consuming generous amounts of these foods have a lower risk of chronic disease than 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 “five portions a day” has been promoted to publicize this idea (90).

Occasionally, free radical 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 be minimized by a generous intake of dietary antioxidants in the form of fruit and vegetables or are supplements needed?

It is generally recognized 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 and chemical plants). 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 as a result, leading to the consequent production of more ROS, which are potentially toxic to cell metabolism.

Of the above groups, smokers are the most widely accessible 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). There was no effect on subsequent disease recurrences among 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)—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. Stich 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 witnessed reductions in leukoplakia and micronuclei from the buccal mucosa. Blot et al. (99) reported a 13% reduction in gastric cancer mortality in people living in Linxian Province, People's Republic of China, after taking 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 positive results of a selenium supplementation study conducted in the United States in patients with a history of basal or squamous cell cancers of the skin (43). Interestingly, the intervention with 200 μ g/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 end-points 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. In addition, results of the Cambridge Heart Antioxidant Study have provided some support for a beneficial effect of vitamin E in individuals who have had a myocardial infarction (100). Recruits to the study were randomly assigned to receive vitamin E (800 or 400 mg/day) or a 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 5 on vitamin E). However, very recently results from the Medical Research Council/British Heart Foundation intervention study in 20 536 patients with heart disease were reported (102). Patients received vitamin E (600 mg), vitamin C (250 mg) and β -carotene (20 mg) or placebo daily for five years. There were no significant reductions in all cause mortality, or deaths due to vascular or non-vascular causes. Thus these antioxidant supplements provided no measurable health benefits for these patients.

In conclusion, some studies have shown that health benefits can be obtained by some people with an increased risk of disease from supplements of antioxidant nutrients. The amounts of supplements used, however, have 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. Therefore, the available evidence regarding health benefits to be achieved by increasing intakes of antioxidant nutrients does not assist in setting nutrient requirements.

8.7 Recommendations for future research

If nutrient intakes are ever to be recommended on the basis of antioxidant properties then more research is needed to gain a better understanding of:

- The optimal plasma or tissue concentrations of nutrients to fully support interactions between antioxidant micronutrients like vitamins E and C, or vitamin E and Se to counter oxidant stress in the tissues.
- The mechanisms whereby micronutrients like vitamins A and C, and minerals iron and zinc are reduced at the time of oxidant stress and the physiological purposes of the changes.

The minimal concentrations of antioxidant nutrients in humans to prevent conversion of benign viruses to their more virulent forms as demonstrated by Beck and colleagues in mice (103).

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