Antioxidants and Atherosclerosis

Hannes B. Stähelin

Geriatric University Clinic, University Hospital Basel, CH-4031 Basel, Switzerland

Cardiovascular disease and stroke resulting from atherosclerosis are the leading causes of death in all countries where life expectancy is high. The incidence of atherosclerosis-associated diseases is strongly correlated with age, age being the most significant risk factor. The strong association of cardiovascular and cerebrovascular diseases and aging suggests a common underlying mechanism of alterations related to aging as well as the atherosclerotic process proper. Thus, the search for this common cause could open up strategies of intervention, delaying the onset of occlusive vascular disease.

One of the important mechanisms inducing aging is the action of reactive oxygen species (ROS) on structures such as DNA, proteins, membranes, and lipids. Experimental evidence suggests that aging is mediated to a large extent by the endogenous as well as exogenous generation of free radicals. Indeed, the lifespan of a given species correlates with antioxidative defense (1).

On close analysis, free radical induced tissue, cell, and molecular damage shows striking similarities to changes observed during the formation of the atherosclerotic lesion. The antioxidant hypothesis of arteriosclerosis was initially based on defensive properties of antioxidants against ROS as demonstrated in vitro and in vivo on arteriosclerosis-like lesions in animals with chronic deficiencies of vitamin C and E, as well as on plasma measurements of essential antioxidants in cross cultural epidemiology (2).

Recent epidemiological studies lend strong support to the protective role of antioxidants in the development of diseases related to atherosclerosis (3).

Atherosclerosis is a multifactorial disease. The concept of risk factors has been firmly established since the Second World War. In the presence of risk factors, the incidence of atherosclerosis increases, when measured either directly by imaging techniques or by major end points such as angina pectoris, myocardial infarction, cardiac death, stroke, or death from stroke. On the other hand, it is quite obvious that clinical events require specific local factors to trigger the formation of thrombi, endothelial dysfunction, and reaction of the vessel wall. Thus, the risk factors for coronary heart disease and stroke differ quite substantially. (Consider the stroke—coronary heart disease paradox (4)) and this makes it difficult to reconcile the observed epidemiology with the known risk factors only. As shown by the MONICA data, a
much better prediction of events is obtained by taking into account the antioxidant status as a protection against atherosclerosis (5).

This chapter deals with the question of antioxidants and nutrition, and the epidemiological evidence linking the protective role of antioxidants to atherosclerosis, and reviews possible mechanisms of action.

ANTIOXIDANTS AND NUTRITION

The body defends itself against free radicals, and for this, it depends on enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GP), which are glutathione and selenium dependent, and catalase (Fig. 1). Essential antioxidants are provided by nutrition. Vitamin C is an important, water-soluble antioxidant and vitamin E is the essential lipid-soluble membrane protective antioxidant. \(\beta\)-carotene and other carotenoids serve as antioxidants, but \(\beta\)-carotene also acts as provitamin A. Recent studies make it clear that nutrients contain other antioxidants summarized under the term nonnutrient bioactive substances. The intake of such nonnutrient bioactive substances (6) may determine subsequent development of disease to a hitherto underrated extent. Observations such as the French paradox of a low cardiovascular mortality rate despite a high plasma cholesterol and LDL concentration are attributed to certain antioxidants such as flavonoids, phenols, and other substances. The requirement of antioxidant micronutrients may depend on the cellular redox state which varies considerably in certain diseases, but also on the susceptibility of other nutrients to oxidation. Thus, a high intake of polyunsaturated fatty acids increases the requirement for antioxidants (5).

The classical recommended daily allowances aiming at preventing overt micronutrient deficiencies are no longer a useful tool in establishing individual requirements

![Figure 1](image-url)  
**FIG. 1.** The body's defense system against reactive oxygen species.
for maintaining health and preventing chronic disease. Other methods for defining the optimum antioxidant status of an individual person need to be developed.

**EPIDEMIOLOGICAL EVIDENCE OF A PROTECTIVE EFFECT OF ANTIOXIDANT INTAKE AGAINST ATHEROSCLEROSIS**

A major end point of epidemiological studies investigating atherosclerosis is organ damage, such as myocardial infarction or stroke. However, the extent of this damage depends on the tissue injury induced by occlusive arterial disease. Thus, clinical manifestation of the disease depends rather on the extent of the tissue injury than on the underlying occlusive event. A protective role of antioxidants could therefore very well be related to protection against tissue damage after arterial occlusion. Indeed, reperfusion injury is mediated, to a large extent, by the formation of free radicals (7). A high antioxidant status decreases reperfusion injury. This mechanism is not considered in detail here.

On the other hand, it is thought that prevention of the occlusive event is crucial in primary and secondary prevention of atherosclerosis-related disease. Thus, a reduction in hard end points (e.g., myocardial infarction, stroke) can also be taken as evidence for the reduction in the formation of atherosclerotic occlusive events. Several large prospective studies have examined the role of antioxidant vitamins in cardiovascular disease, and to some extent also in stroke. They yield to date the strongest support of a beneficial role of antioxidants in preventing coronary heart disease or stroke (8–15) (Table 1). There is an amazing consistency in the overall results as well as some interesting discrepancies. Thus, the U.S. and Finnish studies on very large cohorts exploring the effects of vitamin E and carotene found mostly an effect for vitamin E, with a risk reduction of between 34% and 56%. These studies were based on intake and attribution of risk reduction, for example, to β-carotene, is not very solid because other carotenoids such as lycopene, lutein, and β-kryptoxanthine may be increased concomitantly (5).

The Basel study, measuring the plasma concentrations of carotene as well as of the other vitamins, observed in the 12-year follow-up a significantly increased risk in subjects with carotene values below the first quartile. Particularly striking was the increased risk in subjects with low carotene and concomitantly low vitamin C (Fig. 2) (16). In the 17-year follow-up, low vitamin C became a significant risk factor for ischemic heart disease (relative risk = 2.3); in smokers, the risk ratio even increased to 2.57. Again, the subjects with low carotene and concomitantly low vitamin C had a substantial overmultiplicative increase in risk for ischemic heart disease. In stroke, low carotene was associated with a three-fold increased risk. In the presence of low vitamin C, the risk increased to 3.7. Low vitamin C in itself did not infer an increased stroke risk. The major difference to the above-cited epidemiological studies is a lack of risk association with vitamin E. In contrast to the American and the Finnish population, the Basel plasma vitamin E status was clearly
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RR, relative risk; MI, myocardial infarction; IHD, ischaemic heart disease; CHD, cardiovascular heart disease
above average. Quartile 1 was still higher than the mean plasma values in the above-cited studies. This may be one important explanation why cardiovascular mortality is remarkably low in the Swiss population with, on average, high cholesterol levels. The studies clearly also show that no single antioxidant is crucial but the interplay of the naturally occurring antioxidant appears to be the most important factor (17).

These primary prevention trials are, to some extent, already an intervention trial, because in the Nurses Health study (8), the observed reduction was mainly confined to persons taking vitamin E as supplements. Supplementation was insignificant in the Finnish (14) and the Basel (5) studies. In contrast, the Physicians Health study secondary prevention trial used β-carotene supplements and aspirin alone or in combination (9). The 12-year follow-up showed no benefit of β-carotene supplements alone (18). In an earlier analysis, however, a complete absence of atherosclerosis-related events in the combined group receiving aspirin and carotene was found (9). Newer reports from Los Angeles particularly pertinent to the topic of the workshop come from Steiner (12). In 100 subjects, the combination of vitamin E and aspirin reduced completed stroke or death significantly in subjects after transient ischemic attacks or Prolonged Reversible Ischemic Neurologic Deficit (PRIND).

Investigators looking at the coronary artery lesion progression found that vitamin E supplements enhance the lipid lowering effect on plaque reduction (13). A similar study looking at endothelium-dependent coronary vasomotion observed a synergistic effect of the antioxidant probucol in subjects on a lipid-lowering drug (16). A study by Renaud et al. (14) compared a specific dietary intervention rich in vitamins C, E, and omega-3-fatty acids as well as oleic acid rich in monounsaturated fatty acids in subjects after myocardial infarction and a matched control group. They found an overall reduction of over 70% in the intervention group. This clearly indicates that
several factors are involved. Some are definitely related to the antioxidant properties of the micronutrients, others may relate to nonantioxidant-sensitive mechanisms. Recently, published results strongly supported the protective effect of vitamin E (19,20).

MECHANISM OF ACTION OF ANTIOXIDANTS IN PREVENTING ATHEROSCLEROSIS

Antioxidants interact with gender, other nutrients, and lifestyle, as well as with diseases. Thus, women not only have different nutritional habits, but also show higher plasma carotene levels (21). Estrogens in premenopausal and supplements in postmenopausal women profoundly alter cytokine metabolism which in turn influences endogenous ROS production. For many years, it has been known that smoking reduces plasma vitamin C and carotene. Smokers need a higher daily intake to maintain plasma levels comparable to nonsmokers. The higher turnover is, in part, due to free radical formation by smoking which interacts in very complex ways with free radicals (22). Chronic alcoholism is associated with a low vitamin status as well as low selenium and high peroxidase concentrations. On the other hand, certain grape juices and wines contain flavonoids, phenols, and anthocyanins that exert antioxidant effects (5).

Finally, as the data from the Cretan Diet Intervention study (14) show, the fatty acid composition, particularly the n-6 and n-3 long chain fatty acids, regulates free radical formation and inflammatory responses through different cytokine pathways. Epidemiological data also suggest that antioxidants and drugs may act synergistically or probably in opposite directions. Finding that aspirin and vitamin E, statines and antioxidants in combination significantly improve outcome points to the fact that the effect is not only restricted to one site of action but involves a whole cascade of steps leading to atherosclerosis.

Figure 3 lists some possible interactions of antioxidants with processes associated with atherosclerosis.

In recent years, the hypothesis put forward by Steinberg (23), that oxidized LDL are taken up preferentially by the scavenger receptors of macrophages and induce foam cell formation, attracted much interest. Indeed, foam cell formation is one of the very early events in atherosclerosis. On the other hand, the interaction of oxidized LDL with endothelial cells is free radical dependent and can be modulated by antioxidants, as is the interaction of inflammatory cells with endothelial cells. Thus, the expression of adhesion molecules and modification of vascular tone appear to be antioxidant-dependent. An overview of the protective mechanisms of vitamin C, E, and carotene discussed here is given in the review by Gey (5).

The interesting mechanism of lipid oxidation may be of particular relevance for coronary heart disease. Oxidized LDL can indeed induce a large number of factors and events intimately involved in the development of atherosclerosis. Protection against LDL oxidation by high antioxidants and vitamin E might therefore be one of the reasons for the low coronary heart disease rate in Mediterranean countries.
On the other hand, they are a poor explanation for the protective effects in stroke, because lipids have not been shown to be a substantial risk factor. Therefore, other effects of antioxidants on platelet behavior, expression of adhesion molecules on endothelial cells, etc., are more likely to be mediators in the observed protective action of antioxidants against stroke.

CONCLUSIONS

The evidence of a protective effect of antioxidants against atherosclerosis-related diseases is very strong. In particular, secondary prevention with a combination of
drugs and antioxidants seems highly promising. However, a general recommendation to take antioxidant supplements should be viewed with caution. There is no doubt that high dose antioxidants taken alone can surpass their beneficial effect and become pro-oxidant (5). Nevertheless, the general safety and the possible benefits of postponing and avoiding heart disease and stroke by simple nutritive preventive methods deserve serious evaluation by well designed trials.

Because these compounds are generally cheap and bioactive nonnutrient substances that are probably difficult to test against placebo in a large scale trial, it seems unlikely that the necessary funds to carry out such trials will be found unless the studies are funded by public agencies. Meanwhile, a prudent low dose supplementation of, for example, 100 mg aspirin and 100 mg vitamin E, vitamin C supplements, and a low dose β-carotene supplement (<10 mg/day) may be advocated in persons with increased cardiovascular risk as a rational primary—and particularly secondary—preventive strategy in atherosclerosis. Moderate use of alcohol (i.e., red wine) may on balance be beneficial. The success of this strategy will be enhanced when comcomitant risk factors such as hypertension, hyperlipidemia, and nongenetic homocysteinemia are treated and smoking is abandoned.

REFERENCES

ANTIOXIDANTS ANDATHEROSCLEROSIS


**DISCUSSION**

Dr. Ginsberg: I was intrigued by your slide that showed a relationship between life span and endogenous antioxidative defense. As you well know, there are now transgenic mouse strains available that overexpress, for example, the human superoxide dismutase gene. This gene confers protection against stroke, that is, these animals develop smaller infarcts when the middle cerebral artery is occluded. But I was curious after seeing your slide as to whether their life expectancy would be affected. Do you have any data on that?

Dr. Stähelin: I think in the mouse model, it is not really proven, but there is a recent paper showing that in drosophila you can definitely increase life span (1). In the mouse model, there is always a diet problem because by restricting diet, you have presumably less oxidative stress and you can prolong the life span substantially.

Dr. Ginsberg: I am intrigued by these observations, but the degree of energy restriction that is apparently necessary to increase the life span of a rodent is very severe, isn’t it? You have to reduce the energy consumption by about a third. Is this medically advisable in humans?

Dr. Stähelin: We are in huge experiment on a global scale. Americans are not, at the moment, really indulging in energy restriction. In some other countries, for instance Japan, obesity tends to be much better controlled by social factors. My hypothesis is that the very high life expectancy of the Japanese which, at present, is the highest in the world, is a cohort phenomenon, because people now in the 70- to 80-year age bracket were brought up at a time when they had a much less affluent lifestyle than they have now, and so they had restricted energy intakes, a low cholesterol intake, etc. It is interesting to speculate whether our generation, brought up on a sort of *ad libitum* feeding regimen will, in 30 or 40 years time, have the same life expectancy as our parents now experience, who grew up on a more confined regimen. There is a study in the United States on primates which is showing the same trend as in the rodents but not so dramatically (2).

Dr. Hossmann: To the best of my knowledge, the SOD-overexpressing mouse experiences smaller infarcts only after transient focal ischemia and not after permanent ischemia, and there is protection against reoxygenation injury rather than against ischemic injury or infarct. How far do you think antioxidants are helpful in protecting against reoxygenation injury following an ischemic attack?

Dr. Stähelin: That is an interesting avenue to explore. For instance, after heart surgery,
vitamin C is depleted very rapidly, so these patients experience oxidative stress which could be easily prevented by presurgical administration of sufficient antioxidants. The problem is can you bring antioxidants to the point where they are needed under such circumstances or do you have to take a certain quantity of antioxidants all the time to have as high a level as possible.

Dr. Ginsberg: Chan's group in San Francisco used a model of permanent middle cerebral artery occlusion and showed that antioxidants were protective, but they subsequently showed in the suture occlusion model that it was only in transient occlusion that the treatment worked (3,4). I think the message in this is that in the permanent suture occlusion model, there is really no effective collateral circulation, whereas in permanent occlusion of a middle cerebral artery by other means, this sort of treatment may be effective. This suggests that there is some spontaneous supply from collateral circulation so that even in a permanent occlusion model, some degree of reperfusion still occurs.

Dr. Malnoe: You mentioned pro-oxidant effect of megadoses of antioxidants. I wonder whether you have any evidence for that in vivo. I think this is an important issue because people are taking megadoses of vitamin C or vitamin E and if there is any pro-oxidant effect, I think it should be stressed.

Dr. Stähelin: This is a big problem. For instance, if you have a high vitamin C content, there will of course be a very strong pro-oxidant effect, but to what extent this is relevant to the field of prevention is difficult to say. The paper I referred to was in vitro work where they could show that treatments with 800 mg of vitamin E made low density lipoprotein more easy to oxidize than 400 mg. So there seems to be some sort of a maximum and it probably depends on the balance of other antioxidants present.

Dr. Crozier: As a nutritionist, I was interested in the comments you made that antioxidants come basically from nutrients and enzymes. Would you care to speculate on the relative contribution of nutrients to total antioxidant status or in vivo antioxidant status?

Dr. Stähelin: I think the essential antioxidant micronutrients are simply essential. It is well known that you need a certain safe level at least. The question is rather, do you get a dose-dependent beneficial effect if you increase them? In Basel, we think that you need an optimum cocktail of all the antioxidants to have the beneficial effect, and I think the Cretan diet shows that this could work. To what extent the genetic endowment with these antioxidants—catalase, superoxide, SOD, and the peroxidases—really affect the individual life span is not well explored but I think the tools are now available to look at this.

Dr. Crozier: I recently saw some data where it was shown that by giving n-3 fatty acids the activity of the glutathione handling enzymes could be increased along with the concentrations of glutathione, whereas TBARS were decreased (5). I wonder if there is also a potential for other nutrients to modulate antioxidant status other than antioxidants themselves?

Dr. Stähelin: I think this is a very important possibility and not well explored as yet.

Dr. Guesry: I don't understand why you make such a distinction between coronary heart disease and stroke. If you consider the 80% of stroke which is due to atherosclerosis, I think this is basically the same disease—in other words, it is a vascular disease, not a cardiac disease or a brain disease.

Dr. Stähelin: When you look at it from an epidemiologic point of view, there is a difference. There could be quite different mechanisms that determine whether you record an end point or not. If you have transient occlusions of a vessel in the brain but the brain is well protected, you may not really realise they have occurred, but you still have the disease; the same could be true for myocardial infarction. I think the mechanisms of atherosclerosis are also not quite the same and in this respect, the antioxidants are probably very important because they have
a common effect in stroke and in cardiovascular disease. They are present at a common site in the vessel wall but the injury to the wall may differ. In coronary heart disease, the injury to the vessel wall clearly depends on LDL and on oxidised LDL, there is no question about this today. But in stroke, it depends where the occlusion is and there may be other mechanisms. Despite this, there is still an antioxidant effect, which tells me that the oxidised LDL story, while highly interesting, only explains half the picture, and the other half is the effect on the endothelial cells, on smooth muscle cells, on platelets, and on lymphocytes.

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