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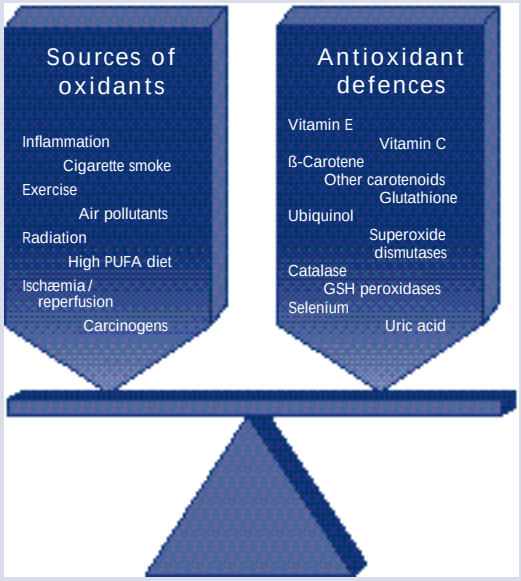

OXIDANTS, ANTIOXIDANTS, AND DISEASE PREVENTION

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OXIDANTS, ANTIOXIDANTS, AND DISEASE PREVENTION

by Lillian Langseth

ILSI Europe

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FOREWORD

Oxygen is essential to human life; without it, we cannot survive. Yet, paradoxically, oxygen is also involved in toxic reactions and is therefore a constant threat to the well-being of all living things. Human beings can tolerate oxygen only because our evolutionary ancestors developed powerful defence mechanisms that minimize its toxic effects. Without this protection, the by-products of our own metabolism would quickly end our lives.

Unfortunately, our natural defences are imperfect; they limit the harm caused by oxygen but do not eliminate it completely. There is some evidence that as the years go by, oxygen-induced damage to body tissues may accumulate. This damage has been hypothesized to be a major contributor to ageing and to many of the degenerative diseases of ageing, including cardio-vascular disease, cancer, cataracts, the age-related decline in the immune system and degenerative diseases of the nervous system.

In the past decade, researchers have made major strides in understanding the link between oxygenated metabolites and human diseases. At the same time, they have learned that it may be possible to prevent, postpone or limit the severity of these diseases by enhancing the body's antioxidant defence mechanisms through improved nutrition.

This monograph was inspired by a symposium held in Stockholm, Sweden, in 1993, which was organised by ILSI Europe. It presents a review of current developments in this rapidly expanding field of medical research. It should be noted that antioxidants are also of importance in other fields, including food technology and preservation. Scientific developments in these areas are beyond the scope of this monograph.

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OXIDANTS AND ANTIOXIDANTS: SOME BASIC CONCEPTS

Oxidants and free radicals

Most of the potentially harmful effects of oxygen are believed to be due to the formation and activity of reactive oxygen species acting as oxidants, that is, compounds with a tendency to donate oxygen to other substances. Many reactive oxygen species are free radicals. A free radical is any chemical species that has one or more unpaired electrons. Many free radicals are unstable and highly reactive. Some of the free radicals and other important oxidants found in living organisms are shown in Table 1.

TABLE 1

Some important reactive oxygen species in living organisms

Free radicals

Hydroxyl radical	$OH^{\bullet}$
Superoxide radical	$O_2^{\bullet -}$
Nitric oxide radical	$NO^{\bullet}$
Lipid peroxyl radical	$LOO^{\bullet}$

Nonradicals

Hydrogen peroxide	H_2O_2
Singlet oxygen	$^1O_2$
Hypochlorous acid	HOCl
Ozone	O_3

TABLE 2

Some sources of free radicals

Internally generated sources

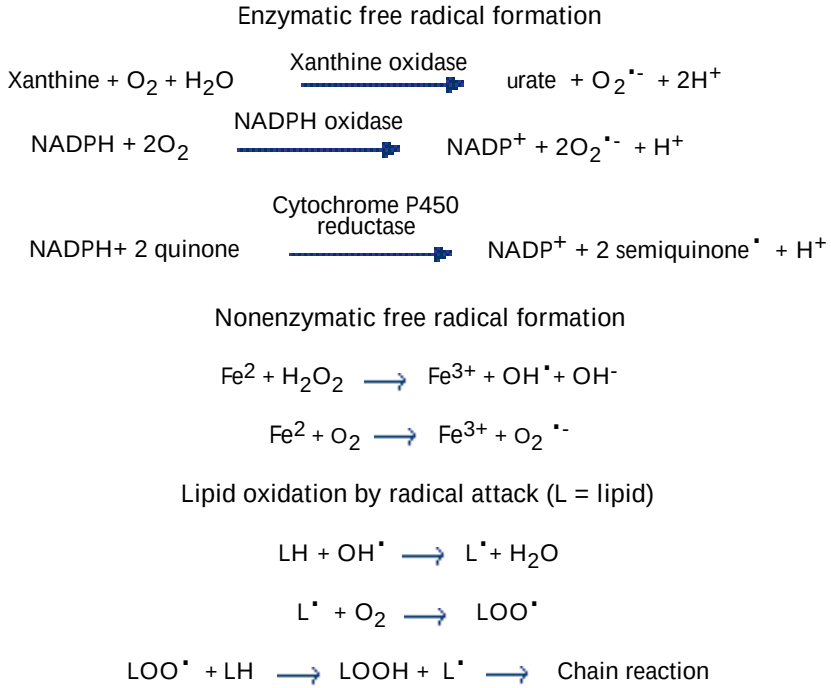
Mitochondria
Phagocytes
Xanthine oxidase
Reactions involving iron and other transition metals
Arachidonate pathways
Peroxisomes
Exercise
Inflammation
Ischæmia / reperfusion

External sources

Cigarette smoke
Environmental pollutants
Radiation
Ultraviolet light
Certain drugs, pesticides, anaesthetics, and industrial solvents
Ozone

Reactive oxygen species are produced continuously in the human body as a consequence of normal metabolic processes. Some reactions that lead to free radical formation are shown in Figure 1. If free radicals are not inactivated, their chemical reactivity can damage all types of cellular macromolecules, including proteins, carbohydrates, lipids, and nucleic acids. Figure 1 shows some of the reactions involved in free radical attacks on lipids. Figure 2 illustrates some of the types of damage that can result from the actions of free radicals. Several of these effects have been implicated in the causation of degenerative diseases and will be described in more detail later in this monograph. For example, destructive

FIGURE 1. Examples of free radical reactions



effects on proteins may play a role in the causation of cataracts, effects on DNA are involved in cancer causation, and effects on lipids apparently contribute to the causation of atherosclerosis.

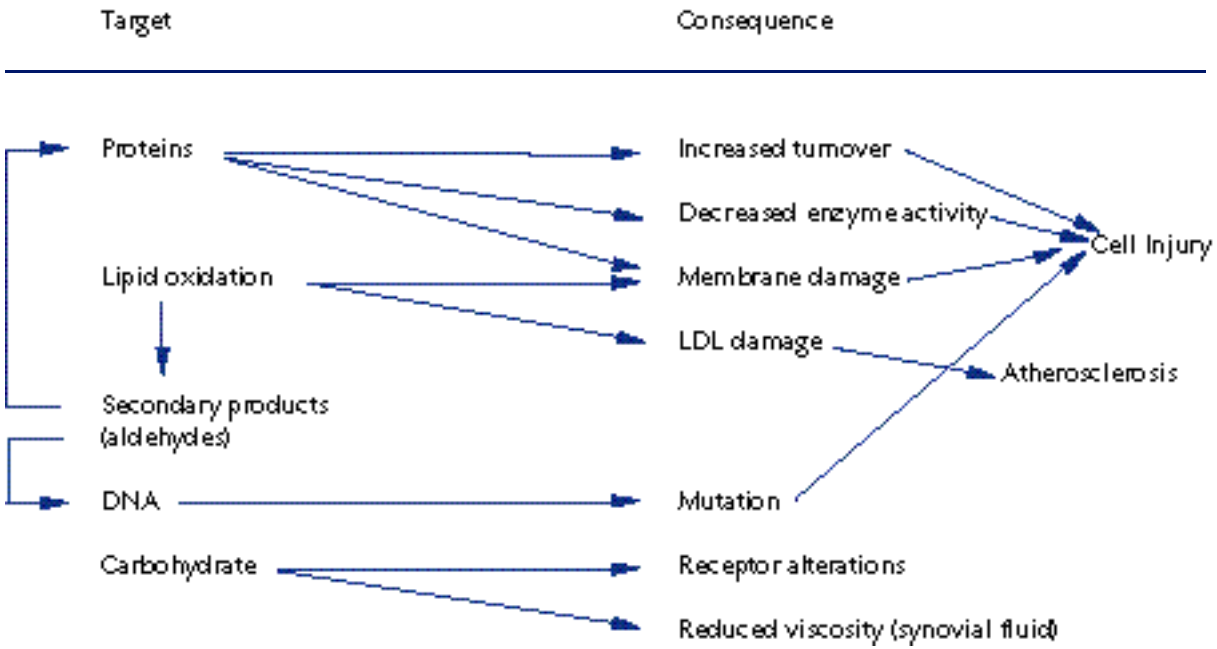
Free radicals and other reactive oxygen species in the human body are derived either from normal, essential metabolic processes or from external sources. Examples of both are shown in Table 2.

Free radicals are not always harmful. It is important to note that they also serve useful purposes in the human body. For example, free radicals play a role in the destruction of disease-causing microbes by specialised blood cells called phagocytes.

Defences against oxidants

The human body has several mechanisms for defence against free radicals and other reactive oxygen species. The various defences are complementary to one another because they act on different oxidants or in different cellular compartments. One important line of defence is a system of enzymes, including glutathione peroxidases, superoxide dismutases and catalase, which decrease the concentration of the most harmful oxidants. Figure 3 shows some of the actions of these enzymes. Superoxide dismutases are a family of antioxidant enzymes which are important in the catalytic decomposition of the superoxide radical to hydrogen peroxide and oxygen. Catalase specifically

FIGURE 2. Free radical damage



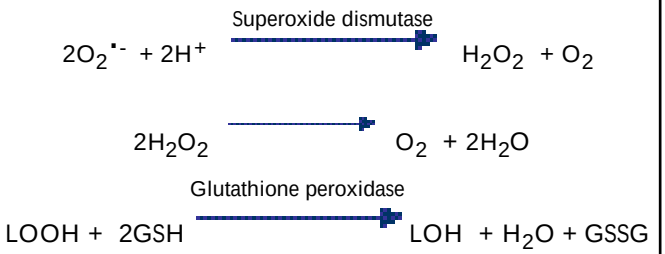
catalyses the decomposition of hydrogen peroxide. Glutathione peroxidases are a family of antioxidant enzymes containing selenium which are important in the reduction of hydroperoxides, for example, those that result from lipid oxidation (Figure 3).

Nutrition plays a key role in maintaining the body's enzymatic defences against free radicals. Several essential minerals including selenium, copper, manganese and zinc are involved in the structure or catalytic activity of these enzymes. If the supply of these minerals is inadequate, enzymatic defences may be impaired.

A second line of defence is small-molecular-weight compounds which act as antioxidants; that is, they react with oxidising chemicals, reducing their capacity for damaging effects. Some, such as glutathione, ubiquinol and uric acid, are produced by normal metabolism. Ubiquinol is the only known fat-soluble antioxidant synthesised by animal cells. It is believed to play an important role in cellular defence against oxidative damage.

Other small-molecular-weight antioxidants are found in the diet, the best known being vitamin E, vitamin C and carotenoids. Some foods also contain other antioxidant substances, as shown in Table 3. Most of the antioxidants found in these foods are phenolic or polyphenolic compounds. Although these substances have no known nutritional function, they may be important to human health because of their antioxidant potency.

FIGURE 3. Enzymatic antioxidant defences (L = lipid)



Oxidative stress

If exposure to exogenous sources of oxidants is high, the body's antioxidant defences may be unable to cope. The result is a condition called oxidative stress, an imbalance between pro-oxidants and antioxidants. Figure 4 illustrates the concept of balance between pro-oxidant factors and antioxidant defences. The diagram shows the normal situation, in which pro-oxidant factors are adequately counterbalanced by antioxidant defences. An increase either in the production of oxidants or in a deficiency in the defence system could disturb this balance, causing oxidative stress.

THE MAJOR ANTIOXIDANT NUTRIENTS

Vitamin-related antioxidants

Although a wide variety of antioxidants in food may contribute to disease prevention, the bulk of the research to date has focused on three antioxidants which are of special interest because they are essential nutrients or precursors of nutrients and are present in significant amounts in body fluids – vitamin E, vitamin C and carotenoids.

The term "vitamin E" is a collective name for numerous different tocopherols and tocotrienols which share the same biological activity. Vitamin E is a fat-soluble substance. It is the major antioxidant in all cellular membranes, and it protects polyunsaturated fatty acids against oxidation.

Vitamin C (ascorbic acid) is a water-soluble substance. It is believed to be the most important antioxidant in extracellular fluids, and it has many known intracellular activities as well.

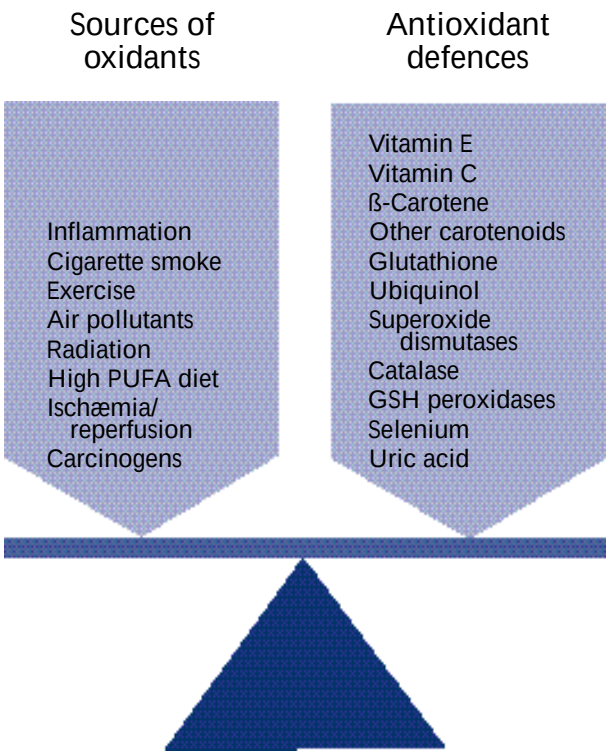
The carotenoids are a group of red, orange and yellow pigments found in plant foods, particularly fruits and vegetables, and in the tissues of animals which eat the plants. Some carotenoids can act as precursors of

TABLE 3

Some foods that contain nonnutrient antioxidants

Product	Antioxidants
Soybeans	Isoflavones, phenolic acids
Green tea, black tea	Polyphenols, catechins
Coffee	Phenolic esters
Red wine	Phenolic acid
Rosemary, sage, other spices	Carnosic acid, rosmaric acid
Citrus and other fruits	Bioflavonoids, chalcones
Onions	Quercetin, kaempferol
Olives	Polyphenols

FIGURE 4. Balance between pro-oxidant factors and antioxidant defences



Adapted from Machlin LJ, Implications for the Biomedical Field, in Williams GM, (ed), Antioxidants: Chemical, Physiological, Nutritional and Toxicological Aspect. Princeton, NJ: Princeton Scientific Publishing Co., 1992;383-387

vitamin A; others cannot. However, this property is unrelated to their antioxidant activity. Important dietary carotenoids include ß-carotene, α-carotene, lycopene, lutein, zeaxanthin and ß-cryptoxanthin.

TABLE 4

Food source of antioxidant vitamins

Vitamin E

Best sources:	vegetable oils, cold-pressed seed oils, wheat germ
Other significant sources:	vegetables, fruits, meat/poultry/fish

Vitamin C

Fruits:	especially citrus fruits, strawberries, cantaloupe melon
Vegetables:	especially tomatoes, leafy greens, cabbage-family vegetables such as broccoli and cauliflower

Carotenoids

ß-Carotene:	yellow-orange vegetables and fruits, dark green vegetables
α-Carotene:	carrots
Lycopene:	tomatoes
Lutein and zeaxanthin:	dark green leafy vegetables, broccoli
ß-Cryptoxanthin:	citrus fruits

Source: Antioxidant Vitamins Newsletter, Nos. 1 and 2; AR Mangels et al. Carotenoid content of fruits and vegetables: an evaluation of analytic data. J Am Diet Assoc 1993;93:284-296

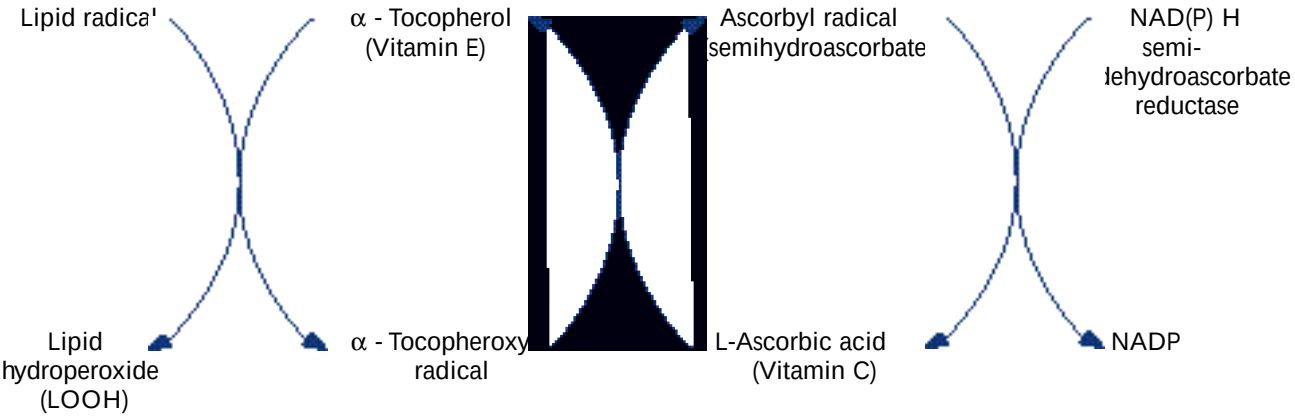
Food sources of antioxidant nutrients

Table 4 lists the best food sources of each of the major dietary antioxidants. As Table 4 indicates, two categories of foods — fruits and vegetables — are particularly important. These foods are the principal sources of both vitamin C and carotenoids. They also contribute substantial amounts of vitamin E to the diet, as well as nonnutrient antioxidants. Authorities in several countries have recommended that everyone should consume at least five servings of fruits and vegetables daily, since population studies have revealed a lower incidence of certain degenerative diseases, such as cardiovascular disease and some forms of cancer, in subjects consuming larger amounts of fruits and vegetables. Unfortunately, the diets typically consumed in many parts of the world, including most of Western Europe and North America, fall far short of this goal.

Interactions between antioxidants

In addition to their individual effects, antioxidants interact in synergistic ways and have "sparing effects" in which one antioxidant protects another against oxidative destruction. For example, vitamin C reinforces the antioxidant effect of vitamin E by regenerating the active form of the vitamin after it has reacted with a free radical (Figure 5). This beneficial interaction has been demonstrated in biological fluids as well as in model systems. There is some evidence that ubiquinol, a fat-soluble antioxidant produced in the human body, may also regenerate vitamin E. There are other types of beneficial interactions as well. For instance, vitamin E can protect the ß-carotene molecule from oxidation and thus may have a sparing effect on this antioxidant. Vitamin E and the mineral selenium appear to act synergistically. In animals, supplementation with one of these nutrients can relieve symptoms caused by a

FIGURE 5. Synergism of vitamin E and vitamin C



Adapted from Papas AM, Oil-Soluble Antioxidants. In Williams GM (ed), Antioxidants: Chemical, Physiological, Nutritional and Toxicological Aspects. Princeton, NJ: Princeton Scientific Publishing Co., 1992;123-149

deficiency of the other. However, they cannot fully replace each other, because selenium, like vitamin E, through its presence in glutathione peroxidase enzymes, functions to protect cell membranes against the action of lipid hydroperoxides.

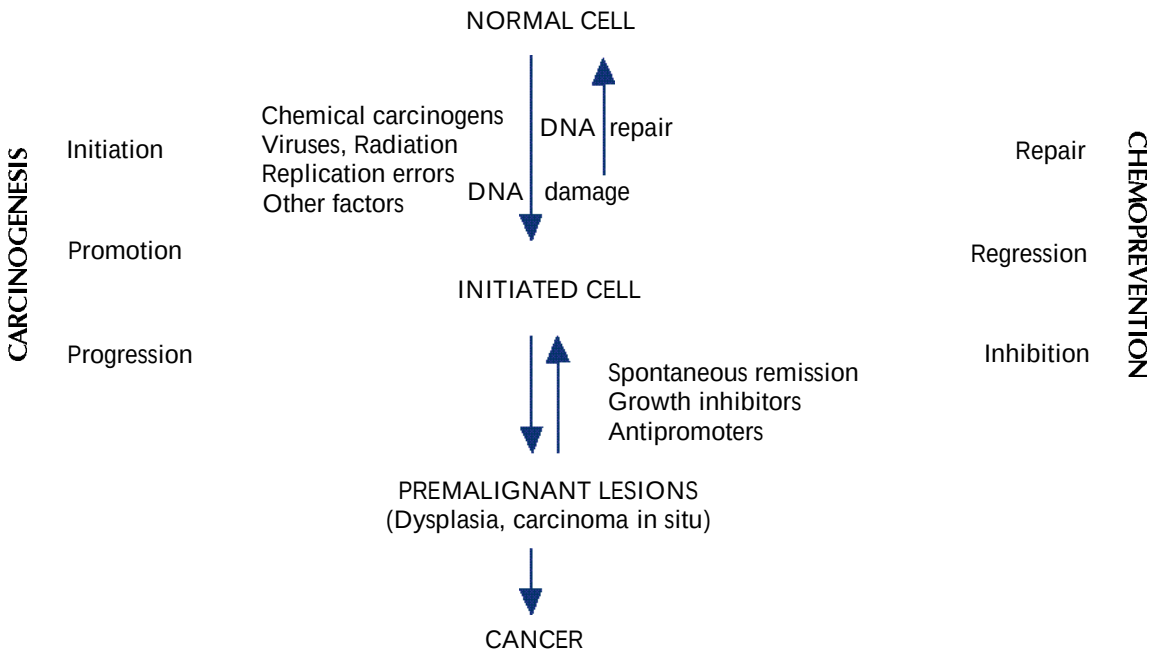
As a result of interactions such as these, combinations of antioxidants may be more effective than larger quantities of any single antioxidant.

ANTIOXIDANTS AND THE PREVENTION OF CANCER

Mechanisms of action

Cancer is the end point of a multistep process involving a sequence of events that occur over a period of years or even decades. Figure 6 summarises current scientific thinking about one possible mechanism by which normal cells may be transformed into cancer cells.

FIGURE 6. A multistep model of carcinogenesis



Adapted from Singh VN, Gaby SK, Premalignant Lesions: Role of Antioxidant Vitamins and ß-Carotene in Risk Reduction and Prevention of Malignant Transformation, Am J Clin Nutr 1991: 53-386S-390S. © American Society for Clinical Nutrition.

DNA damage is considered to be one of the most important contributors to cancer. Much of this damage is oxidative in nature. A marker of mutagenic DNA damage would be useful in the estimation of cancer risk of various populations and in monitoring the effects of chemoprevention. Much of this damage is oxidative in nature. It has been estimated that a typical human cell experiences about 10.000 oxidative "hits" to its DNA each day. DNA repair enzymes remove most of this damage, but not all of it. Oxidative lesions to DNA accumulate with age, and so does the risk of cancer.

If a cell containing damaged DNA divides before its DNA can be repaired, the result is a permanent genetic alteration, the first step in carcinogenesis. Cells that divide rapidly are more susceptible to carcinogenesis than slowly dividing cells because there is less opportunity for DNA repair before cell division.

Oxidants and antioxidants may also play a role in the later stages of cancer development. There is increasing evidence that oxidative processes contribute to the promotion stage of carcinogenesis, although the mechanisms for this are not well understood. Antioxidants may be able to cause the regression of premalignant lesions or inhibit their development into cancer. Preliminary studies have indicated that some antioxidants, particularly ß-carotene, may be of benefit in the treatment of precancerous conditions such as oral leukoplakia (which may be a precursor of oral cancer).

Some antioxidant nutrients may protect against cancer through mechanisms other than their antioxidant properties. For example, carotenoids may both enhance immune function and increase gap junctional communication (a type of interaction between cells that inhibits cell proliferation); both of these actions may be relevant to cancer prevention.

Evidence for a protective effect of fruit and vegetable consumption

An impressive body of scientific evidence shows that people with high dietary intakes of fruits and vegetables are less likely to develop cancer than people who have low dietary intakes of these foods. Approximately 200 epidemiologic studies of fruits, vegetables and cancer have been published, and their results are remarkably consistent. Table 5 summarises the results of these studies.

In general, high intakes of fruits and vegetables are associated with a protective effect against cancer, whereas low intakes are associated with increased risks of cancers at most body sites except the prostate. Of 156 dietary studies of cancers at sites other than the prostate, 128 demonstrated statistically significant associations. The evidence is strongest for epithelial cancers, such as cancers of the lung, larynx and oesophagus, and less convincing for hormone-dependent cancers, such as breast cancer.

Fruits and vegetables are the principal sources of vitamin C and carotenoids, and it is believed that these antioxidants are major contributors to the apparent cancer-protective effects of these foods. Fruits and vegetables also contain vitamin E as well as other nutrients including folate (a B complex vitamin) and fibre, which may protect against cancer by other mechanisms. All of these nutrients, and perhaps some nonnutrient components, may be involved in cancer prevention.

Tissue levels of antioxidants

Some epidemiologic studies have used biomarkers — physiological indicators of antioxidant status — rather than dietary data as measures of exposure. The most commonly used short-term biomarker is the concentration of an antioxidant in blood plasma.

TABLE 5

Epidemiological studies of fruit and vegetable intake and cancer prevention

Cancer site	Number of studies [a]	Significant protective effects	Significantly increased risk
All sites including prostate	170	132	6
All sites except prostate	156	128	4
Lung	25	24	0
Larynx	4	4	0
Oral cavity, pharynx	9	9	0
Oesophagus	16	15	0
Stomach	19	17	1
Colorectal	27	20	3
Bladder	5	3	0
Pancreas	11	9	0
Cervix	8	7	0
Ovary	4	3	0
Breast	14	8	0
Prostate	14	4	2
Miscellaneous[b]	8	6	0

Source: Block G, Patterson B, Subar A. Fruit, Vegetables, and Cancer Prevention: A Review of the Epidemiological Evidence, Nutr Cancer 1992:18:1-29. © Lawrence Erlbaum Associates. Adapted by permission.

[a] Only studies that reported results in terms of relative risk and included 20 or more cancer cases at the site in question are included in this table.

[b] Melanoma, thyroid, biliary tract, mesothelioma, endometrial, and childhood brain tumours.

The findings of a major Chinese study support the concept that antioxidants may be protective against cancer. This study was conducted in 65 Chinese counties with an extraordinary diversity of disease patterns. Blood samples were collected from 100 randomly selected residents of each county; samples from members of the same community were then pooled for analysis. There was a consistent inverse correlation between cancer death rates in the 65 counties and blood levels of ß-carotene, vitamin C and selenium. For a wide range of cancers, associations between low death rates and high vitamin C levels were stronger than those for any other nutrient. High ß-carotene levels were associated with a reduced risk of stomach cancer.

The EURAMIC study (European Community multicentre study on antioxidants, myocardial infarction and breast cancer), now in progress, is using adipose tissue concentrations of vitamin E and ß-carotene and toenail concentrations of selenium as biomarkers of antioxidant status. These biomarkers were chosen because they are believed to be an integrated measure of exposure over months rather than days. Epidemiologic studies in six European locations (Berlin, Germany; Coleraine, United Kingdom; Granada, Spain; Malaga, Spain; Zeist, The Netherlands; and Zürich, Switzerland) will compare levels of these biomarkers in breast cancer patients with those in otherwise similar women without the disease who live in the same areas. Results of the myocardial infarction arm of the study are presented in the cardiovascular intervention trials section of this monograph.

Effects of individual carotenoids

In a few recent studies, epidemiologists have attempted to distinguish effects of specific carotenoids, rather than focusing on total carotenoids or ß-carotene. The results of these studies have suggested that several different carotenoids may be associated with reduced cancer risks. For example, in a study conducted in Hawaii, high dietary intakes of ß-carotene, α-carotene and lutein were each associated with reduced risks of lung cancer. Another study in the United States found that blood levels of total carotenoids, α-carotene, ß-carotene, cryptoxanthin and lycopene, but not lutein, were lower among women who later developed cervical cancer than among those who remained healthy. In the same U.S. population, blood levels of five major carotenoids were all found to be lower among individuals who later developed oral cancer than among controls.

Vitamin E

The evidence linking vitamin E and cancer risk is less extensive than that for vitamin C and carotenoids. Until recently, a lack of reliable information on the vitamin E content in foods has impeded epidemiologic studies of dietary vitamin E intake. Instead, most researchers have used blood vitamin E levels as a biomarker of vitamin E nutriture. The findings of these blood studies have been inconsistent; some have shown an inverse association between vitamin E levels and cancer risk, whereas others have found no association.

A large multicentre case-control study conducted by the U.S. National Cancer Institute associated the use of vitamin E supplements with a 50% reduction in oral cancer risk. Dietary vitamin E and multivitamins had no significant effect. Dietary vitamin E intake in the United States is generally less than 15 international units (IU)/day (11 mg α-tocopherol equivalents [ATE]/day); multivitamins provide 30 IU/day (22 mg ATE/day); and most single-entity supplements sold in the United States contain at least 100 IU (74 mg ATE). This suggests the possibility that vitamin E may show a "plateau effect", meaning that below a certain critical dose it may not have a detectable inverse association with cancer risk. The plateau dose is likely to be greater than that which can be obtained through diet alone. It is also possible, however, that the relationship between

vitamin E supplementation and oral cancer seen in this study may not have been causal; people who chose to use supplements may have differed from nonusers in other ways that might affect oral cancer risk. At present, the use of supplements is an individual decision, and consumers receive little guidance about appropriate types and doses. In general, individuals who choose to take supplements tend to make other efforts to improve their health as well, reflecting a generally health-conscious lifestyle.

Intervention trials

Several clinical intervention trials designed to evaluate the effect of antioxidant supplementation on cancer risk are now in progress (see Table 6). Studies of this type are the best way to establish definitively the effects of a specific substance. However, cancer prevention intervention trials must involve large numbers of subjects and lengthy periods of treatment. Therefore, intervention trials are usually undertaken only when substantial evidence from other types of investigations suggests that an effect is likely to be demonstrated.

Four intervention trials of antioxidant nutrients have already been completed. Two were conducted in Linxian (a rural area in China with very high oesophageal and stomach cancer rates and a high prevalence of subclinical nutritional deficiencies), one in Finland and one in the United States.

The larger of the Linxian trials was designed to determine the effect of nutrient supplementation on cancer risk in the general population. Participants in the trial either received various combinations of nutrient supplements or received placebos daily for 5 years. One of the supplements (a ß-carotene / vitamin E / selenium combination) was found to reduce stomach cancer deaths by 21%, total cancer deaths by 13% and total mortality by 9%. Thus, one or more of these antioxidants appear to be protective against cancer in the population studied. However, the results of this trial may not be directly applicable to Western populations, where both dietary habits and disease patterns are drastically different from those of this nutritionally deprived Chinese community.

The other Linxian trial evaluated the effects of multivitamin and ß-carotene supplementation on oesophageal cancer risk in individuals who already had oesophageal dysplasia, a precancerous condition. More than 3.000 subjects received either supplements or placebos daily for 6 years. The results of the study were negative; supplementation did not reduce oesophageal cancer risk.

The Finnish trial, conducted by the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, evaluated the effect of vitamin E and ß-carotene on the incidence of lung cancer and other cancers in 29.000 men who had smoked for an average of 35.9 years. Men were randomly assigned to one of four regimens: ß-carotene (20 mg / day), vitamin E (50 mg / day as dl-α-tocopheryl acetate), both supplements together at the same levels, or an inactive placebo. Follow-up continued for 5 to 8 years, with lung cancer as the primary endpoint.

Subjects taking ß-carotene either alone or with vitamin E were compared with subjects not taking ß-carotene. Contrary to expectations, the men who were taking ß-carotene showed a statistically significant 18% increase in lung cancer incidence. There was no evidence of an interaction between vitamin E and ß-carotene with respect to lung cancer. ß-Carotene had some effect on the incidence of cancers other than lung cancer.

The unexpected results of the Finnish trial should receive serious consideration, but the findings of a single study must always be placed in appropriate perspective. More studies are required.

TABLE 6

Some major long-term cancer prevention trials

Scientist/ country	Cancer site	Study group	Sample size	Treatment	Projected completion
Blot/China	Oesophagus, stomach, and overall mortality	General population of a high-risk area	30.000	Various combinations of nutrients	Study completed. See text for discussion of results.
Li/China	Oesophagus	Subjects with oesophageal dysplasia	3.300	Multivitamin plus ß-carotene	Study completed. See text for discussion of results.
Heinonen/Finland (ATBC Cancer Prevention Study)	Lung	Male long-term smokers	29.000	ß-Carotene and vitamin E	Study completed. See text for discussion of results.
Greenberg/USA	Colorectal	Subjects with a colorectal adenoma removed surgically	751	ß-Carotene vitamin E plus C, or all three nutrients together	Study completed. See text for discussion of results.
Hennekens/USA	All	Male physicians	22.000	ß-Carotene	1996
Goodman/USA (CARET)	Lung	Smokers and asbestos-exposed workers	17.000	ß-Carotene and vitamin A	1998
Green/Australia	Skin (cancer and actinic keratoses)	General population	1.800	ß-Carotene	1998
Mayne/USA	Head and neck	Subjects with previous cancers removed surgically	600	ß-Carotene	1998

Source: Antioxidant Vitamins Newsletter, June 1992 and January 1993. Updated and reprinted with permission.

Another cancer prevention trial, completed in 1994 in the United States, failed to show that a 4-year period of antioxidant treatment could prevent the occurrence of new colorectal adenomas in patients with a history of past adenomas. The 751 patients participating in this trial were randomly assigned to receive daily either ß-carotene (25 mg), vitamin E (400 mg) plus vitamin C (1 g), all three nutrients together, or an inactive placebo.

Cancer: summary

In summary, both biochemical and epidemiologic studies have indicated that antioxidant nutrients and the foods that contain them may have important protective effects in the prevention of human cancer. The evidence for a beneficial effect of fruits and vegetables is overwhelming. The evidence for protective effects from individual antioxidant nutrients is less definitive, and therefore other constituents of a diet may also play an important role. One major intervention trial, conducted in a non-Western population, has shown a beneficial effect of combined supplementation with ß-carotene, vitamin E and selenium. In two recently completed trials in Western populations, however, one showed no benefit of supplementation with ß-carotene, vitamin E or vitamin C on prevention of colorectal adenomas, whereas in the other, supplementation with ß-carotene increased the risk of lung cancer in heavy smokers. More solid evidence on the relationship between antioxidant nutrients and cancer may be forthcoming within the next few years from other intervention trials in Western countries.

ANTIOXIDANTS AND CARDIOVASCULAR DISEASE

Mechanisms of action

Current theory suggests that oxidation may play a role in cardiovascular disease in two ways, one involving the long-term development of atherosclerosis and the other involving the immediate damage that occurs during a heart attack or stroke. Antioxidants may help counteract both of these processes.

LDL oxidation and atherogenesis

An increasing body of scientific evidence supports the concept that oxidation, possibly mediated by free radicals, may contribute to atherogenesis by transforming low-density lipoprotein (LDL) into an oxidised form. Oxidised LDL has been found in damaged arterial walls and has been shown to have several actions that could contribute to the initiation and progression of arterial damage.

There is evidence from human studies that associates the extent of LDL oxidation with the extent of atherosclerosis. For example, in a study of Swedish heart attack victims, it was found that increased susceptibility of LDL to oxidation was associated with increased severity of atherosclerosis. Similarly, in a Finnish study, men with accelerated progression of atherosclerosis were found to have higher levels of antibodies to oxidised LDL than men who showed no progression of atherosclerosis. Additional evidence comes from short-term intervention trials in human volunteers, as described below in the Intervention Trials section.

Ischæmia/reperfusion injury

Another way in which oxidants and antioxidants may influence cardiovascular disease is through the process of ischæmia / reperfusion injury — the immediate cause of tissue damage during a heart attack or stroke. All living tissues of the human body need oxygen to survive. If their supply of blood and oxygen is cut off (a situation called ischæmia) they begin to die. Irreversible damage can be prevented only by reperfusion — the restoration of blood flow and reintroduction of oxygen. Ironically, although reperfusion is necessary for recovery, it can damage tissues still further because harmful oxygen free radicals are formed during the reperfusion process.

The role of free radicals in heart attacks has been assessed in experimental model systems. Free radicals have been found to accumulate in isolated heart muscle that has been subjected to a temporary interruption of blood flow. In some experimental models, treatments that inhibit the accumulation of free radicals have been shown to reduce the severity of damage to the heart muscle. In one human study, pretreatment with vitamin C was apparently of benefit to patients who underwent long periods of cardiac arrest during cardiopulmonary bypass surgery. The release of enzymes associated with ischæmia was strikingly decreased in those patients who received vitamin C, indicating a reduction in cell damage.

Epidemiologic evidence

An increasing body of epidemiologic evidence links high intakes of antioxidants with reduced risks of cardiovascular diseases. The evidence is strongest for vitamin E, limited but promising for ß-carotene, and inconsistent for vitamin C.

Vitamin E

The most notable findings on vitamin E come from two large epidemiologic studies conducted in the United States. Key findings of these studies are summarised in Table 7. In an ongoing study of almost 40.000 male health professionals, it was found that those in the top fifth of the group in terms of vitamin E intake showed a statistically significant 40% reduction in heart attack risk. In a parallel study of 87.000 female nurses, those in the top fifth of vitamin E intake had a 34% reduction in heart attack risk. In both groups, the association was attributable mainly to vitamin E consumed in supplement form. Dietary vitamin E had no significant effect, presumably because vitamin E intakes from food are far lower than those achievable through supplementation. Daily use of single-entity supplements, generally containing at least 100 IU (74 mg ATE) of the vitamin, for 2 or more years was associated with a 37% decrease in heart attack risk among men and a 41% decrease in women. Supplement doses greater than 100–250 IU / day (74–185 mg ATE / day) were not associated with further reductions in risk.

Although these findings are impressive, they do not constitute definitive proof that vitamin E supplementation causes a reduction in heart disease risk. These were not intervention trials; they were observational studies of people who chose for themselves whether or not to use supplements. It is possible that supplement users may have differed from nonusers in other ways that might affect heart disease risk and that these other factors, rather than vitamin intake itself, were responsible for the inverse association between supplement use and coronary risk. However, other aspects of the data argue against this explanation.

If supplement use were merely a marker for other aspects of a healthy lifestyle, one would also expect to see effects associated with the use of other types of supplements, including vitamin C. But this was not the case. In both

TABLE 7

Vitamin E intake and heart disease risk (findings from two U.S. epidemiological studies)

Men	
Reduction in heart disease risk associated with high vitamin E intake (top fifth of the cohort, median intake 419 IU / day)	40%
Reduction in heart disease risk associated with vitamin E supplementation (100 IU / day or more for at least 2 years)	37%
Women	
Reduction in heart disease risk associated with high vitamin E intake (top fifth of the cohort, median intake 208 IU / day)	34%
Reduction in heart disease risk associated with vitamin E supplementation (use of single-entity supplements for 2 or more years)	41%

men and women, vitamin C supplementation was not associated with decreased coronary risk. This fact tends to strengthen the case for a specific effect of vitamin E.

The idea that vitamin E might protect against heart disease is also supported by other studies. A cross-cultural study of 16 European population groups (the MONICA study) has shown a strong inverse correlation between blood vitamin E levels and heart disease risk. A study conducted in Scotland showed an inverse correlation between the incidence of angina and blood levels of vitamin E in men. Also, as described below, several trials have shown that high-dose vitamin E supplementation reduces the susceptibility of LDL to oxidation.

The EURAMIC study, referred to previously, provides additional evidence about the relationship of vitamin E and other antioxidants to cardiovascular risk. In one arm of this study, vitamin E and ß-carotene concentrations were measured in 683 men who had recently experienced a myocardial infarction and in 727 control men who remained healthy. Coordinated investigations were conducted in nine European centres (Berlin, Germany; Edinburgh, United Kingdom; Granada, Spain; Helsinki, Finland; Malaga, Spain; Moscow, Russia; Sarpsborg, Norway; Zeist, The Netherlands; and Zürich, Switzerland) and in Jerusalem, Israel. The researchers found that low adipose tissue vitamin E concentrations were not associated with increased myocardial infarction risk. The lack of effect of vitamin E suggests that protection may be seen only in supplement users. The amounts of vitamin E obtained from foods may be insufficient for protection against myocardial infarction.

ß-Carotene

The EURAMIC study, however, showed that low adipose tissue ß-carotene concentrations were associated with significantly increased risk of myocardial infarction, a risk that was mainly confined to current smokers.

The same study of male health professionals that showed an inverse relationship between vitamin E intake and coronary risk also associated high dietary ß-carotene intakes with reduced coronary risk in smokers. As Table 8 shows, current smokers in the top fifth of ß-carotene intake showed a 70% reduction in heart disease risk and former smokers showed a 40% reduction. A preliminary analysis of data from the

TABLE 8

Reduction in heart attack risk associated with high carotene intake (results from the Health Professionals Follow-up Study)

All subjects combined	29% decrease
Current smokers	70% decrease
Former smokers	40% decrease
Lifelong nonsmokers	No significant effect

Source: Eric B Rimm et al. Vitamin E Consumption and the Risk of Coronary Heart Disease in Men. New England Journal of Medicine 328(20) :1450-1456 (May 20, 1993)

parallel women's study, reported only in abstract form, also suggests an inverse association between ß-carotene intake and coronary risk. Further results from the ongoing study are expected shortly. However, these preliminary data show that those in the top fifth of ß-carotene intake (smokers and nonsmokers combined) showed a 22% reduction in heart disease risk.

Vitamin C

As noted above, the U.S. studies of female nurses and male health professionals did not find any association between vitamin C supplementation and coronary risk. However, several other studies have shown effects of vitamin C. For example, the Basel Prospective Study showed that Swiss men with low blood vitamin C levels had an increased risk of dying from a heart attack during 12 years of follow-up compared with men with normal blood vitamin C levels. Studies comparing different European populations indicate that coronary heart disease mortality is higher in those with blood vitamin C levels that are almost in the deficient range. An analysis of a 10-year follow-up study of a representative sample of the U.S. population found that men with the highest vitamin C consumption (at least 50 mg/day from diet plus regular use of supplements) had a 42% lower rate of death from cardiovascular diseases and women had a 25% lower rate. Further research is needed to resolve the discrepancy in epidemiologic findings on vitamin C intake and heart disease risk.

Other dietary antioxidants

A small number of studies have suggested that dietary antioxidants other than vitamin E, vitamin C and carotenoids might be protective against heart disease. For example, in a study conducted in The Netherlands, high intakes of flavonoids (found in black tea, onions, and apples) were associated with decreased coronary mortality in a group of elderly men. Garlic also contains antioxidants, and a U.S. study indicated that garlic supplementation can reduce the susceptibility of LDLto oxidation. It has also been suggested that the apparent protective effect of red wine against heart disease, which is believed to contribute to the relatively low cardiovascular death rate in France, may be due not to the alcohol content of the wine but to its antioxidant content. In vitro, antioxidants isolated from red wine have been found to inhibit the oxidation of LDL. The activity of the natural antioxidant ubiquinol is currently under investigation. All of these studies provide intriguing leads for future research.

Intervention trials

As Table 9 indicates, three major intervention trials of antioxidants and cardiovascular disease are now in progress, two in the United States and one in the United Kingdom (the MRC/BHF Heart Protection Study, starting date 1994). These studies are likely to provide definitive evidence on the effects of ß-carotene supplementation on cardiovascular risk, and two of the trials should provide valuable information about vitamin E as well.

TABLE 9

Cardiovascular disease prevention trials

Scientist/ country	Endpoint	Study group	Sample size	Treatment	Projected completion
Heinonen/Finland	Cardiovascular events	Male smokers	29.000	ß-Carotene and vitamin E	Study completed. See text for discussion of results.
Hennekens/USA	Cardiovascular events	Male physicians	22.000	ß-Carotene (with and without aspirin)	1996 (Preliminary results for subgroup already reported – see text)
Buring/USA	Cardiovascular events	Women over age 50	45.000	ß-Carotene and vitamin E (with and without aspirin)	1998
Peto and Collins/ UK	Cardiovascular events	Subjects at 5-year risk of myocardial infarction	20.000	ß-Carotene, vitamin E and vitamin C (with and without simvastatin)	2000

Source: Antioxidant Vitamins Newsletter, June 1992 and January 1993. Updated and reprinted with permission.

A fourth study, the Alpha-Tocopherol, Beta Carotene Cancer Prevention Trial, conducted among 29.000 male smokers in Finland and designed primarily to study the effect of vitamin E and ß-carotene supplementation on lung cancer, was recently completed (see the cancer section of this monograph). The Finnish smokers who received vitamin E supplements appeared to have lower risks of death from ischæmic heart disease and ischæmic stroke, but an increased risk of death from hæmorrhagic stroke. There were also more deaths from ischæmic heart disease among subjects who were taking ß-carotene.

To obtain preliminary information about the effect of ß-carotene on cardiovascular risk, the investigators conducting the study of U.S. male physicians analysed the data for one small subgroup of their study population — the 333 men with a history of angina. In this high-risk group, those taking ß-carotene (50 mg on alternate days for 2 years or more) showed a 54% reduction in new cardiovascular events compared to those taking a placebo.

Valuable information has also been obtained from short-term trials designed to assess the effect of antioxidant supplementation on the susceptibility of blood lipoproteins to oxidation. In several trials of this type, daily consumption of high-dose vitamin E supplements (800 IU [592 mg ATE] or more), usually for a period of several weeks, has been shown to decrease the susceptibility of LDL from these subjects to in vitro oxidation by copper sulphate. This action appears to be specific to vitamin E; high doses of ß-carotene, another fat-soluble antioxidant, do not have the same effect. Various combinations of antioxidant nutrients have also been shown to be effective in decreasing the oxidation of LDL, but further research is needed to determine whether all of the antioxidants contribute to the effect or whether vitamin E is the only active agent.

Cardiovascular disease: summary

In summary, biochemical studies, epidemiologic investigations and preliminary findings from most intervention trials suggest that antioxidant nutrients may play a role in reducing cardiovascular risk. The evidence is strongest for vitamin E. However, protective effects of vitamin E may be evident only at high doses — much more than can be obtained from a normal diet. Further research is needed to confirm the role of vitamin E and determine the optimal intake. The current evidence on ß-carotene is limited but promising. Clinical trials already in progress should provide definitive answers on the role of this nutrient within the next few years. The evidence on vitamin C and cardiovascular disease is inconsistent, and more study of this nutrient is needed. There is also some limited preliminary evidence suggesting that certain antioxidants other than micronutrient vitamins and minerals in foods may have beneficial effects on cardiovascular health.

EYE DISEASES

Age-related diseases of the eye are major health problems around the world. In technologically developed countries, the treatment of cataracts is one of the largest contributors to total health care costs for the elderly. In less developed countries, where treatment is often unavailable, cataracts are a major cause of blindness in older adults. World-wide, approximately 50 million people are blind from cataracts.

In Western countries, the most common cause of new cases of blindness in the elderly is not cataracts but an age-related disorder of the retina called macular degeneration or maculopathy. Oxidative processes have been implicated in the causation of both cataract and age-related maculopathy. Recent evidence suggests that high dietary intakes of antioxidants may help delay or prevent these disorders.

Cataracts

Cataracts occur when transparent material in the lens of the eye becomes opaque. Much of the material in the lens consists of extremely long-lived proteins, which can become damaged over the decades of a human lifetime. Since there is no direct blood supply to the lens, nutrients enter and waste products are removed by a simple diffusion process which is slow and inefficient. Oxidation, induced primarily by exposure to light, is believed to be a major cause of damage to the proteins of the lens. When these proteins become oxidised, they clump together and precipitate, causing portions of the lens to become cloudy.

The eye has defence systems which protect the lens from oxidative damage. Antioxidants and antioxidant enzymes inactivate harmful free radicals, and proteases (enzymes which break down proteins) selectively remove damaged proteins from the lens. However, these

defence systems cannot always keep pace with oxidative damage. As a result, oxidised proteins may accumulate. As people age, the defence systems grow less effective, and damage to lens proteins may become irreversible.

Several recent epidemiologic studies conducted in Western countries have associated high intakes or blood levels of antioxidant nutrients with reduced rates of cataract. All three of the major dietary antioxidants — vitamin C, vitamin E and carotenoids — have been associated with decreased cataract risk.

For example, a Finnish study showed that people with low blood levels of vitamin E or ß-carotene had higher risks of developing cataracts during a 15-year follow-up period. The increase in risk was greatest among individuals who had low blood levels of both of these antioxidant nutrients.

In the ongoing study of female U.S. nurses, it was found that intake of carotene was inversely associated with cataract risk. In the same study, the risk of cataract was decreased by 45% among women who had used vitamin C supplements for 10 years or more.

A small Canadian epidemiologic study indicated that nonusers of vitamin C supplements were 3.3 times more likely than users to develop cataracts. Similarly, nonusers of vitamin E supplements were 2.3 times more likely than users to develop cataracts.

A multicentre U.S. study found that use of multivitamins was associated with a decreased risk of each of four major types of cataract; high intakes of fruits and vegetables also appeared to be protective. In another U.S. study, low blood vitamin C levels were associated with an 11-fold increased risk of one type of cataract, and low blood carotenoid levels were associated with a 7-fold increased risk of another type of cataract. Low intakes of fruits and vegetables were also associated with increased cataract risk.

In contrast, 2 studies conducted in the Orient had negative results. An epidemiologic study conducted in fishing communities of Hong Kong found no significant associations between blood levels of antioxidant nutrients and cataract. Also, in the recent nutrition intervention trial in Linxian, China, supplementation with ß-carotene / vitamin E / selenium or with vitamin C / molybdenum did not lead to reductions in cataract risk. However, it should be noted that particularly in the Hong Kong study, the subjects were probably exposed to high levels of ultraviolet radiation — a major causative factor in cataract. Interestingly, a riboflavin / niacin supplement did show a significant protective effect, which may be indirectly related to antioxidant defence mechanisms. Riboflavin is a cofactor for the activity of several essential antioxidant enzymes, and intakes of this vitamin in Linxian are marginal at best. The riboflavin / niacin supplement may have exerted its effect by correcting a subclinical riboflavin deficiency, thus enhancing the activity of antioxidant enzymes. However, a similar benefit of riboflavin supplementation would not be expected in Western populations, where intakes of this vitamin are generally ample. The difference between the Western and Asian findings may reflect differences in overall nutritional status and cataract risk factors in the two types of populations.

Age-related macular degeneration

Some scientific evidence suggests that excessive exposure to light and the resulting production of oxidants contribute to the causation of age-related maculopathy. In particular, exposure to blue light has been implicated in this disorder. If blue light is involved, carotenoids might be protective, since they can absorb blue light.

An analysis of data from a national U.S. survey showed an inverse correlation between age-related maculopathy

TABLE 10

Some clinical conditions in which oxygen free radicals are thought to be involved

Brain and nervous system
Parkinson's disease
Tardive dyskinesia
Amyotrophic lateral sclerosis
Actions of neurotoxins
Vitamin E deficiency
Hyperbaric oxygen
Hypertensive cerebrovascular injury
Aluminium overload
Allergic encephalomyelitis (demyelinating diseases)
Potentiation of traumatic injury

Eye
Cataract
Age-related macular degeneration
Photic retinopathy
Ocular haemorrhage
Retinopathy of prematurity

Heart and cardiovascular system
Atherosclerosis
Adriamycin cardiotoxicity
Keshan disease (selenium deficiency)
Alcohol cardiomyopathy

Kidney
Metal ion-mediated nephrotoxicity
Aminoglycoside nephrotoxicity
Autoimmune nephrotic syndromes

Reproductive functions
Sperm abnormalities
Germ-line mutations leading to congenital malformations
Childhood cancer
Hypertensive complications of pregnancy

Gastrointestinal tract
Nonsteroid-anti-inflammatory-drug– induced GI tract lesions
Oral iron poisoning
Endotoxin liver injury
Diabetogenic actions of alloxan
Halogenated hydrocarbon liver injury
Free-fatty-acid–induced pancreatitis

$Alpha_1$-antitrypsin deficiency

Lung
Bronchopulmonary dysplasia
Mineral dust pneumoconiosis
Bleomycin toxicity
Hypoxia
Cigarette smoke effect
Emphysema
Adult respiratory distress syndrome (some forms)
Effects of oxidant pollutants (ozone, SO_2, NO_2)

Red blood cells
Falconi's anaemia
Sickle cell anaemia
Favism
Malaria
Protoporphyrin photo-oxidation

Cancer

Alcohol-related diseases

Ageing

Radiation injury

Inflammatory disorders/immune function
Age-related decline in immune function
Autoimmune diseases
Rheumatoid arthritis
Glomerulonephritis
Vasculitis (hepatitis B virus)

Leprosy

Iron overload
Nutritional deficiencies
Dietary iron overload
Idiopathic hemochromatosis

Ischæmia-reperfusion
Stroke/myocardial infarction
Organ transplantation

Exercise-induced oxidative stress

Adapted from Aruoma OI, Kaur H, Halliwell B, Oxygen Free Radicals and Human Diseases, Journal of the Royal Society of Health 1991;111:172–177, with additional information from more recent publications.

and the consumption of "vitamin A–rich" fruits and vegetables. The "vitamin A" found in these foods actually consists of carotenoid precursors of the vitamin rather than preformed vitamin A (retinol).

A large multicentre U.S. case-control study that focused on the most severe form of age-related maculopathy found markedly reduced risks in individuals with high blood carotenoid levels. Significant associations were detected for the sum of all carotenoids and for four of five individual carotenoids (ß-carotene, α-carotene, cryptoxanthin and lutein/zeaxanthin, but not lycopene). Vitamin C and vitamin E did not show significant effects.

In contrast, a much smaller British study that included all types of age-related macular degeneration found no association between serum carotenoid or vitamin E levels and the risk of macular degeneration. The difference between these findings and those of the U.S. study may reflect differences in sample size or differences in the pathogenesis of the different types of maculopathy.

In summary, there is evidence suggesting that oxidative processes may play a role in causing age-related disorders of both the lens and the retina of the eye. All of the published epidemiologic studies of antioxidants and cataract in Western populations have shown significant associations for at least one antioxidant nutrient. The overall body of evidence suggests that all three major dietary antioxidants — vitamin C, vitamin E and carotenoids — may be beneficial in reducing cataract risk in Western populations. Less is known about age-related macular degeneration, but some epidemiologic evidence suggests an inverse association with carotenoids. Further research is needed to determine conclusively whether improved nutrition may reduce the risk of these very prevalent eye disorders in older adults.

OTHER DISEASES AND PATHOLOGICAL PROCESSES

Although most research on oxidants, antioxidants and disease has focused on cancer, cardiovascular disease and degenerative disorders of the eye, oxidative processes are also implicated in a wide variety of other clinical conditions and pathological processes, as shown in Table 10. A few examples of particular interest are described below.

Neurological disorders

Biochemical studies suggest that oxidation may play a role in the causation of several disorders of the brain and nervous system. Therefore, it has been hypothesized that antioxidants might be helpful in ameliorating the symptoms or in slowing the progression of some neurological disorders.

Some studies have shown beneficial effects of vitamin E supplementation in decreasing the severity of tardive dyskinesia; others have not found an effect. Tardive dyskinesia is a disorder involving involuntary movement. It occurs as a side effect of long-term treatment with certain antipsychotic drugs.

An open pilot trial indicated that supplementation with vitamins C and E might be of benefit in slowing the progression of Parkinson's disease. However, a larger placebo-controlled trial found no benefit from vitamin E therapy.

Sperm damage, birth defects and childhood cancer

The children of fathers who smoke cigarettes have increased rates of congenital malformations and

childhood cancer. These problems may be related, at least in part, to increased oxidative damage to sperm cells caused by oxidants in cigarette smoke. Cigarette smoking is associated with reduced sperm count and poor sperm quality; it is also associated with decreased blood vitamin C levels. Vitamin C supplementation has been shown to improve sperm quality in heavy smokers. Ample intakes of vitamin C have also been shown to reduce oxidative damage to sperm DNA. Further studies are needed to determine whether improved antioxidant status, particularly with respect to vitamin C, is of benefit in reducing infertility and germ-line mutations in men who smoke cigarettes or who are exposed to oxidative stress from other causes.

Exercise-induced oxidative stress

Because exercise leads to increased oxygen consumption, it causes an increase in the production of oxygen-initiated free radicals. Some studies have reported that antioxidant supplementation reduces evidence of exercise-induced muscle damage. Antioxidants may also play a role in reducing muscle soreness after overexertion. However, it is unclear whether antioxidants can directly enhance physical performance.

Inflammatory disorders

Free radicals and oxidative stress may play a role in inflammatory diseases. Rheumatoid arthritis is one example. The products of free radical reactions have been detected in the blood and joints of patients with this disease. Other lines of evidence also suggest the involvement of oxidative stress in rheumatoid arthritis and in other inflammatory diseases such as glomerulonephritis.

Decreases in immune function

Several aspects of immune function show a marked decline with increasing age. Preliminary studies in elderly people have indicated that this decline can be partly offset by dietary antioxidant supplementation. The age-associated decrease in cell-mediated immunity may be due to a decreased level of small-molecular-weight antioxidants and decreased activity of antioxidant enzymes.

OXIDANTS, ANTIOXIDANTS, AND DISEASE: IMPLICATIONS OF THE SCIENTIFIC EVIDENCE

Although much remains to be learned, there is now convincing evidence for the involvement of free radicals in a number of diseases which constitute major public health problems throughout the world. Free radicals and oxidative processes are believed to play important roles in the pathogenesis of many age-related disorders. The evidence implicating them in the causation of cancer, atherosclerotic cardiovascular disease and cataracts is especially strong.

The human body has a complex system of natural enzymatic and nonenzymatic antioxidant defences which counteract the harmful effects of free radicals and other oxidants. Protection against free radicals can be enhanced by ample intakes of dietary antioxidants, of which the best studied are vitamin E, vitamin C and carotenoids. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention. Efforts should be made to ensure optimum intakes of foods containing these important molecules. There is a need for improvement in the quality of the diet,

especially with respect to increased consumption of fruits and vegetables. However, other strategies, including optimisation of food processing, selective fortification of foods and the use of safe nutritional supplements, may also need to be considered. All three of the major antioxidant nutrients — vitamin E, vitamin C and ß-carotene — are safe even at relatively high levels of intake. There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term.

Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, there is a potential for substantial savings in the cost of health care delivery.

More research is needed to clarify and extend scientific understanding of the health effects of antioxidants. Basic research should continue, and additional large-scale randomised trials and clinical studies should be undertaken. Funding for research in this field is urgently needed.

At the same time, efforts should also be made to communicate to the general public existing information about the importance of protective nutrients in fruits and vegetables. Government agencies, health professionals and the news media should work together to promote the dissemination of scientifically sound information about this aspect of nutrition to all segments of the population.

GLOSSARY

α-Carotene: A carotenoid found in carrots and certain other vegetables. Its provitamin A activity is weaker than that of ß-carotene.

Antioxidant: Any substance which can delay or inhibit oxidation.

ß-Carotene: The most abundant of the carotenoids. ß-Carotene has strong provitamin A activity. Unlike vitamin A itself, ß-carotene is a strong antioxidant.

ß-Cryptoxanthin: A carotenoid pigment found in citrus fruits.

Biomarker: An indicator of an exposure or an effect in a biological system.

Carcinogenesis: The complex, multistep process of cancer causation.

Carotenoids: A group of red, orange, and yellow pigments found in plant foods and in the tissues of organisms that consume plants. Carotenoids have antioxidant activity, and some, but not all, can act as precursors of vitamin A.

Cataract: A disorder in which the lens of the eye becomes partly or completely opaque as a result of the precipitation of proteins.

Epidemiology: The study of the causes and distribution of disease in human populations.

Extracellular: Inside the body, but not within cells.

Free radical: Any chemical species capable of independent existence that contains one or more unpaired electrons. Most free radicals are unstable and highly reactive.

Glutathione: A small-molecular-weight antioxidant molecule produced naturally in the human body and present in some foods.

Glutathione peroxidases: A family of antioxidant enzymes containing selenium which are important in the reduction of different hydroperoxides.

In vitro: From the Latin meaning "in glass". The term is applied to biological processes studied experimentally in isolation from the organism, as distinct from in vivo, which refers to the study of processes in the living organism.

Intracellular: Within cells.

Ischæmia: Reduced or inadequate blood supply to a part of the body.

Low-density lipoprotein (LDL): A complex of lipid and protein found in the blood which is important for the transport of cholesterol around the body. Raised levels are a risk factor for coronary heart disease.

Lutein: A carotenoid found primarily in green leafy vegetables. Lutein has no provitamin A activity.

Lycopene: A carotenoid found primarily in tomatoes. Lycopene has antioxidant activity but does not act as a precursor of vitamin A.

Oxidative stress: A condition in which the production of oxidants and free radicals exceeds the body's ability to inactivate them.

Placebo: A harmless and pharmacologically inactive substance, usually disguised, given to compare its effect with that of an active material.

Reperfusion: The reoxygenation of tissue that has been deprived of adequate oxygen (ischæmia) as a result of either surgical procedures or physiological dysfunction. Vital organs can tolerate only a brief period of oxygen deprivation before cell injury and death occur. Subsequent reperfusion, however, can also cause tissue damage. Ischæmia reperfusion damage can be prevented or decreased in the presence of antioxidants.

Tocopherols, tocotrienols: Forms of vitamin E.

Ubiquinol: An antioxidant produced by normal metabolism. Ubiquinol is the only known fat-soluble antioxidant synthesised by animal cells. It is believed to play an important role in cellular defence against oxidative damage. Ubiquinol can also contribute to the antioxidant defence system by regenerating the active form of vitamin E after that vitamin has reacted with a free radical. (The water-soluble vitamin C can also perform this function).

Vitamin A: Retinol or its fatty acid ester.

Vitamin C: Ascorbic acid, ascorbate. A major water-soluble antioxidant.

Vitamin E : A collective name for 8 tocopherols and tocotrienols. A major fat-soluble antioxidant.

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