Nutrition Research, Vol. 20, No. 3, pp. 449–459, 2000

0271-5317/00/$–see front matter

PII: S0271-5317(00)00138-X

ANTIOXIDANTS AND DISEASE: MORE QUESTIONS THAN ANSWERS

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ABSTRACT

Reactive oxygen species are widely believed to be involved in the etiology of many diseases as indicated by the signs of oxidative stress seen in those diseases. Conversely, antioxidants are believed to be protective. An important part of the supporting evidence is the consistently-seen inverse association between, on the one hand, intake of ß-carotene and vitamin C and of fruit and vegetables, and, on the other hand, risk of cancer and coronary heart disease (CHD). However, the failure of supplemental β-carotene to prevent these diseases in intervention trials suggests that the associations for that nutrient reflect confounding rather than cause and effect. With respect to other antioxidants there is inconsistent evidence that supplements of vitamin E may have some ability to prevent cancer and CHD while selenium may prevent cancer. Overall, the role of oxidative stress in disease, especially cancer and CHD, has probably been overstated; other components of the diet (other nutrients, phytochemicals and dietary fiber) likely play a significantly greater role. The possible benefits of supplements are discussed.

Key Words: Antioxidant, Cancer, Coronary Disease, Oxidative Stress, Vitamin E

INTRODUCTION

Various evidence indicates that oxidative stress is closely associated with a diverse assortment of diseases (1). From this it is generally inferred that antioxidants will therefore prevent those diseases. The evidence is re-evaluated.

In this paper a nutrient refers to any substance which is essential (such as a vitamin) while a phytochemical is a plant-based substance which may have a beneficial effect in the body but is non-essential. Antioxidants may be in either class. For instance, β-carotene is a nutrient whereas various other carotenoids also act as antioxidants but do not have vitamin A activity and are therefore not

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essential nutrients but phytochemicals.

THE BIOCHEMISTRY OF OXIDATIVE STRESS

At a meeting of the World Health Organisation in the mid-1960s the Russian toxicologist, Professor Sanojki, referred to various degenerative diseases as being "rusting diseases" and linked their etiology to reactive oxygen species (ROS) (2).

The biochemistry of ROS and oxidative stress has been reviewed elsewhere (3-5). ROS include singlet oxygen, the superoxide anion radical, the peroxide anion, and the hydroxyl radical. They are highly reactive free radicals produced from molecular oxygen. They can be generated during normal cellular respiration, by activated leucocytes as part of the immune response, and by exogenous oxidants such as air pollution and cigarette smoke. They have an undoubted capability to be harmful by their action on vital cellular components including lipids, proteins and DNA.

The body has several defence systems to counteract oxidative stress. These comprise endogenous enzymes (including catalase, glutathione reductase and superoxide dismutase), endogenous factors (including glutathione, urate and coenzyme Q), and nutritional factors (principally the antioxidant nutrients, especially β-carotene and other carotenoids, vitamin C, vitamin E and selenium).

OXIDATIVE STRESS AND DISEASE

The body is normally in a steady state condition with free radicals being continuously generated and quenched. However, the accumulated long-term damage done by free radicals is implicated in numerous degenerative diseases. Evidence from many has heavily implicated oxidative stress in a spectrum of diseases and of states of body dysfunction. Oxidative stress has been shown variously as depressed levels of antioxidant substances (e.g., vitamin E, urate), low levels of enzymes which form part of the antioxidant defence system, and increased levels of oxidation products (e.g., malondialdehyde, DNA damage).

A well-known example of an oxidation product apparently leading to disease is oxidized cholesterol in low-density lipoprotein (LDL). This is more atherogenic than native LDL, thereby implicating oxidative stress in atherosclerosis and coronary heart disease (CHD) (6).

The following is a partial list of the conditions considered to be associated with oxidative stress: an impaired immune system and increased risk of infectious disease (7); cancer (8); diabetes (both noninsulin-dependent and insulin-dependent diabetes) (9,10); autoimmune conditions including rheumatoid (11) and ankylosing spondylitis (9); various respiratory diseases (12); eye disease, including cataracts (13) and retinal damage leading to age-related macular degeneration (14); Alzheimer's disease (15); and schizophrenia (16).

FRUIT, VEGETABLES AND CANCER

The purported close association between a state of oxidative stress and disease implies that antioxidants will be protective against these same diseases. Particularly important in this regard is the strong inverse relationship seen between intake of fruit and vegetables and the risk of cancer (17) with an overall risk reduction of between 30 and 50% (18). If these impressive benefits are a result of the intake of antioxidants, then the obvious protective substances may be vitamin C and the carotenoids.

Epidemiological data link vitamin C intake with reduced risk of several cancers, especially oral cavity, esophagus, stomach and, to a lesser extent, colon and lung (19,20). Likewise, the epidemiological evidence clearly shows a strong inverse association between the intake of β-carotene and the risk of several cancers, especially lung and stomach (21). Some attention has been paid to other carotenoids. Epidemiological studies have reported that α-carotene has an inverse association with cancer of a similar strength to that seen for β-carotene (22). Lycopene, a carotenoid present in tomatoes, has attracted much attention recently; it shows a strong inverse relationship with several types of cancer, especially prostate, lung and stomach (23). A weaker association has been described for lutein (22). Each of these substances is an antioxidant. It must be stressed, however, that "association does not prove causation." In reality, vitamin C and carotenoids may be acting merely as surrogate measures of fruit and vegetables and it is other components of these foods that prevent cancer. The crucial evidence - the gold standard - is a controlled clinical trial. But the results of three such trials provided no evidence of cancer prevention by supplements of β-carotene (24-28).

There is some evidence of protection against cancer by supplemental β-carotene based on early end-points. One study reported significant reversal of leukoplakia, a precancerous oral lesion (29). Similarly, another study observed partial regression of precancerous changes of the stomach (30). A trial on Filipino betel nut chewers reported a reduction in numbers of buccal mucosa cells with micronuclei (31). This indicates the prevention of precancerous changes of the oral cavity.

Let us now address the question as to why trials using β-carotene failed to prevent cancer. Possibilities that have been suggested include: the wrong carotenoid was given, or it was given at the wrong dose, or for an insufficient duration, or at the wrong stage of carcinogenesis. However, another very real possibility is that antioxidants are not the common denominator between fruit, vegetables and the prevention of cancer. Other factors that may offer a partial explanation are:

1. There is a strong inverse relationship between the intake of dietary fiber and colon cancer (32). There is also evidence suggestive of an inverse relationship between fiber and breast cancer (33). However, as vegetables (and, to a lesser extent, fruit) are a major source of fiber, part of this association may represent confounding by associated substances.

2. Cruciferous vegetables - broccoli, cabbage, cauliflower, brussels sprouts, and others - contain phytochemicals which induce the synthesis of detoxifying enzymes and may thereby be anticarcinogenic (34). This helps explain the epidemiological evidence indicating a protective relationship between these vegetables and colon cancer (35).

FRUIT, VEGETABLES AND CHD

Law and Morris (36) recently estimated that an increase in consumption of fruit and vegetables could potentially reduce levels of CHD by 15%.

Although the data lack consistency, a negative association has been reported between intake of vitamin C and carotenoids and the risk of CHD (6). Kritchevsky *et al.* (37) have reported a negative relationship between intake of carotenoids with vitamin A activity and carotid artery plaques. As with cancer these associations might easily reflect confounding by associated substances present in fruit and vegetables. Intervention studies have indicated that supplemental β-carotene does not protect against CHD (25,27,28).

Fruit and vegetables are, of course, a major source of not only antioxidants but also of other dietary factors. The latter may explain why fruit and vegetables help prevent CHD. Potassium is protective against elevated blood pressure, a major risk factor for CHD (38,39). In addition, evidence which has only recently emerged indicates that folate is protective against CHD and other cardiovascular diseases (40,41). The mechanism appears to be that folate lowers the blood level of homocysteine (42,43), which is a risk factor for CHD (41,44,45). The evidence for these associations, however, is inconsistent (46).

We cannot therefore conclude that fruit and vegetables prevent CHD because of their content of antioxidants. Indeed, Law and Morris (36) recently argued that folate and potassium fully explain the protective action of fruit and vegetables against CHD.

SELENIUM, VITAMIN E AND DISEASE

In addition to vitamin C and carotenoids there are other antioxidants in food that may have anticarcinogenic action. Animal experiments have demonstrated that selenium functions as an antioxidant (47). There is much evidence from international correlation studies and from animal experiments that selenium is protective against cancer (30). Data from the Health Professionals Follow-Up Study indicate a strong inverse association between selenium status and risk of prostate cancer (48). One controlled intervention study has been carried out and this reported a dramatic 50% fall in total cancer mortality using a supplement of 200 μg/day (i.e., several times greater than the RDA) (49). Clearly, this exciting observation merits further study.

Turning to vitamin E the epidemiological evidence is inconclusive for a protective role in cancer though this antioxidant does appear to be negatively associated with colorectal adenomas (22). In the ATBC Cancer Prevention Study a dose of 50 mg/day apparently reduced the incidence of prostate and colorectal cancer by 36% and 16%, respectively (24). Vitamin E (400 IU/day) caused partial regression of precancerous changes of the stomach (30). One interpretation of these seemingly contradictory findings is that vitamin E becomes anticarcinogenic only at pharmacological doses (i.e., when the intake is several times greater than the US RDA [8-10 mg/day]).

Evidence both from international and prospective studies has indicated that vitamin E intake is negatively related to the risk of CHD (6). In two of the prospective studies the strongest association

was with total intake of vitamin E (diet plus supplements) (50,51). Intervention trials, by contrast, have failed to show a protective benefit of supplemental vitamin E (36). Other data indicate that the vitamin reduces the susceptibility of LDL to oxidation (52-54) and this may be expected to be protective against atherosclerosis. Studies on experimental animals have also shown that vitamin E can be protective against atherosclerosis (6). Taking this clearly inconclusive evidence as a whole vitamin E may have a mild preventive action against CHD, especially at intakes of over 50 mg/day.

Other work has indicated a possibly beneficial role for supplemental vitamin E in diabetes based on lowered serum levels of glycosylated hemoglobin and triglyceride (55). Supplemental vitamin E at a dose of 60-800 IU/day reportedly improves immune function in elderly subjects (56), while a single dose of 1 g helped prevent oxidative damage of DNA (57).

If vitamin E and selenium are indeed protective against disease, this has little relevance to the question of how fruit and vegetables prevent disease as these foods are a minor source of those nutrients, especially of the high doses that appear most effective.

HOW IMPORTANT ARE ANTIOXIDANTS?

The possible benefit of vitamin C and β-carotene has been studied in other conditions apart from cancer and CHD. Epidemiological evidence suggests that vitamin C protects against cataracts (13), asthma (12), and a decline in pulmonary function (58). As emphasized earlier such associations must be viewed cautiously. However, in the case of vitamin C and asthma, there is also some supporting evidence from intervention studies (12). Supplemental doses of β-carotene and of vitamin C each help prevent oxidative damage of DNA (57,59), while ß-carotene also improves immune function (60).

Studying the relationship between antioxidant status and disease has proven to be a highly profitable line of research. It has expanded our knowledge concerning the etiology of numerous diseases and the means by which they might be prevented. But it is essential to take a balanced perspective and avoid the danger of over-enthusiasm for the potential of antioxidants.

The importance of the association between oxidative stress and disease should not be exaggerated. Halliwell *et al.* (3) pointed out that disease processes can give rise to oxidative stress (in addition to the reverse). Halliwell (5) also noted that: "In most human diseases oxidative stress is a secondary phenomenon, not the primary cause of the disease." Likewise, Dusinska *et al.* (9) caution that the role of oxidative DNA damage in carcinogenesis has not been proven and that there are many inconsistencies in the relationship. Red wine has significant antioxidant activity (61) but evidence from case-control and prospective studies indicates that it has a no greater protective association with CHD than any other type of alcoholic beverage (62).

Taking the evidence as a whole it is difficult to escape the lack of convincing evidence that places oxidative stress at the center of any disease process or gives antioxidants a major role in the prevention of disease. While many studies have shown associations between intake of antioxidants and disease risk, very few studies have provided evidence that antioxidants actually prevent any disease. Conversely, there is strong evidence that fruits and vegetables prevent cancer, CHD and possibly other diseases. We cannot at this time say how much of this, if any, is due to antioxidants and how much to nutrients and phytochemicals.

One line of investigation that should prove profitable is intervention studies using fruit and vegetables (63). Such studies can expand our knowledge concerning the protective relationship of these foods against cancer, CHD and other diseases such as hypertension. It may be more intellectually satisfying to determine which nutrient or phytochemical is most effective in disease prevention but that approach can be hit-and-miss as we have seen with the β-carotene trials.

THE POSSIBLE BENEFITS OF SUPPLEMENTS

Should we take supplements? While we have overwhelming evidence for advising a generous intake of fruit and vegetables, the evidence is scanty and contentious that supplements of the antioxidant nutrients and phytochemicals provided by these foods (vitamin C and the carotenoids) will prevent disease. This confirms the conventional wisdom that the best source of nutrients and phytochemicals is food not supplements.

We must bear in mind that whole grains are also of great importance. Evidence from many case-control studies on cancer (64,65) and a prospective study on CHD (66) indicates that a generous intake of whole grains reduces the risk of both diseases by one third. For the above reasons, therefore, it is highly unlikely that any one-a-day pill presently available will have the health-giving properties of whole foods.

However, there is some positive evidence in support of supplements. Taking the evidence as a whole, vitamin E, which is mainly obtained from oils, may have a mild protective action against CHD and certain cancers. The benefit seems most likely at intakes only obtainable from supplements (typically 50-500 mg/day). While more research is clearly needed, especially from intervention studies which look at incidence and death from CHD and cancer, supplementation with 50 mg/day of vitamin E may well commend itself as a general prophylactic.

Selenium may also prove to be a potent cancer-preventive agent but this requires further study. It is essential that the dose is not excessive (maximum of 100-200 μg/day) due to the risk of toxicity.

The evidence obtained from studies on vitamin E and selenium indicates the potential for substances which provide safe, effective and cheap means to prevent degenerative diseases and to help preserve body function well into old age.

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Accepted for publication July 21, 1999.