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Diet and Antioxidant Status*

A. M. PAPAS

Eastman Chemical Company, PO Box 1974, Kingsport, TN 37662-5230, USA

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Abbreviations: BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; LDL = low density lipoprotein; PG = propyl gallate; PUFA = polyunsaturated fatty acid; SOD = superoxide dismutase.

Introduction

The link of diet and chronic disease is very well documented. Poor diet combined with lack of exercise is the second leading cause of death in the United States, accounting for over 300,000 deaths every year (McGinnis and Foege, 1993). Heart disease, the leading cause of death in the United States and many industrialized countries, is greatly influenced by diet, especially by the amount and type of fat. As much as one-third of all cancers are related to our diet.

Nutritionists, dieticians and health professionals disagree on many issues related to nutrition and disease. They are, however, practically unanimous in their recommendation of diets rich in fruits and vegetables for good health and for reducing the risk of heart disease and some cancers. Many epidemiological and limited clinical studies provided the basis for this recommendation (Block *et al*., 1992; Gillman *et al*., 1995). Particularly prominent were studies that documented a striking lower incidence of heart disease and cancer in the Mediterranean countries than in northern Europe and North America (Fig. 1). The Mediterranean diet, which is rich in fruits and vegetables and low in saturated fats (Trichopoulou *et al*., 1993; Willett *et al*., 1995), appeared to account for most of the difference. Other studies documented diet-related differences in chronic disease in the people of North America and Europe as compared with people of Japan and some other Asian countries. This lower incidence of disease has been attributed, in large part, to higher consumption of soy and fish (Adlercreutz *et al*., 1995; Anderson *et al*., 1995; Daviglus *et al*., 1997; Messina, 1995) and lower consumption of meat and saturated fat (Weisburger, 1997).

The beneficial effects of fruits and vegetables and the Mediterranean diet are very likely due to many of their components such as fibre, micronutrients, and others. Antioxidants, which are among their major components, have been proposed, but not yet confirmed, as the principle active agents for reducing the risk of chronic disease (Block, 1992). For soy, the suggested beneficial agents include isoflavones such as daidzein and genistein (Adlercreutz *et al*., 1995). These are phenolic compounds with several biological functions including antioxidant activity.

Diet has a profound effect on antioxidant status and ranks among the top factors under our control. Unlike other factors such as smoking, stress and disease, that have only a prooxidant effect, diet, depending on its components, can have antioxidant or prooxidant effects.

This section will discuss the major dietary factors affecting antioxidant status. The term 'diet' in this section will include natural foods and drinks, additives and nutritional supplements.

Dietary factors affecting the antioxidant status

The overall effect of the diet, whether antioxidant or prooxidant, is determined by its components and related factors including:

- Antioxidant and prooxidant nutrient and non-nutrient components
- Absorption and bioavailability
- Food processing and storage
- Food additives and nutritional supplements
- Chemical, chiral form and formulation of addi tives and supplements.

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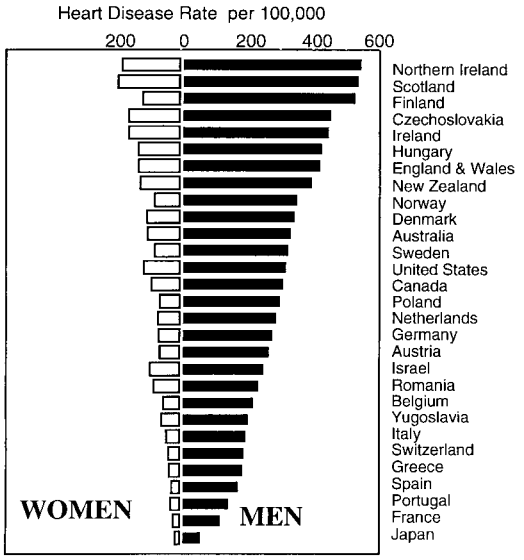

Fig. 1. Country differences in the incidence of heart disease. Diet is believed to be among the major factors accounting for these differences. (Data from the World Health Organization.)

- Some of the above factors such as the major dietary antioxidants (vitamins E and C, carotenoids, flavonoids and other phytochemicals, alcohol) are discussed in separate sections. This section will focus on the remaining factors.

Dietary antioxidants and prooxidants

Dietary antioxidants

Our diet contains antioxidants recognized as essential nutrients and others, which have antioxidant activity even though they are not essential nutrients.

Nutrient antioxidants

Nutrient antioxidants in the diet include:

- Vitamin E (tocopherols and tocotrienols)
- Vitamin C or ascorbic acid
- Vitamin A and its non-nutrient precursor β-carotene
- Nutrients essential for normal function of endogenous antioxidant systems. For example, the minerals Cu, Mn, Zn, Se, Fe and the vitamin riboflavin are important cofactors of antioxidant enzyme systems.

Non-nutrient antioxidants

The diet also contains enzymes, peptides, proteins and other compounds similar to those present in our body. Glutathione, coenzyme Q_{10}, catalases, superoxide dismutases (SOD), albumins and others are good examples. From these, small peptides, such as glutathione, may escape hydrolysis and can be absorbed intact. Larger proteins, however, such as catalases, SOD and albumins are denatured and hydrolysed in the digestive system and thus, little, if any, is absorbed in the active form.

Table 1. Major classes of phytochemicals with antioxidant activity

Class of phytochemicals	Example compounds
Carotenoids	lycopene, lutein, astaxanthin
Bioflavonoids	genistein, diadzein, quercetin
Phytosterols	sitosterol, stigmasterol, oryzanol
Tannins	catechins and other polyphenol compounds
Chlorophylls	chlorophyll A and chlorophyllin
Terpenoids	limonin and limonene
Allylic compounds	diallyl sulfide and disulfide
Indoles	indole-3-carbinol

Finally, a large number of phytochemicals, not recognized as essential nutrients, apparently play an important antioxidant role in the body. Practically all diets contain at least some phytochemicals. Fruits, vegetables and herbs are particularly rich sources (Lin, 1995). Many phytochemicals are now studied extensively for their potential role in reducing the risk and even preventing and treating some diseases (Steele *et al*., 1994). Major classes of phytochemicals with potential for antioxidant activity are listed in Table 1.

Dietary components that may function as prooxidants

Dietary components, which can be easily oxidized or can promote oxidation, have a major impact on the antioxidant status. The following have major practical significance.

Polyunsaturated fatty acids (PUFAs)

Our diets contain significant amounts of lipids such as triglycerides, phospholipids, cholesterol and others. Lipids are susceptible to attack by free radicals and their oxidation can be very damaging because, as discussed earlier, it proceeds as a chain reaction. Lipids containing PUFA are particularly prone to attack by free radicals. In recent decades, consumption of PUFA-rich vegetable oils has been increasing because saturated fats have been associated with a higher risk of heart disease and cancer (Weisburger, 1997). The ω-3 PUFA (α-linolenic, eicosapentaenoic and docohexaenoic), believed to be the beneficial agents in fish (Simopoulos, 1991), are now added to infant formula and other foods in other countries. They are also available in many countries including the United States as nutritional supplements. In the absence of appropriate antioxidants, PUFA form free radicals and can have a significant prooxidant effect leading to significant depletion of vitamin E and increased oxidation products (Brown and Wahle, 1990; Meydani, 1996; Meydani *et al*., 1991; Wander *et al*., 1996). It is important to note, however, that PUFA-rich vegetable oils are also good sources of tocopherols and/or tocotrienols and other fat-soluble phytochemicals that have antioxidant function.

Transition metals

Iron (Fe) and copper (Cu) are considered as transition metals because they have variable oxidation numbers, namely, Fe^{2+} or Fe^{3+} and Cu^{+} or Cu^{2+}. Because they can accept or donate electrons, they are major promoters of free radical reactions. The reaction of Fe^{2+} salts with H_2O_2, known as the Fenton reaction, yields Fe^{3+} and the extremely reactive hydroxyl radical ($HO^{\cdot}$). Fe^{3+} and Cu^{2+} can be reduced to Fe^{2+} and Cu^{+}, respectively, by ascorbate (vitamin C). Iron also hydrolyses lipid hydroperoxides to alkoxy and hydroxy radicals. These radicals are major initiators of lipid peroxidation.

Transition metals may act as prooxidants in the food, especially in stored fats and oils (Papas, 1993), in the digestive tract (Chadwick *et al*., 1992; Nelson, 1992; Stone and Papas, 1997) and, after absorption, in our tissues (Halliwell *et al*., 1995; Herbert *et al*., 1994; Lynch and Frei, 1993). Their practical importance, however, is generally higher in foods and the digestive tract, because absorbed Fe and Cu is almost completely bound to proteins and enzymes. For example, haemoglobin contains 55–60% of all body iron, followed by ferritin/haemosiderin with 30%, myoglobin with 10%, and transferrin with 0.1%. Fe and Cu bound to proteins and enzymes are shielded from surrounding media and do not act as prooxidants. Actually, caeruloplasmin, the major protein containing Cu, inhibits lipid peroxidation.

Absorbed Fe is carried by transferrin, a glycoprotein with high affinity for Fe^{3+}. Excess binding capacity (70–80%) of transferrin assures that concentration of free Fe^{3+} in blood is maintained at extremely low levels (less than 10^{-12} M). Inside the cell, Fe concentration is also very low and is regulated by the expression of the transferrin receptor and ferritin. Oxidative stress causes release of Fe primarily from ferritin. Superoxide and nitric oxide probably mediate this release. Iron is also released from haemoglobin and other proteins and enzymes when red blood cells are haemolysed or tissue is injured. In people suffering from the hereditary diseases idiopathic haemochromatosis and thalassaemia (haemoglobin is defective) excess unbound Fe catalyses oxidation and production of free radicals (Lynch and Frei, 1993; Stohs and Bagchi, 1995).

Faeces have high levels of bile pigments (such as bilirubin, biliverdin), which can chelate iron in a form capable of supporting the superoxide-driven Fenton reactions. In our diet, heme iron, available primarily in animal products and especially red meat, is better absorbed at rates of 10–35%. Most of our dietary Fe, however, is derived from plants in the insoluble non-heme Fe^{3+} form and commonly complexes with oxalates or phenolic compounds. In order to be absorbed, it requires reduction to the more soluble Fe^{2+} ion. This is achieved by the action of the stomach hydrochloric acid, which aids solubilization of Fe^{3+} and thus allows reducing agents, such as vitamin C, to reduce it to the Fe^{2+} form. Thus, vitamin C and other organic acids such as lactic and citric, enhance the absorption of Fe (non-heme Fe is absorbed at rates ranging from 2 to 20%).

This effect is highly desirable when dietary Fe is low and has low bioavailability (non-heme form) especially for menstruating women, and children, who have high nutritional requirements. If, however, the amount of dietary Fe is high, the effect of vitamin C and other reducing agents may be harmful because it may lead to excessive Fe absorption. There is serious concern that in people suffering from idiopathic haemochromatosis, thalassaemia, or other conditions causing iron overload, vitamin C may have a prooxidant effect by further increasing absorption, but may also cause ferritin to release Fe^{2} (Herbert *et al*., 1994). The role of vitamin C, however, as a prooxidant even in conditions of iron overload, remains highly controversial. Specifically it is suggested that, at high serum levels, reduced ascorbic acid drives through the pores of the ferritin protein shell to the inside surface, where it converts the Fe^{2} to catalytic Fe^{3}, which then leaks out of the pores and generates free radicals (Herbert *et al*., 1996). In addition, it was suggested that high levels of vitamin C inhibit the antioxidant activities of caeruloplasmin and, specifically, the conversion of Fe^{2} to Fe^{3}. Other researchers, however, reported results showing that this is unlikely; however, oral supplementation with vitamin C can raise plasma levels sufficiently to inhibit caeruloplasmin (Berger *et al*., 1997). They also dispute the prooxidant effect of vitamin C *in vivo*, even in conditions of Fe overload (Gutteridge, 1991).

While this discussion focused on Fe, the same considerations apply to Cu and other transition metals. Because Cu levels in our body and diet are much lower than Fe, its practical significance as prooxidant may be lower, even though Cu is more reactive. The adult male body contains about 4.5 g Fe and only about 75 mg Cu. The daily Fe recommended daily dose (RDA) for adults is 10–15 mg and only 1.5–3.0 mg for Cu. It is estimated that 1 mg Fe is absorbed daily. A large part of dietary iron is not absorbed and is concentrated in faeces at a level calculated to be 10-fold greater than in most tissues.

We proposed that the antioxidant status of the digesta has a direct effect on the production of free radicals and possibly the development of colon cancer (Stone and Papas, 1997). The amount of Fe and its ionic form are important considerations. In addition to vitamin C, flavonoids and superoxide ions reduce Fe^{3+}, thus increasing the lipid peroxidation and other harmful reactions. Other phytochemicals, such as tannins, bind metals including Fe and reduce their absorption. Vitamin C appears to

counteract the inhibition of phenolic compounds (Siegenberg *et al*., 1991).

Phytochemicals

Many phytochemicals have antioxidant properties (Cao *et al*., 1997; Lin, 1995), which are discussed in several other sections. Phytochemicals may also have prooxidant effects. For example, phenolic flavonoids, which have been shown to have lipid antioxidant properties, can also form hydroxyl radicals either by autoxidation or when complexed with metals such as Cu or Fe (Cao *et al*., 1997).

Some phytochemicals affect the antioxidant status directly, others indirectly, and some may have both direct and indirect effects. For example, phytates, tannins, and other phenolic compounds may have a direct antioxidant effect by scavenging free radicals. They also bind metals such as Fe, Cu, Zn, Mn and reduce their absorption (Tuntawiroon *et al*., 1991). It has been suggested that the effect of phenolic compounds in Fe absorption depends on their content of iron-binding galloyl groups, whereas the phenolic catechol groups seem to be of minor importance (Brune *et al*., 1989). If such binding causes a deficiency of these essential antioxidant enzyme cofactors the net effect would be prooxidant. If, however, there is sufficient amount for optimal enzymatic activity, binding of the Fe and Cu will prevent them from acting as prooxidants.

Food processing

Storage, handling, processing and cooking of food can result in oxidative damage. The degree of damage and the resulting oxidation products depend on a variety of factors including temperature, exposure to light and air, amount and saturation of lipid material, presence of oxidation promoting metals, and other factors (Halliwell *et al*., 1995; Papas, 1993). Peroxides and prooxidants in processed foods affect antioxidant status.

Processing has a major effect on absorption and bioavailability. Carotenoids provide excellent examples. Absorption of lycopene from fresh tomatoes and β-carotene from fresh carrots is significantly lower than from tomato juice or cooked carrots and carrot juice. Heat processing of tomato and carrot juice further increases bioavailability (Erdman *et al*., 1993; Stahl and Sies, 1992). Processing to produce juice and heating breaks the carotenoid protein complexes. In addition, heating causes isomerization such as conversion of *cis* to *trans* β-carotene which as discussed below, may affect bioavailability. Excessive heating may decrease absorption and bioavailability by promoting oxidation or formation of complexes of antioxidants with carbohydrates and proteins.

Absorption

Absorption and bioavailability determine whether dietary antioxidants and prooxidants have a direct effect on the blood and other tissues in addition to their effects on the digestive tract. From the many factors affecting absorption and bioavailability of antioxidants, the following have major significance.

Dietary fat

Antioxidant fat-soluble vitamins and phytochemicals are absorbed as micelles. For example, bile emulsifies the tocopherols incorporating them into micelles along with other fat-soluble compounds, thereby facilitating absorption. Lipases are required to hydrolyse esterified antioxidants such as vitamins A and E. Dietary fat stimulates the secretion of bile and lipases and has direct effect on the absorption of fat-soluble antioxidants (see example of β-carotene). When dietary fat is replaced by non-lipid fat replacers such as Olestra®, which do not stimulate production of lipases and bile, absorption of fat soluble antioxidants is reduced. It is for this reason that Olestra is fortified with some fat-soluble nutrients and phytochemicals (Cooper *et al*., 1997; Schlagheck *et al*., 1997).

Physiological conditions or diseases, which reduce the secretion of bile or production of lipases, have a direct effect on absorption (Sokol, 1993). Examples include alcoholic hepatitis, which damages liver function; cholestasis, a genetic disease, which impairs bile secretion; and cystic fibrosis, a genetic disorder which impairs the function of the pancreas and production of lipases.

Even though excess dietary fat is a major public health issue, there are small segments of the population where very low dietary fat impairs absorption of major antioxidants but also of some prooxidants.

Interactions between dietary antioxidants and nutrients

Some of the well-known interactions of minerals affect the antioxidant status. For example, high calcium levels in the diet reduce the absorption of other minerals such as iron, copper, manganese, zinc and selenium. The net effect of impaired absorption on the antioxidant status of the blood and other tissues may be harmful if it leads to deficiency for normal enzyme and other physiological functions; their effect, however, may be neutral or positive if they prevent excessive absorption. Their effect on the antioxidant status of the digesta is different. When absorption is reduced, these minerals remain in the gut and their effect depends on the degree and type of binding to other compounds.

Physiological conditions, disease, and drugs

The absorption of some nutrient and non-nutrient antioxidants in babies, especially premature babies, and the elderly are discussed in other sec-

tions. Disease conditions, especially those causing inflammation of the gut or significantly change its microflora, may impair the absorption of nutrients. Of particular current interest is the malabsorption occurring in advanced stages of AIDS primarily due to colonization of the gut by pathogenic fungi and diarrhoea (Koch *et al*., 1996). Many AIDS patients develop steatorrhaea, a condition associated with serious fat malabsorption including fat-soluble antioxidants (Lambl *et al*., 1996). Some drugs have a direct effect on absorption of nutrients including antioxidants (Blumberg and Suter, 1991; Halpner and Blumberg, 1995). Others, especially antibiotics, have direct effect on microflora and thus may affect absorption.

Intestinal microflora

Intestinal microflora is concentrated primarily in the lower gut; their number increases by six orders of magnitude between the ileum and the colon. The abundance of gut microflora is illustrated by the fact that they constitute about 40–55% of the dry weight of faeces (Stephen and Cummings, 1980). Significant interactions of the microflora with components of the diet and with gastric secretions directly affect absorption and the antioxidant status.

Major examples of such effects include the hydrolysis by intestinal microflora of the non-absorbable glucoside forms of lignans, flavonoids and other phytochemicals to their absorbable aglucone form (Borriello *et al*., 1985; Xu *et al*., 1995). For example, the isoflavones diadzein and genistein are absorbed in their aglucone form after they are hydrolysed with the aid of gut microflora.

Carbohydrates, proteins and lipids can be modified by gut microflora with direct effects on absorption. Cellulose, a major component of the fibre in our diet, not digestible by mammalian enzymes, is partially hydrolysed and metabolized by bacteria to short-chain fatty acids including butyric, propionic and acetic which are readily absorbed even from the lower gut. Butyric acid and, to a lesser extent, propionic acid, have been associated with lower risk of colon cancer (Gamet *et al*., 1992; Velazquez *et al*., 1996). Other carbohydrates such as starch and sugars can be fermented by gut bacteria to short-chain fatty acids. Proteins are also partially hydrolysed to fatty acids and ammonia. PUFA are partially hydrogenated by anaerobic gut bacteria. Because easily digestible food components such as starch, sugars, proteins and lipids are largely digested and absorbed before they reach the lower gut, the effect of microflora is rather minimal.

The respiratory activity of bacteria in the gut produces superoxide radicals ($O_2^{\cdot-}$) that, in the presence of chelated Fe, generate hydroxyl radicals. These radicals cause lipid peroxidation and other reactions, which modify nutrients and other dietary components and non-dietary compounds such as drugs (Blakeborough *et al*., 1989; Chadwick *et al*., 1992; Van Tassell *et al*., 1990).

Bioavailability

Bioavailability is discussed separately from absorption in order to emphasize their respective importance in understanding the role of dietary components on antioxidant status. The following example of tocopherols and tocotrienols, all compounds of the vitamin E family, discussed in detail in an earlier section, illustrates this point. α-Tocopherol and γ-tocopherol are equally well absorbed. However, α-tocopherol is preferentially secreted by the liver into the blood lipoproteins. The naturally occurring RRR stereoisomer and the synthetic *all-rac*-α-tocopherol are also equally well absorbed, yet levels of α-tocopherol in the blood and tissues increase significantly more with RRR than *all-rac*. A tocopherol binding protein is responsible for incorporating preferentially α-tocopherol over γ- and other tocopherols into nascent very-low-density lipoproteins entering the blood. The same mechanism has been proposed for the preference of RRR over *all-rac*-α-tocopherol (Kayden and Traber, 1993). It has been suggested that tocopherols and particularly non-α-tocopherol and *all-rac*-α-tocopherol, are secreted into the intestine via bile. Tocotrienols appear in the blood and tissues at significantly lower levels than tocopherols even when ingested at equivalent or higher amounts (Hayes *et al*., 1993). It is unlikely that significantly lower absorption accounts for this difference suggesting other mechanisms are involved.

Other examples abound: *cis*-β-carotene appears in blood and tissues at significantly lower concentrations than the corresponding *trans* form even when ingested at equivalent or higher amounts. Using labelled compounds, Parker and his associates showed that, after absorption, the *cis* form β-carotene is converted to the *trans* form (Parker, 1996). This conversion increases the apparent bioavailability of the *trans* form at the expense of the *cis* form (Parker, 1996). Other compounds may be rapidly modified after absorption to compounds that have similar or opposite antioxidant effects. It is thus apparent that understanding absorption and bioavailability is essential for evaluating the effects of the diet on antioxidant status (Stahl *et al*., 1995).

Food additives, fortified foods and nutritional supplements

Some of our foods contain food additives that have a direct and/or indirect effect on the antioxidant status. The following are examples with practical significance.

Food antioxidants

Synthetic and natural food antioxidants are used routinely in some foods especially those containing oils and fats. Lipids in foods and particularly those containing PUFA are easily oxidized in a chain reaction. Natural tocopherols, the synthetic α-tocopherol, and other phenolic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and *tert*-butylhydroquinone (TBHQ) are effective chain-breaking antioxidants (Papas, 1993). Rosemary extract is also used as an antioxidant.

Water-soluble antioxidants such ascorbic and citric acids are also used extensively. Ascorbic acid is a synergist for tocopherols because it regenerates oxidized tocopherols (Niki *et al*., 1982; Packer *et al*., 1979). Some compounds have an indirect antioxidant effect. Citric acid, lecithin and others bind prooxidant metals such as Fe and Cu or (lecithin and melanoidins) decompose hydroperoxides to stable products.

The effect of food antioxidants is not restricted in the food. Natural antioxidants such as tocopherols and antioxidant phytochemicals have a major impact on the antioxidant status in the whole digestive tract. They are also absorbed, at least in part, and have an antioxidant effect in our body. Synthetic antioxidants and their metabolites are also absorbed and may have some tissue effects. For example, it was reported that BHA is largely absorbed (70–100%) and excreted in the urine as the glucuronide conjugate of BHA or TBHQ. The strong scrutiny of synthetic antioxidants, and particularly BHA and BHT, for their potential toxic effects at very high levels (Papas, 1993), overshadowed several reports of beneficial effects directly related to their antioxidant function (Arroyo *et al*., 1992; Black and Mathews-Roth, 1991; Slaga, 1995; Williams and Iatropoulos, 1996). Food antioxidants may have contributed to the dramatic decrease in stomach cancer during the last 60 years.

Fortified foods

Antioxidant vitamins and other antioxidants have been routinely added to foods. The traditional fortification of foods such as cereals, milk and flour with vitamins and minerals is now expanding to many new products. Fortification is expanded and includes antioxidant phytochemicals such as carotenoids, flavonoids and others. Examples of such products are drinks fortified with carotenoids, vitamins and minerals; orange juice fortified with vitamins C, E and calcium; snack bars fortified with a variety of vitamins, minerals and phytochemicals; margarines fortified with β-carotene and sterols. Of course, fortification provides both antioxidants and prooxidants such as Fe and Cu and some phytochemicals can act as prooxidants under certain conditions (Cao *et al*., 1997).

Nutritional supplements

National surveys indicate that 40–50% of Americans take vitamin supplements, mostly in the form of tablets and capsules (Dickinson, 1998). New formats such as drinks and snack bars, which blur the differences from fortified foods, are also becoming popular. A smaller percentage regularly takes supplements of individual vitamins such as E (10%), C (17%), or groups of nutrients such as B vitamin complex (6%), mixture of antioxidant vitamins plus selenium, ω-3 fatty acids, etc. In addition, consumption of herbs, herb extracts and phytochemicals is rapidly increasing. Japan has been leading the way in the use of nutritional supplements and with new product formats such as drinks, teas and herbal extracts. These products are widely available, including in vending machines. Europeans and people in developing countries have been using herbs and their extracts extensively but their use of tablets and capsules has been significantly lower than in the United States and Japan.

Intake of antioxidant nutrients from nutritional supplements is usually in excess of the RDAs. For example, the most popular doses of vitamin E are 400 and 200 and 800 IU (RDA is 10–15 IU and US RDA 30 IU) and of vitamin C, 500, 1000 and 250 mg (RDA is 60 mg).

Chemical, chiral form and formulation

Chemical and chiral form

In the past, the role of chirality and chemical form on antioxidant activity received little attention. It has now come to the forefront as a result of major studies. Vitamin E provides an excellent example. The differences of various chemical forms of vitamin and particularly α- and γ-tocopherols were discussed briefly above and will be discussed in detail later. Also, the major differences between the naturally occurring RRR stereoisomer and the synthetic racemic mixture of α-tocopherol which approaches the ratio of 2:1 for blood and some tissues shows the dramatic effect of chirality (Acuff *et al*., 1994; Burton *et al*., 1998; Kayden and Traber, 1993). Other examples of β-carotene and lycopene were mentioned above and will also be discussed later. Even simple changes in chemical form can have major impact on physical characteristics, stability and antioxidant effect. For example, the palmitate ester of ascorbic acid has some solubility in oil while ascorbic acid and sodium ascorbate are insoluble. The acetate ester of α-tocopherol is yellow oil while the succinate ester is white solid. Both esters are extremely stable in storage while the free tocopherol is easily oxidized when exposed to air, heat, and especially in the presence of oxidizing agents such as Fe and Cu. In our body, these esters function as antioxidants only after hydrolysis and

release of the free tocopherol. Thus, the tocopheryl esters do not function as antioxidants in food, the oral cavity, the oesophagus, the stomach and the duodenum because their active hydroxyl group is blocked. Esters must by hydrolysed by pancreatic lipases prior to absorption of the α-tocopherol, a major consideration for people with cystic fibrosis, premature infants and the elderly.

Formulation

Formulation can dramatically change absorption and bioavailability of antioxidants. Carotenoids provide excellent examples. Less than 10% β-carotene in raw carrots is absorbed. Absorption is higher in cooked carrots and carrot juice (Erdman *et al*., 1993; Parker, 1996). Commercial β-carotene, in viscous oil form, is absorbed at approximately 10–20%. In contrast, absorption of β-carotene microencapsulated with gelatin, is significantly higher (Gaziano *et al*., 1995).

The effect of formulation on solubility and other physical characteristics can be dramatic. Microencapsulated β-carotene is a white solid completely dispersible in water while extracted β-carotene is very viscous oil. The importance of chemical form and formulation is illustrated in the following example. In the major ATBC intervention study on the role of α-tocopherol and β-carotene in lung cancer in smokers, the daily dose of β-carotene was 20 mg of microencapsulated synthetic form with estimated absorption exceeding 90%. The estimated average daily intake of β-carotene is 3.0 mg; thus the dose used would be 6.7 times the daily intake. If, however, we factor the approximate absorption of 10% from food sources and 90% from the supplement, then the effective dose is 18 mg (20 mg × 90%) *v*. 0.30 mg from the food (3.0 mg × 10%). Thus the effective dose was 60 times higher.

Unfortunately, in many clinical studies the chemical and chiral forms and formulation are not considered, thus creating major difficulty in the interpretation of the results.

Antioxidant function in foods, *in vitro* and in humans: a new look

The relative ability of compounds to prevent oxidation in foods or *in vitro* systems is often extrapolated to their antioxidant function in the tissue. Such extrapolations are usually inaccurate and not very meaningful. Tocotrienols were reported to prevent oxidation of low-density lipoproteins (LDL) *in vitro* at least equal to or several-fold better than α-tocopherol (Kamal-Eldin and Appalqvist, 1996; Serbinova *et al*., 1991). α-Tocopherol, however, is by far the most abundant antioxidant in LDL and is the principal antioxidant for LDL (Esterbauer *et al*., 1993) and in cell membranes. Similarly, on the basis of their function as food antioxidants and from some *in vitro* systems, β-, γ- and δ-tocopherols and synthetic compounds such as trolox, BHA, BHT, TBHQ and PG are effective chain-breaking antioxidants, yet their role in humans for preventing LDL oxidation is very low compared with α-tocopherol. These apparent contradictions may be due to several reasons. For example, α-tocopherol is preferentially secreted into the lipoproteins over the other tocopherols and tocotrienols. Furthermore, while natural phenolic and synthetic antioxidants share the same active group they lack the phytyl chain, which provides tocopherols their unique ability to be positioned in the cell membrane.

Because antioxidants act as components of a complex system, comparisons based on single test or criterion are not very meaningful. Although γ-tocopherol may play a lesser role than α-tocopherol in preventing lipid oxidation in LDL, it may play a more important role in neutralizing nitrogen radicals. The major sites of its action may also be different (Stone and Papas, 1997). Glutathione, coenzyme Q_{10} and vitamin C have additive, synergistic effects or regenerate tocopherols (Halliwell *et al*., 1995; Niki *et al*., 1982; Packer *et al*., 1979). In addition, unique physical and chemical properties such as solubility, enzymatic activity and others allow individual antioxidants to perform specialized functions. The water-soluble vitamin C plays a critical role in the cytoplasm while the lipophilic vitamin E is an important antioxidant in membranes but both are essential components of the antioxidant system. Coenzyme Q_{10} is a critical component of the electron transfer system in the mitochondria, the basic process for generating energy in the cell. In the absence of vitamin E in the mitochondrial membrane or vitamin C in the cytoplasm this process would not function efficiently.

Conclusion

The diet is one of the leading factors under our control affecting the antioxidant status. The effect of the diet must be considered in its totality and should include the digestive system. For this reason, our view of absorption and bioavailability of food components must be expanded to include their effect in the digestive and urinary systems. These considerations are particularly important when using advanced techniques of food processing and nutrient formulation, which change dramatically their absorption and bioavailability characteristics. Similarly they must be considered in the design of clinical trials evaluating the health effects of foods, nutrients and phytochemicals. Major changes in the diet can have diverse and even opposing effects on the antioxidant status. Lower fat in the diet or fat substitutes reduce the amount of fatty acids, especially PUFA, available for oxidation. Very low

fat diets, however, reduce the absorption of fat-soluble nutrients and phytochemicals including many antioxidants.

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