OVERVIEW

This report focuses on vitamin C, vitamin E, selenium, and β-carotene and other carotenoids (α-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin). These compounds have frequently been called dietary antioxidants since in some cases they counteract oxidative damage to biomolecules (Halliwell, 1996), and the possibility exists that increased intakes of these compounds may protect against chronic disease. Although the term dietary antioxidants is a convenient description, these compounds are multifunctional, and some of the actions observed in vivo may not represent an antioxidant function, even though the compounds have been classified as antioxidant nutrients (Sies and Stahl, 1995).

Therefore, in this report the above compounds were evaluated with respect to their role in human nutrition, without limiting the investigation to antioxidant properties. Information was reviewed regarding the minimum amount of these nutrients required to prevent deficiency diseases, as well as the amounts that might impact on chronic diseases, regardless of whether the effect was an antioxidant effect or not. Resolution of any impact of these compounds on chronic disease will require evaluation of the many human intervention trials that are still under way (Table 2-1).

Four main tasks were assigned to the Dietary Reference Intakes Panel on Dietary Antioxidants and Related Compounds. The first task was to develop a definition of a dietary antioxidant; the second
### TABLE 2-1 Intervention Trials: Antioxidants and Chronic Diseases

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Study Type&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Study Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin Cancer Prevention Study</td>
<td>U.S.&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Secondary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>1,805 men and women with recent nonmelanoma skin cancer; aged 40–89 y</td>
</tr>
<tr>
<td>Linxian Cancer Prevention Study</td>
<td>China</td>
<td>Primary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>29,584 poorly nourished men and women, aged 40–69 y</td>
</tr>
<tr>
<td>α-Tocopherol, β-Carotene Cancer</td>
<td>Finland</td>
<td>Primary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>29,133 male cigarette smokers, aged 50–69 y</td>
</tr>
<tr>
<td>Prevention Study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Polyp Prevention Study</td>
<td>U.S.</td>
<td>Secondary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>864 men and women with recent nonmelanoma skin cancer</td>
</tr>
<tr>
<td>(Greenberg et al., 1994)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The β-Carotene and Retinol Efficacy</td>
<td>U.S.</td>
<td>Primary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>14,254 heavy smokers and 4,060 asbestos workers, aged 45–69 y</td>
</tr>
<tr>
<td>Trial (Omenn et al., 1996)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cambridge Heart Antioxidant Study</td>
<td>U.K.&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Secondary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>2,002 patients with coronary atherosclerosis, mean age 62 y</td>
</tr>
</tbody>
</table>
| Duration of Treatment (y) | Daily Dose | Primary Disease | Outcome | Results $^b$
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>50 mg β-carotene</td>
<td>Skin cancer</td>
<td></td>
<td>No effect on occurrence of new nonmelanoma skin cancers</td>
</tr>
<tr>
<td>5.25</td>
<td>15 mg β-carotene, 30 mg α-tocopherol, 50 mg selenium</td>
<td>Cancer</td>
<td></td>
<td>9% reduction in total mortality; 13% decrease in cancer mortality; 21% decrease in stomach cancer deaths; 10% decrease in cerebrovascular mortality (nonsignificant)</td>
</tr>
<tr>
<td>6</td>
<td>50 mg α-tocopherol and/or 20 mg β-carotene</td>
<td>Lung cancer</td>
<td></td>
<td>50% increase in hemorrhagic stroke deaths among vitamin E group; 11% increase in ischemic heart disease deaths among β-carotene group; 18% increase in lung cancer among β-carotene group; no effect of vitamin E on lung cancer</td>
</tr>
<tr>
<td>4</td>
<td>25 mg β-carotene, 1,000 mg vitamin C, 400 mg α-tocopherol</td>
<td>Colorectal cancer</td>
<td></td>
<td>No reduced incidence of adenomas</td>
</tr>
<tr>
<td>4</td>
<td>30 mg β-carotene, 25,000 IU retinol (as retinyl palmitate)</td>
<td>Lung cancer</td>
<td></td>
<td>28% increase in lung cancer; 26% increase in CVD $^d$ (nonsignificant); 17% increase in total mortality among treatment group</td>
</tr>
<tr>
<td>1.4</td>
<td>400 or 800 IU (268 or 537 mg) α-tocopherol</td>
<td>CVD death or nonfatal MI</td>
<td></td>
<td>77% decrease in risk of subsequent nonfatal MI; no benefit on cardiovascular mortality</td>
</tr>
</tbody>
</table>

continued
<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Study Type&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Study Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physicians’ Health Study (Hennekens et al., 1996)</td>
<td>U.S.</td>
<td>Primary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>22,071 male physicians, aged 40–84 y</td>
</tr>
<tr>
<td>Nutritional Prevention of Cancer Study (Clark et al., 1996, 1998)</td>
<td>U.S.</td>
<td>Secondary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>1,312 men and women with history of basal or squamous cell carcinoma; aged 18–80 y</td>
</tr>
<tr>
<td>GISSI-Prevention Trial (GISSI-Prevenzione Investigatori; 1999)</td>
<td>Italy</td>
<td>Secondary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>11,324 patients with recent MI</td>
</tr>
<tr>
<td>Women’s Health Study (Lee et al., 1999)</td>
<td>U.S.</td>
<td>Primary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>39,876 healthy women, aged ≥45 y</td>
</tr>
<tr>
<td>Heart Outcomes Prevention Evaluation Study (HOPE Study Investigators, 2000)</td>
<td>Canada</td>
<td>Secondary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>9,541 high-risk men and women, aged ≥55 y</td>
</tr>
<tr>
<td>MRC/BHF Heart Protection Study (MRC/BHF, 1999)</td>
<td>U.K.</td>
<td>Secondary prevention trial</td>
<td>20,536 high-risk men and women, aged 40–80 y</td>
</tr>
</tbody>
</table>

<sup>a</sup> Study Type: Primary prevention = prevention of coronary events in healthy persons; secondary prevention = prevention of coronary events in persons with established coronary disease; randomized = randomized, double-blind, placebo-controlled intervention; MI = myocardial infarction.
<table>
<thead>
<tr>
<th>Duration of Treatment (y)</th>
<th>Daily Dose</th>
<th>Primary Disease</th>
<th>Outcome</th>
<th>Results&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>50 mg β-carotene (alternate days)</td>
<td>Cancer, CVD</td>
<td>No effects on CVD or cancer including among smokers</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>200 µg selenium</td>
<td>Skin cancer, prostate cancer</td>
<td>No effect on incidence of skin cancer; 63% reduction in prostate cancer incidence; reduction in total cancer mortality and total cancer incidence</td>
<td></td>
</tr>
<tr>
<td>3–5</td>
<td>300 mg α-tocopherol and/or 1 g ω-3 PUFA&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Total mortality</td>
<td>No benefit from vitamin E; 15% decrease in risk of death, nonfatal MI, and stroke from ω-3 PUFA</td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>50 mg β-carotene (alternate days)</td>
<td>MI, stroke, or CVD death</td>
<td>No effect on incidence of cancer, CVD, or total mortality</td>
<td></td>
</tr>
<tr>
<td>4–6</td>
<td>400 IU (268 mg) α-tocopherol, ACE&lt;sup&gt;h&lt;/sup&gt; inhibitor</td>
<td>MI, stroke, or CVD death</td>
<td>No benefit from vitamin E</td>
<td></td>
</tr>
<tr>
<td>≥5</td>
<td>20 mg β-carotene, 600 mg α-tocopherol, 250 mg vitamin C</td>
<td>Total mortality</td>
<td>No results yet</td>
<td></td>
</tr>
</tbody>
</table>

*continued*
<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Study Type&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Study Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women’s Antioxidant Cardiovascular Study</td>
<td>U.S.</td>
<td>Secondary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>8,000 women with prior CVD event or ≥3 coronary risk factors, aged ≥40 y</td>
</tr>
<tr>
<td>(Manson et al., 1995)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women’s Health Study</td>
<td>U.S.</td>
<td>Primary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>39,876 healthy women, aged ≥45 y</td>
</tr>
<tr>
<td>(Buring and Hennekens, 1992)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physician's Health Study II</td>
<td>U.S.</td>
<td>Primary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>15,000 healthy male physicians, aged ≥55 y</td>
</tr>
<tr>
<td>(Hennekens, 1998)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUVIMAX</td>
<td>France</td>
<td>Primary prevention; randomized, double-blind, placebo-controlled intervention</td>
<td>12,735 men and women, aged 35–60 y</td>
</tr>
<tr>
<td>(Hercberg et al., 1998)</td>
<td></td>
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</tr>
</tbody>
</table>

<sup>a</sup> A primary prevention trial is one in which the study participants have no history of the disease outcome being investigated. Participants in a secondary prevention trial have had a prior occurrence of the outcome being investigated.

<sup>b</sup> Unless noted otherwise, results are statistically significant.

<sup>c</sup> U.S. = United States.

<sup>d</sup> CVD = cardiovascular disease.

task was to select, in addition to vitamin C, vitamin E, and β-carotene, other food components which might prove to be antioxidants and play a role in health; the third task was to assess the role of these compounds in health; and the fourth task was to develop Dietary Reference Intakes (DRIs) for the selected nutrients. The panel was asked to evaluate vitamin C, vitamin E, and β-carotene and other antioxidants. Since other dietary carotenoids share many,
| Duration of Treatment (y) | Daily Dose                                                                 | Primary Disease Outcome | Results
<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>50 mg β-carotene, 600 IU α-tocopherol, 500 mg vitamin C (alternate days)</td>
<td>CVD</td>
<td>No results yet</td>
</tr>
<tr>
<td>NR</td>
<td>600 IU α-tocopherol, 100 mg aspirin (alternate days)</td>
<td>MI, stroke, or CVD death</td>
<td>No results yet</td>
</tr>
<tr>
<td>NR</td>
<td>50 mg β-carotene, 400 IU α-tocopherol (alternate days), 500 mg vitamin C, multivitamin (centrum silver)</td>
<td>CVD, cancer, eye diseases</td>
<td>No results yet</td>
</tr>
<tr>
<td>8</td>
<td>6 mg β-carotene, 30 mg α-tocopherol, 120 mg vitamin C, 100 µg selenium, 20 mg zinc</td>
<td>Cancer, ischemic heart disease</td>
<td>No results yet</td>
</tr>
</tbody>
</table>

* U.K. = United Kingdom.
* MI = myocardial infarction.
* PUFA = polyunsaturated fatty acid.
* ACE = angiotension-converting enzyme.
* NR = not reported.

Although not all of the properties of β-carotene, additional carotenoids (α-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin) were added as related nutrients that would be investigated. In addition, because of the important role that selenium plays as a cofactor for oxidant defense enzymes, it was also included as a related nutrient.
Definition and Criteria for a Dietary Antioxidant

Definition of a Dietary Antioxidant

The definition was based on several criteria: (1) the substance is found in human diets; (2) the content of the substance has been measured in foods commonly consumed; and (3) in humans, the substance decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species in vivo. Thus, the definition of a dietary antioxidant is as follows:

*A dietary antioxidant is a substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological function in humans.*

This stringent definition, with supporting information, was slightly modified from the proposed definition published earlier (IOM, 1998). The earlier report stated that the nutritional recommendations that will be presented in the final report for some of these dietary components may not be determined by or related to their possible action as antioxidants. The primary indicators for developing the Dietary Reference Intakes (DRIs) are based on specific actions and effects of the compounds that may or may not be attributed to their antioxidant activity. In particular, criteria chosen upon which to base EARs and thus RDAs for any of the compounds investigated are not related specifically to ameliorating chronic diseases because a scientific justification for such a requirement was not found.

The European Commission Concerted Action on Functional Food also adopted stringent criteria regarding the relationship between free-radical events and human disease, and whether antioxidants are capable of modulating these events and thus reducing the risk of disease (Diplock et al., 1998). The summary report concluded that, “there is at present insufficient evidence available on which to base a firm conclusion that antioxidants are capable of reducing risk of disease” (Diplock, 1998). Recommendations for the nutrients in this report are similar. However, it should be pointed out that a large number of human intervention studies are under way using the compounds discussed in this report, and the results of these studies may give rise to new conclusions.
Are Vitamin C, Vitamin E, Selenium, and β-Carotene and Other Carotenoids Dietary Antioxidants?

Vitamin C functions physiologically as a water-soluble antioxidant by virtue of its very strong reducing power (high redox potential) and facile regeneration via ubiquitous reductants such as glutathione, nicotinamide adenine dinucleotide, and nicotinamide adenine dinucleotide phosphate. The primary method used to estimate the requirement relates to the vitamin C intake needed to maintain near-maximal neutrophil ascorbate concentration with minimal urinary excretion. Because smokers suffer increased oxidative stress and metabolic turnover of vitamin C, their requirement is increased. Although there is ample evidence that vitamin C administration can result in decreases in markers of oxidative stress, a Recommended Dietary Allowance (RDA) derived from a direct antioxidant function of vitamin C could not be calculated because of the lack of a quantitative relationship between this antioxidant function and a health-related endpoint. Since vitamin C reduces markers of oxidative stress, it meets the definition of a dietary antioxidant.

Vitamin E functions as a chain-breaking antioxidant that prevents the propagation of lipid peroxidation. To estimate the requirement, data were examined on the intake of vitamin E that would prevent hydrogen peroxide-induced lysis of erythrocytes. Under these circumstances, vitamin E is acting as an ex vivo antioxidant, maintaining a normal physiological function in humans. Although it is not yet possible to relate vitamin E intake to a lowering of chronic disease risk, it still meets the definition of a dietary antioxidant.

Selenium functions through selenoproteins, several of which are oxidant defense enzymes. The criterion used to estimate the requirement for selenium relates to the intake needed to maximize the activity of the plasma selenoprotein glutathione peroxidase, an oxidant defense enzyme. It is not clear if the diseases associated with selenium deficiencies, Keshan disease or Kashin-Beck disease, are due to oxidative stress. The selenium in several selenoproteins has a biochemical role in oxidant defense, thus maintaining normal physiological function, and as such plays a role as a dietary antioxidant.

β-Carotene and other carotenoids function as sources of vitamin A and, due to this provitamin A activity, can prevent vitamin A deficiency. Because no other specific nutrient functions have been identified at this time, no requirements have been established for any of the carotenoids. β-Carotene and the other carotenoids display in vitro antioxidant activity, but the evidence that they act as in vivo...
antioxidants in humans is still controversial. Therefore, β-carotene does not meet the definition of a dietary antioxidant. Similar conclusions have been drawn for α-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin.

**OXIDATIVE STRESS, ANTIOXIDANTS, AND CHRONIC DISEASE**

*Oxidative Stress*

There have been many proposals as to how oxidative stress can be defined. Because all cells are exposed to oxidants, generated either endogenously from metabolism or exogenously from a variety of environmental insults, the problem arises as to what constitutes an oxidative stress. Furthermore, different cells can be exposed to the same level of oxidants, but depending on the level of antioxidants or protective mechanisms available to the cell, they may or may not experience an oxidative stress. Sies (1985) defined oxidative stress as “a disturbance in the prooxidant-antioxidant balance in favor of the former.” Others have amplified this definition to include “short- and/or long-term disturbance of the prooxidant-antioxidant balance resulting in adverse effects that are due either to impaired antioxidation or to favored prooxidation” (Biesalski et al., 1997). Here, oxidative stress is defined as an imbalance between the production of various reactive species and the ability of the organism’s natural protective mechanisms to cope with these reactive compounds and prevent adverse effects.

The primary reactive species include reactive oxygen species (ROS) and reactive nitrogen species (RNS). These in turn react in the body and generate radical intermediates of lipids, proteins, and nucleic acids that ultimately form the chemical end products of oxidative stress. The physiological consequences of these end products have been hypothesized to be the causes of many chronic diseases as well as the natural aging process (Ames, 1998; Halliwell, 1997). The protective mechanisms include protective enzymes, antioxidant or quenching compounds produced by the organism, and similar compounds made available in the diet. The evidence that chronic disease results from an imbalance between formation and removal of reactive species is discussed below.

The primary defensive compounds are antioxidants that can interact with and quench reactive radical species, and enzymes that can inactivate these species or their products. All of the compounds in this report—vitamin C, vitamin E, selenium in the form of selenoproteins, and β-carotene and other carotenoids—are in vitro
antioxidants, and some evidence exists that they have in vivo antioxidant actions. The protective enzymes include, among others, superoxide dismutase, catalase, and selenium-containing glutathione peroxidases and thioredoxin reductase. Other antioxidant mechanisms include stimulation of the expression of antioxidant or repair enzymes, as well as chelation of transition metals. A recent review of the relationship between antioxidant supplementation and oxidative damage concludes that, with the exception of vitamin E and possibly vitamin C reducing markers of lipid peroxidation, the evidence is insufficient that antioxidant supplementation leads to a material reduction in oxidative damage in humans (McCall and Frei, 1999).

**Biomarkers of Oxidative Stress**

There have been numerous tests described to evaluate the level of markers of oxidative stress in living systems. Many of these markers represent oxidative breakdown products of normal tissue components and metabolites. Products derived from oxidized lipids include age pigments (lipofuscin), aldehydes, alkanes, and prostanoids such as the F2-isoprostanes derived from unsaturated fatty acids. Oxidation of proteins produces protein carbonyls and amino acid derivatives such as methionine sulfoxide and nitrotyrosine, derived from the reaction of peroxynitrite (ONOO\(^-\)) with tyrosine. Purine and pyrimidine metabolites that are derived from oxidized nucleic acids can be detected in tissues and in urine. Since virtually all tissues are exposed to ROS and RNS, there will always be a baseline production of these biomarker molecules. What is important in evaluating the extent of oxidative stress is the change in the level of the biomarker compared to a baseline or steady-state level.

It is quite clear that dietary change can alter the levels of some of the biomarkers described above, and this phenomenon has been reported when the dietary intake of fruits and vegetables has been increased. Examples of such studies include reductions in biomarkers of deoxyribonucleic acid (DNA) damage (Pool-Zobel et al., 1997) or of lipid peroxidation (Miller et al., 1998), following consumption of diets rich in fruits and vegetables. Many of these studies have been reviewed recently (Halliwell, 1999).

A major problem with the use of biomarkers such as those described above is not knowing whether they reflect processes in the initiation of a disease state or whether they are products of a disease state. The validity of biomarkers, either intermediate or final, will not be settled until they are evaluated as part of intervention trials.
A causal relationship between the formation of a biomarker of oxidant stress and a chronic disease has not yet been validated.

Some attempts have been made to determine a more global measure of oxidative stress, such as TRAP (total radical-trapping antioxidant capability) (Wayner et al., 1985), ORAC (oxygen radical absorbance capacity) (Cao et al., 1993), TEAC (trolox equivalent antioxidant capacity) (Miller et al., 1993), and FOX (ferrous oxidation/xylenol orange) (Jiang et al., 1992) assays. However, because these markers do not measure the same oxidants or antioxidant defenses (Cao and Prior, 1998), it is difficult to validate them as useful markers of oxidant stress.

**Evidence of Oxidative Stress and Chronic Disease**

Aerobic metabolism produces energy, waste products such as carbon dioxide, and a small, but steady stream of radical by-products that are capable of reacting with all of the body’s constituents to form oxidative damage products (Ames et al., 1993; Chance et al., 1979). Calculations have been made of the number of radical species formed per cell per day, but it is not at all clear how these numbers relate to diseases. Since the entire population is exposed to oxidative stresses and only a small fraction develops a chronic disease, it is clear that at this time, it is not understood how to evaluate the role of oxidative stress in the development of chronic disease. The potential role of oxidative stress in six chronic disease areas and aging is described briefly below.

**Cancer**

DNA is subject to damage, and either an exogenous or an endogenous mutagen can produce damage at a faster rate than the normal protective process of enzymatic repair (Ames et al., 1995). Under these circumstances, elevated levels of excision products will appear in the urine. The observation that 8-oxy-7,8-dihydro-2′-deoxyguanosine (8-oxodG) is a major urinary product of both oxidative damage to DNA and damage by ionizing radiation that produces ROS, has served as the basis for the hypothesis that oxidative DNA damage is carcinogenic (Ames et al., 1995). The presence of 8-oxodG in DNA would lead to a guanine-to-thymine transversion that would result in a DNA mutation and, depending on its location, an altered gene product. 8-OxodG is also produced by ionizing radiation, and since this process can be carcinogenic, it has been assumed that any condition that produces 8-oxodG will also
be carcinogenic. However, a causal relationship between oxidative stress to DNA and cancer in humans has not yet been established (Poulsen et al., 1998).

A great deal of evidence based on epidemiological studies indicates that consuming diets rich in fruits and vegetables is associated with both a decrease in oxidative damage to DNA (Halliwell, 1998) and a lower risk of a number of common cancers. There are several mechanisms that could account for these observations, in addition to antioxidant components that scavenge radical intermediates. Other mechanisms include the modification of carcinogen activation by the inhibition of phase 1 enzymes, modification of carcinogen detoxification by phase 2 enzymes, and suppression of the abnormal proliferation associated with preneoplastic lesions (Wargovich, 1997).

Cardiovascular Disease

Of all the chronic diseases in which excess oxidative stress has been implicated, cardiovascular disease has the strongest supporting evidence. A coherent pathogenetic mechanism has been developed to account for the earliest stage in atherogenesis, namely, the development of the fatty streak lesion. Hypercholesterolemia and, particularly, increased concentrations of low-density lipoproteins (LDLs) cause the accumulation of cholesterol-loaded “foam cells” beneath the endothelial lining of major arteries, which in turn develop into a fatty streak. This lesion is clinically benign but is the precursor of later lesions (the fibrous plaque and the complex lesion) that ultimately give rise to clinical manifestations (angina pectoris and myocardial infarction) (Steinberg and Witztum, 1990).

An elevated LDL cholesterol level sufficiently increases the risk of cardiovascular disease. Brown and Goldstein (1986) demonstrated that the molecular defect in individuals with familial hypercholesterolemia was the absence of functional LDL receptors. The histological lesions in these patients cannot be differentiated from that of lesions in individuals who have normal LDL receptors. The implication therefore is that normal LDL taken up by way of the normal LDL receptor cannot be the basis for the formation of foam cells. In other words, the LDL must first be modified somehow and the modified form must be taken up into the monocytes or macrophages via one or more alternative receptors ultimately and develop into foam cells. One such modification, and the most extensively studied, is LDL oxidation, and several macrophage receptors have been shown to take up oxidatively modified LDL (oxLDL) and
thus develop foam cells (Navab et al., 1995; Steinberg, 1997; Steinberg and Witztum, 1990; Steinberg et al., 1989; Steinbrecher, 1997).

In addition to its ability to cause foam cell formation, oxLDL can contribute to atherogenesis by virtue of a number of properties that differ from those of normal LDL (reviewed in Steinberg, 1997). The key question is whether these potentially proatherogenic properties of oxLDL are of sufficient importance that inhibition of the generation of oxLDL by antioxidants will have a significant impact on the rate of progression of atherosclerotic lesions.

The finding that a relationship existed between LDL levels and the incidence of cardiovascular disease was followed by the observation that oxLDL was associated with the development of atherosclerotic lesions in experimental animals. This association led to the hypothesis that oxLDL is the causative agent in the development of cardiovascular disease (Steinberg et al., 1989). This hypothesis has served as the basis for a number of human intervention trials, testing whether antioxidant agents capable of decreasing the extent of oxidation of LDL and thus decreasing oxLDL concentration might prove useful in decreasing the incidence of cardiovascular disease.

Several biomarkers of oxidative stress have been used to evaluate the extent of oxLDL formation and/or the extent of oxidative damage to lipids in general. OxLDL formation has been assessed after treating individuals with dietary antioxidants and evaluating the presence of oxLDL. In addition there have been reports of a potent effect of supplementary antioxidants in lowering the extent of ex vivo oxLDL formation. The results are not consistent, and as such, it is still not possible to conclude that dietary antioxidants prevent the formation of oxLDL in vivo. In addition, this modulation of LDL oxidation still must be validated as a marker of risk for cardiovascular disease (Zock and Katan, 1998).

The appearance of F₂-isoprostanes in urine has been suggested by a number of investigators as a reliable index of in vivo free radical generation and oxidative lipid formation. There is very strong evidence from animal studies that F₂-isoprostanes increase in plasma and urine as a result of oxidative stress, and in humans, these products are elevated in smokers (Morrow et al., 1995). Evidence is gradually accumulating that supplementary antioxidants, and vitamin E in particular, can both affect the level of F₂-isoprostanes in animal models of atherosclerosis and decrease the extent of arterial wall lesions (Pratico et al., 1998). Similar results have been obtained in humans (Hodis et al., 1995). Although some reviews point out that the protective role of antioxidants in animal models of atherosclerosis is only partially confirmed in human studies (Faggiotto et al.,
1998), others are much more supportive of a protective role for vitamin E in coronary heart disease (Pryor, 2000).

Cataracts

There have been a number of observational epidemiological studies of the risk of developing cataracts in humans. Many of these studies indicate that the risk of cataracts may be inversely proportional to the serum level of antioxidants (Knekt et al., 1992; Taylor et al., 1995) or may be reduced by supplement use (Jacques et al., 1997). These studies, however, have been considered to be inconclusive (Christen et al., 1996a; Leske et al., 1998). In the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, 5 to 8 years of daily supplementation with either 50 mg of vitamin E or 20 mg of β-carotene or both resulted in no difference in the prevalence of cataracts in the men in this study (Teikari et al., 1997).

Age-Related Macular Degeneration

This irreversible disease, which is the major form of blindness in the elderly in the United States, Canada, and Europe, has been related to antioxidants found in the diet. This is because the pigment in the macular region of the normal retina consists of the two xanthophyll carotenoids, lutein and zeaxanthin (Bone et al., 1988; Handelman et al., 1988). This observation, coupled with the epidemiological observations of an inverse relationship between the risk of age-related macular degeneration (AMD) and the ingestion of fruits and vegetables (Goldberg et al., 1988), led a number of groups to propose that the basis of this chronic disease was a nutritional deficiency of green, leafy vegetables and yellow and orange fruits and vegetables that were rich in lutein and zeaxanthin (EDCCSG, 1992; Seddon et al., 1994; Snodderly, 1995). Another association was the observation that smokers, who have lower plasma levels of carotenoids, also have a lower macular pigment (lutein and zeaxanthin) density (Hammond et al., 1996) and an increased risk of developing AMD (Christen et al., 1996b). However, all of these reports are associative in nature and do not demonstrate a causal relationship between deficiencies of lutein and zeaxanthin and development of AMD.
Central Neurodegenerative Diseases

There is increasing evidence that a number of common neurodegenerative diseases, such as Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, and amyotrophic lateral sclerosis may include adverse responses to oxidative stress. Small intervention trials in patients with these diagnoses have reported some improvement with either vitamin E (Muller, 1994; Sano et al., 1997) or vitamin C (Morris et al., 1998; Riviere et al., 1998), but it is still too early to draw any conclusions as to the usefulness of these compounds in these diseases, or to their ability to delay onset of the disease.

Diabetes Mellitus

Cardiovascular complications are the major causes of death in diabetes. The incidence of coronary heart disease in type II diabetes is significantly higher than that in the general population (Kannel and McGee, 1979). In addition, individuals with diabetes experience microvascular complications (retinopathy, neuropathy, and nephropathy) secondary to their hyperglycemia. Thus, oxidative processes also may play an important role in the development or progression of diabetes mellitus.

In vitro oxidation of LDL from patients with diabetes mellitus proceeds at an accelerated rate, which suggests that they are more susceptible to the atherogenic process (Chisolm et al., 1992; Nishigaki et al., 1981; Reaven et al., 1995; Tsai et al., 1994). The several ways in which oxLDL is potentially more atherogenic than native LDL have been discussed above (see section “Cardiovascular Disease”).

Diabetics tend to have smaller, denser LDL (associated with hypertriglyceridemia) and these LDLs are more susceptible to oxidative modification ex vivo (Feingold et al., 1992). The TRAP of plasma from patients with insulin-dependent diabetes is decreased (Tsai et al. 1994), which may account in part for the greater susceptibility of their LDL to oxidation.

Microvascular complications are believed to be the ultimate consequences of nonenzymatic glycosylation and the progressive accumulation of advanced glycosylation end products (AGEs). There is evidence that the formation of these complex carbohydrate-protein and carbohydrate-lipid complexes is accompanied and accelerated by oxidative processes, which may lead to diabetic complications (Brownlee et al., 1988; Dyer et al., 1993; Hunt et al., 1988; McCance et al., 1993; Mullarkey et al., 1990). The glycosylation process is associated with increased formation of free radicals, and the possi-
bility that treatment with antioxidants might slow the development of AGEs is under investigation.

The issue of whether modifications observed in plasma and tissue proteins in patients with diabetes are due to an oxidative stress or a stress from reactive carbonyls has been discussed by Baynes and Thorpe (1999). A number of studies have tried altering the pathobiology of diabetes by treating patients with either single antioxidant-type compounds or combinations of compounds with antioxidant properties. The results have been inconclusive, which may reflect the fact that the underlying pathology is not caused exclusively by an oxidative stress, but by an inability to metabolize and inactivate reactive carbonyls appropriately. Under these circumstances, the oxidative damage may be exacerbated, resulting in an increase in many of the markers associated with oxidative stress but not caused directly by oxidative stress (Baynes and Thorpe, 1999).

**Aging**

Aging is not in itself a chronic disease, but rather is characterized by the active or passive presence of a chronic disease (cardiovascular disease, cancer, cataracts, Alzheimer’s disease, etc.). It is not clear if an accumulation of chronic insults and weakened defenses renders the aging individual more susceptible to various diseases. Do antioxidants play a role in preventing aging or prolonging life? There is no direct evidence in humans for such an effect, although vitamin E supplementation appears to improve some immune responses in the elderly (Meydani et al., 1997). There have been suggestions that supplementing older animals with antioxidants may improve various physiological functions (Hagen et al., 1999), but the only experimental intervention that has resulted in prolongation of the life span of the animals has been the drastic reduction of food consumption (Pariza and Boutwell, 1987). Whether such a protocol would delay aging in humans has not yet been studied. Whether dietary antioxidants can lead to healthier aging remains to be proven.

**CONCLUSIONS**

There is little doubt that an imbalance in the production of free radicals and other reactive species and the natural protective systems available to organisms can lead to the production of oxidized products of lipids, nucleic acids, and proteins. These oxidation products, or biomarkers of this imbalance, may be related to early
events in certain chronic diseases. However, they have not yet been adequately validated as markers of the onset, progression, or regression of any chronic diseases. Although vitamin C, vitamin E, and selenium have been shown to decrease the concentrations of some of the biomarkers associated with oxidative stress, the relationship between such observations and chronic disease remain to be elucidated. As a consequence, it has not been possible to establish that dietary antioxidants or other nutrients that can alter the levels of these biomarkers are themselves causally related to the development or prevention of chronic diseases.

REFERENCES


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