Recent Advances in Oxidative Stress and Antioxidants in Medicine.

John Smythies, M.D., F.R.C.P.[1]

Introduction

Until quite recently most physicians adhered to the belief that the only function of vitamins was to prevent deficiency diseases such as scurvy and pellagra. When Linus Pauling founded orthomolecular medicine twenty-five years ago by suggesting that vitamins might have other functions than this, he was greeted with scorn, derision and outright hostility by the orthodoxy. Today the situation is entirely transformed. There is now an enormous literature on the role of vitamins in the body other than their prevention of deficiency diseases. This is based largely on the discovery of the wide role that oxidative stress plays in many diseases and of the role of antioxidant vitamins in preventing and combating these diseases. The only point of disagreement in main stream medicine today is whether the right amount of protective antioxidant vitamins can be obtained by improving one's diet alone – i.e. by eating more fruits and vegetables – or whether supplements are needed.

Oxidative Stress and Antioxidant Defenses

Oxidative stress arises because the life-giving oxygen molecule is easily converted into toxic reactive oxygen species or ROS (also called free oxygen radicals). The predominant ROS are the superoxide radical ion, hydrogen peroxide and the hydroxyl radical ion. The latter is the most toxic and will attack and damage proteins, lipids and DNA. ROS are made by a variety of different enzymes such as the enzymes of the electron chain in the mitochondria (that generate energy by synthesizing ATP), prostaglandin H synthase (the rate limiting enzyme on the prostaglandin synthesis pathway), nitric oxide synthase, and other oxidases. Life would be impossible unless the cell developed powerful antioxidant defenses against ROS. ROS have normal roles in the body as intracellular signals and in controlling gene expression. Trouble only results if there are excess ROS or defective antioxidant mechanisms.

Antioxidant defenses are of two kinds – small molecules that bind or scavenge the ROS and enzymes that turn them into harmless products. The small molecules are again of two types – water-soluble and lipid-soluble. The principle water soluble antioxidant molecules in the body are ascorbate (vitamin C) and glutathione (GSH). The principle lipid soluble antioxidants are vitamin E and a variety of carotenes, which are relatives of vitamin A. Plants also contain a large number of other important antioxidants called phytochemicals that are not vitamins. The body cannot make them but lack of them does not lead to deficiency diseases. These include the flavonoids and polyphenols. Other important antioxidants are vitamin B_3 and coenzyme Q10. The antioxidant enzymes are catalase, superoxide dismutase and glutathione peroxidase.

It is important to note here that these antioxidants act in synergism. When vitamin E, for example, neutralizes a toxic ROS it itself is oxidized. This oxidized form has to be converted back to vitamin E to deal with more ROS. This conversion is carried out by vitamin C or by glutathione, which in turn become oxidized. The oxidized vitamin C is converted back to the protective form by NADH, a complex that contains vitamin B_3. The antioxidants coenzyme Q10 and lipoic acid also play a role in this process. Vitamin C and glutath-

1. Brain and Behavior Laboratory, Center for Human Information Processing U.C.S.D. La Jolla. CA. 92093-0109 e-mail smythies@psy.uscd.edu

ione have other protective actions, e.g. by scavenging ROS themselves, that do not depend on this recharging of vitamin E.

Oxidative stress occurs whenever the production of damaging ROS exceeds the capacity of the antioxidant defenses to cope with them. Oxidative stress is involved in a large number of diseases some of which I will describe briefly. More details on all this information may be found in my new book *Every Person's Guide to Antioxidants* shortly to be published by Rutgers University Press.

Coronary Heart Disease

One factor in the development of atherosclerosis is the oxidation of fats by ROS. When macrophages attempt to phagocytose oxidized fats they fail to do so properly and become bloated with lipid (as 'foamy cells'). These foamy cells play an important role in forming the atheromatous plaque that leads to coronary obstruction and myocardial infarction. A very large number of epidemiological, plus a few double-blind controlled, studies have shown that diets high in vitamin E, and/or vitamin E supplementation, are protective against heart attacks. Vitamin C is less effective and seems to work mainly as a 'helper' of vitamin E by the recharging mechanism described earlier. This is not surprising as vitamin C is a water-soluble antioxidant and the damage in the heart vessel wall occurs in a very lipophilic environment. Carotenes are also less effective and in some studies have been reported actually to increase heart attacks. Coenzyme Q10 has been shown to be very effective especially during an actual heart attack. The effective level of vitamin E necessary to protect against heart attacks is between 400-800 mg/day of the natural form. The synthetic form is much less effective. The former consists of a number of stereoisomers of alpha tocopherol, whereas the latter consists of only one. This difference is important. It is difficult or impossible to get this level of vitamin E intake from the diet without eating far too much fat, so supplements are needed. Every person at risk for coronary disease should take vitamin E supplements at this level plus one aspirin tablet a day. This combination has been shown to act better than either alone. As we have seen, these antioxidants act as a team, so this level of vitamin E intake requires an adequate intake of the other members of the team especially vitamin C (500 mg/day) and vitamin B_3 (100 mg/day).

However, oxidative stress is only one factor in the complex process that leads to atherosclerosis. Two other recently discovered factors, besides cholesterol and a diet high in saturated fats, are inflammation and high homocysteine levels. The latter is due mainly to deficiencies in the diet of folic acid and vitamins B_6 and B_{12}. So the diet should contain adequate amounts of these. The last factor is now considered so important that steps are being taken to add these vitamins to flour. The Work Study Group on Diet, Nutrition and Cancer of the American Cancer Society has estimated that about one third of the half million deaths each year in the US arise from faulty diet. There is now absolutely no doubt that a diet high in fruit and vegetables (three helpings of vegetables and two of fruit a day) and low in calories, fat, smoked foods and nitrites leads to a large reduction of the incidence of many cancers, particularly of the upper respiratory and digestive tracts. Cancers of the colon seem to be affected more by the level of fiber in the diet. Cancers of the breast, uterus and prostate are affected more by hormonal factors than diet. Cancers of the lymphatic and blood systems do not appear to be affected by diet at all. But it is still uncertain what the responsible factors in the diet are. Four studies that measured blood levels of vitamin E and/or betacarotene showed in all cases that low levels of these were associated with increased incidence of cancer. But it is probable that the flavonoids and polyphenols in the fruits and vegetables

also play an important role. There are also other anticancer agents in fruits and vegetables, such as isoprenes, that are not antioxidants. Moreover, the well documented effect of vitamin C in preventing gastric cancer may be linked with its ability to neutralize carcinogenic nitrosamines in the diet rather than to its antioxidant properties.

In two recent studies of heavy smokers beta-carotene has been reported to increase the risk of lung cancer. In the first study in the US (CARET)[1] it was given together with vitamin A and so which was responsible is hard to say. In the second study in Finland (ATBC)[2] it was given along with 50 mg/day of synthetic vitamin E. Both studies reported a significant rise in lung cancer in the vitamin treated group as compared with the placebo group, and in the second there was also a 75% increase in myocardial infarcts. Clearly beta-carotene should not be given to heavy smokers. In any case it is a poor antioxidant and other carotenes are probably more effective. Vitamin E in contrast is quite non-toxic except in cases of vitamin K deficiency.

Diabetes

Insulin-dependent diabetes is an autoimmune disease in which the beta cells of the pancreas are destroyed by the immune system which uses ROS as part of its cytotoxic armamentarium. Vitamins C and E inhibit lipid oxidation in diabetics. Tests of vitamin C in human diabetics have shown that it improves vascular performance, leads to a fall in blood levels of LDL (bad) cholesterol and a rise in protective glutathione levels. In other tests the antioxidant B_3 vitamin (nicotinamide at 3 g/day) cut the need to give insulin to diabetics. It is obviously important to try and give the antioxidants before the beta cells have been destroyed. So in a recent study[3] of 22 children at high risk for developing diabetes, 8 were given placebo and 14 children 3 g/day of nicotinamide. All 8 of the children on placebo had developed frank diabetes by the end of the study but only one of the children on nicotinamide did.

In another study[4] of 80 diabetic patients with damage to the retina and peripheral nerves, 20 were given the antioxidant lipoic acid, 20 vitamin E, 20 selenium, and 20 placebo. The first two groups showed a significant improvement. Lipoic acid is currently being used in the treatment of diabetic nerve damage. A very recent study of a double-blind, placebo-controlled trial of the antioxidant amino acid L-arginine in human diabetes found that it strongly reduced the levels of lipid oxidation.

Two studies have shown that pregnant diabetic rats fed vitamin E have a much lower level of offspring with congenital abnormalities than do untreated diabetic rats. In humans the birth defects associated with diabetes can be prevented by a strict adherence to a treatment and dietary regimen to prevent raised blood sugar levels but antioxidant supplements may make this treatment more effective. Thus antioxidant therapy seems promising in diabetes especially if the prediabetic stage can be treated.

Respiratory Diseases

The lung is particularly vulnerable to oxidative stress due to its heavy exposure to oxygen. Risk factors for asthma have been shown to include low dietary intake of vitamins C and E and selenium, high body iron and exposure to environmental toxins. Vitamin C is the major antioxidant in the fluid that covers the surface of the lung where it protects against environmental oxidants including toxic nitrogen oxides in smog. Asthmatics also have low blood levels of the antioxidant enzyme GSHpx and selenium. The current evidence suggests that antioxidants should protect against asthma and alleviate its symptoms. A recent double-blind placebo-controlled study[5] of 17 asthmatic adults showed that supplementation with vitamin E (400 mg/day) and vitamin C (500 mg/day) led to a

significant reduction in their sensitivity to ozone. In acute respiratory distress syndrome the antioxidant defenses in the blood are lowered and there is a fall in the protective elements of the plasma. Clinical trials have shown that antioxidant therapy is very effective in this condition.[6] Other lung diseases associated with oxidative stress are hypersensitivity pneumonitis, chronic obstructive pulmonary disease, emphysema and pneumoconiosis due to mineral dust. In these conditions vitamin C can improve lung function.

Exposure to excessive amounts of smog and to tobacco smoke lead to severe oxidative stress owing to the toxic oxides of nitrogen that they contain. This leads to a reduction in antioxidant defenses especially vitamin C and glutathione. Smokers need at least 200 mg/day of vitamin C just to maintain minimal acceptable antioxidant function. But how much better to give up smoking!

Alzheimer's Disease

There is now much evidence to show that oxidative stress produced by the abnormal pro-oxidant protein beta-amyloid plays a key role in this disease. Measures of oxidative damage in the brain are raised and antioxidant defenses are defective. This is basically an inflammatory disease of the brain and there is now considerable evidence to suggest that non-steroidal antinflammatory agents, such as ibuprofen may prevent or delay the onset of symptoms. Out of 16 clinical trials of non-steroidal antiinflammatory agents on this topic reported by Pat and Edith McGeer of the University of British Columbia, 15 had positive results.[7]

In a recent double-blind, placebo-controlled study[8] of vitamin E (2 g/day), or the drug selegiline (which cuts down the production of ROS in the brain), or a combination of the two, in 341 cases of Alzheimer's disease of moderate severity showed a very significant therapeutic effect for both vitamin E and for selegiline in preventing the further development of the illness. But the combination of the two did not confer added any added benefit.

Schizophrenia

Recent research has shown that schizophrenics are low in antioxidant defenses. Plasma levels of vitamin C are low and more vitamin C is needed to raise blood levels to a given level than normal suggesting an increased usage in combating oxidative stress. Glutathione levels in blood are also low. There is also a strong correlation between low levels of the antioxidant enzyme GSHpx and the degree brain damage in schizophrenia. Many years ago Abram Hoffer, Humphry Osmond and I demonstrated that schizophrenia may also be associated with a diminished capacity for dealing with toxic quinone metabolites in the brain produced from the oxidation of catecholamines. Recent trials of antioxidants have been promising.[9]

Parkinson's Disease

This disease is associated with severe oxidative stress in the substantia nigra and destruction of its neuromelanin-containing dopaminergic neurons. Neuromelanin is a complex polymer made up of units of metabolites of dopamine quinones. It is normally neuroprotective by preventing the build-up of toxic dopamine quinones and because it is a powerful chelator of toxic heavy metals particularly iron. However, in excess, it becomes neurotoxic and leads to the destruction of the cell. In the disease the cellular levels of glutathione fall to very low levels. This leads also to failure to store dopamine properly in its vesicles so the dopamine is left unprotected by antioxidants in the cytoplasm and its increased conversion to toxic quinones results which damages the cell.

There have been several trials of antioxidants in Parkinson patients but the results are conflicting. However, as we saw

in the case of diabetes, antioxidants are likely to work much better before the cells have been destroyed by oxidative attack. Unfortunately this attack starts at least five years before any symptoms develop. So the only way to protect against the development of Parkinson's disease is to take antioxidant supplements from aged 40 on.

Ischemia/reperfusion

In heart attacks and stroke, and during organ transplants, a period of ischaemia (lack of blood) is followed by reperfusion when blood carrying oxygen re-enters the damaged area or organ. This produces severe oxidative stress and lowering of intracellular glutathione levels. Several studies have shown that effective protection can be given by antioxidants during transplant surgery. A recent study of coronary artery bypass surgery showed that preoperative administration of vitamin E (four weeks) resulted in fewer abnormalities in the electrocardiogram and fewer infarctions around the time of the operation.[10]

The Common Cold

Linus Pauling claimed 20 years ago that large doses of vitamin C would benefit sufferers from the common cold. Recent studies show that there is no change in the number of colds; but there are significant falls in the duration and severity of the symptoms.[11] Many of the symptoms of a cold are due to the ROS produced by the macrophages to kill the virus, attacking the cells of the mucous lining of the nasal cavity instead. The antioxidant properties of vitamin C could protect against this.

Pancreatitis

Pancreatitis is associated with severe oxidant stress and depletion of antioxidant defenses–beta-carotene and vitamins C and E. Antioxidant defenses are low in pancreatitis. One clinical trial of the antioxidant NAC has produced promising results.

Systemic Sclerosis

This is a connective tissue disease (also called scleroderma) due to overproduction of collagen that leads to vascular damage and Raynaud's syndrome (repeated attacks of vascular spasm leading to gangrene of the extremities). It is marked by repeated ischaemia and reperfusion in the tissues with resulting oxidative tissue damage. Many organs are involved including the heart, lung, gut and kidneys. These patients have low plasma levels of vitamin C but normal levels of vitamin E. The low level of vitamin C is not due to dietary deficiencies nor maladsorption. It is not clear if the low plasma levels of vitamin C is a cause or result of the oxidative stress. Clearly antioxidants might be effective in this disease.

Cystic Fibrosis

Cystic fibrosis patients have very low beta-carotene and vitamin E levels and high levels of oxidized fats. Giving supplements of beta-carotene help normalize the levels of lipid oxidation. The lower the levels of the protective beta-carotene, the higher are the levels of oxidation of the fats. Therefore supplements containing beta-carotene could be therapeutic in cystic fibrosis.[12] Patients with this disease who have low blood levels of vitamin C (below 40μ m/L) have high indices of inflammation. Whereas those with high levels (above 80μ m/L) have lower values of these inflammation levels.

However it is theoretically possible that antioxidants might benefit the bacterium more than the patient. The bacterium already produces one antioxidant itself (slimy alginate) and the antioxidant might interfere with the oxidant attack used by the white blood cells to kill the bacterium, as happens in eye infections.

Renal Dialysis

The red blood cells in these patients show evidence of oxidative stress with reduced levels of the antioxidant enzymes

SOD, CAT and GSHpx. This is results in fragile red cells with more rigid cell membranes and increased lipid oxidation. So this is another area in which antioxidants added to the perfusion fluid would be expected to be advantageous.

Rheumatoid Arthritis

The fluid in the affected joints in these patients has raised levels of ROS and free iron. The more severe the disease the higher are measures of lipid oxidation in the blood. Many patients with rheumatoid arthritis are marginally deficient in vitamins C and E. A low blood level of vitamin E, beta-carotene and selenium is associated with an eight-fold increase in the risk for the disease.

ß-thalassemia

These patients suffer from severe iron overload from the repeated blood transfusions necessary, which leads to damage to many organs. The excess iron induces severe oxidative stress. The total antioxidant potential in blood is significantly reduced.

Amongst other diseases where oxidative stress is involved are myotonic dystrophy, hyperthyroidism, malaria, inflammatory bowel disease, motor neuron disease and collagen and mitochondrial diseases.

Safety of Antioxidants

In general, antioxidants are very safe but there are a few things to watch out for. Vitamin C is contraindicated in patients with iron overload as in the presence of free iron vitamin C turns into a pro-oxidant. In practice this means that patients with hemachromatosis, thalassemia and similar conditions should not be given large doses of vitamin C. There have also been some theoretical worries that vitamin C might increase the chance of getting an oxalate kidney stone as oxalate is a metabolite of vitamin C. However, as far as I know, there has never been a clinical report of such an event. Nevertheless the physician should bear this possibility in mind in dealing with patients with this condition. In a recent report[13] Dr. Anthony Diplock of Guy's Hospital states that the 'stone story' has proven on critical examination to be "without foundation". If excess vitamin C is ingested it is excreted as such not as oxalate. He criticizes earlier studies that seemed to show that increasing vitamin C intake led to increased oxalate output to technical errors in the estimation. In fact he concludes that vitamin C in the doses normally used in supplements is entirely free from side effects. Some other reports suggest that vitamin C should be used with caution in elderly patients with cataract.

Vitamin E is quite safe except in patients with vitamin K deficiency. As we have seen beta-carotene should not be given by itself or with vitamin E or vitamin A to heavy smokers. As far as I know there have been no reports of toxicity in the case of coenzyme Q10 and alpha lipoic acid. However, it is always important to give a full range of antioxidants, and not just one or two, since they work together in a very complex system. Giving large amounts of just one or two antioxidants will upset this delicate mechanism and may have undesirable effects. The only circumstance when this rule may be broken is if a blood level profile shows a deficiency of one or more particular antioxidants (see further below).

Effective Therapy with Antioxidants

It is no longer an effective strategy in disease prevention, nor is it rational therapy, to advise that all people should do nothing more than increase their intake of fruit and vegetables to recommended levels. Nor is it advisable simply to take an antioxidant supplement mixture from the shelf at the supermarket without any kind of medical supervision. This is because there are great differences between individuals as to their need for particular antioxidants. We now have accurate ways of measuring the blood levels of all important antioxidants and so it should be a part of

the routine medical checkup for everyone to have their antioxidant profile taken just as no doctor would neglect to take blood sugar levels. Furthermore, in the case of all diseases in which oxidative stress is involved, it is obviously important to keep track of the state of the patient's antioxidant defenses by doing this profile. In this manner, both people who wish to minimize their risk of being damaged by oxidative stress, and people who wish to have the optimum treatment for any disease that they be suffering from in which oxidative stress plays a role, will be more likely to have their antioxidant deficiencies put right.

Conclusion

Linus Pauling would be very pleased to see his brain child rapidly becoming part of mainstream medicine. There have been over 4,000 papers published available on Medline on oxidative stress and antioxidants since 1993. On an average twenty new papers are published every week on this topic. The drug industry is busy looking for new natural and synthetic antioxidants. Interestingly many herbs used in traditional medicine contain potent antioxidants that may be responsible for their effectiveness. As I said earlier the only dispute today is between those who say that all we need to do is to eat more fruits and vegetables and those who say that this good advice is simply not going to work. The reason for this is that at present only 10% of the population of the USA follow these guide lines about three helpings of vegetables and two of fruit a day and all attempts to increase this percentage have failed, so hard to shift are ingrained nutritional habits. So the opposition claim that it is better to take a well balanced and inclusive antioxidant supplement than do nothing other than subsist on the usual junk food diet. The purists claim that this will mean that supplement takers will miss out on the many important flavonoids, polyphenols, isoprenes and similar ingredients of fruits and vegetables that are not usually found in supplements. Therefore, it is encouraging that some supplement manufacturers have started adding flavonoids and polyphenols to their formulas. It is also encouraging that the National Cancer Institute has started a joint venture with the agricultural industry to try to alert the public to the vital need to eat more fruits and vegetables.

Some purists also make the claim that the standards used to evaluate new drugs – double-blind, placebo-controlled trials – should always be used in the case of antioxidants before any supplements be taken. The mistakes in this argument have been pointed out cogently by Dr. Gladys Block[14] and Dr. Jeffrey Blumberg.[15] Since antioxidants work as a team such trials as are presently being conducted of only one or two antioxidants are badly designed for the reasons I have outlined in this review. Moreover, in the case of the attempt to prevent the development of chronic diseases such as atherosclerosis, cancer or Parkinson's disease. we are dealing with a pathology that is spread out over many years, so that these sort of trials are in practice impossible to carry out.

Dr. Blumberg says: "It is unrealistic and unnecessary to wait until the clinical trials are complete before applications for disease prevention are endorsed." He adds that the evidence should be judged in toto and not just on the standards applied to new drugs i.e. prospective, randomized, double-blind placebo-controlled trials. He also comments that antioxidants "appear remarkably benign even at high supplementary intakes" and that they are inexpensive to boot. He concludes "Recommendation to wait until every conceivable study has been designed and conducted to achieve a level of absolute certainty will result in the continuing cost of the disease to the individual and society."

Dr. Block lists a series of fallacies in the purist approach and says these trials usually:
• Select persons at high risk for a disease;
Rarely test more than one or two substances and usually at one dose.
•Test only the efficacy of an agent given

for a limited time usually late in life.
• Tell us little about prevention of long-term chronic diseases.
• Tell us nothing about whether the agent at high dose might reduce the risk of chronic diseases if taken throughout a lifetime.
• Tell us nothing about the combination of antioxidants that we have seen to be so important.
• Do nothing to resolve the questions that interest us, which involve persons with no unusual risk of disease, a lifetime exposure to noxious and protective agents involving an enormously complex interaction among nutrients, and the effects of these nutrients on hundreds of diseases, many uncommon.

Clinical trials, she says, simply cannot answer these questions. What we needed is a solid examination of the laboratory and epidemiological evidence. Unfortunately, at the moment, the purists are in the majority, at least on the Committees that decide on whether the RDAs of antioxidants should be raised. The evidence I present in my book offers strong support to the claim that the RDAs for vitamin E and vitamin C at least should be raised immediately. How can one expect people to increase their intake of fruits and vegetables if the official RDAs still reflect the completely exploded theory that all that vitamins do in the body is to prevent deficiency diseases? Moreover the very concept of an RDA that can be applied to everybody is not in keeping with the fact that people's needs vary and need to be evaluated on an individual basis by a individual dietary survey plus a blood antioxidant profile.

References

I. Omenn GS, Goodman GE, Thornquist MD et al: Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med,* 1996; 334: 1 1 5 O- 1 1 5 5

2. *The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers.* N Engl J Med, 1994; 330: 1029-1035.

3. Elliott RB, Chase HP: Prevention or delay of type 1 (insulin dependent) diabetes mellitus in children using nicotinamide. *Diabetologia,* 34: 362-365.

4. Packer L, Witt EH, Tritschler HJ: Alpha-lipoic acid as a biological antioxidant. *Free Rad Biol Med,* 1995; 19: 227-250.

5. Voelker R: Antioxidants and asthma. *JAMA,* 1997; 277,:1926.

6. Sawyer MAJ, Mike JJ, Chavin K et al: Antioxidant therapy and survival in ARDS. *Crit Care Med,* 1989; 17: S153.

7. McGeer PL, Schulzer M, McGeer EG: Arthritis and anti inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiological studies. *Neurology,* 1996; 47: 425-432.

8. Sano M, Ernesto C, Thomas RG et al: Controlled study of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. *N Engl J Med,* 1997; 36: 1216-1222.

9. Kanofsky JD, Sandyk R: Antioxidants in the treatment of schizophrenia. *Internat J Neurosci,* 1992; 62: 97 100.

10. Sisto T, Paajanen H, Metsä-Ketelä T et al: Pretreatment with antioxidants and allopurinol diminishes cardiac onset events in coronary bypass grafting. *Ann Thorac Surg,* 1995; 59: 1519-1523.

11. Hemilä H, Herman ZS: Vitamin C and the common cold: a retrospective study of Chalmers' review. *Am J Clin Nutr,* 1996; 14: 116-123.

12. Ramsey BW, Farrell PM, Pencharz P et al: Nutritional assessment and management in cystic fibrosis: a consensus report. *Am J Clin Nutr,* 1992; 55: 108-116.

13. Diplock AT: Safety of antioxidant vitamins and ß-carotene. *Am J Clin Nutr,* 1995; 62 (suppl..): 1510S-1516S.

14. Block G. Are clinical trials really the answer? *Am J Clin Nutr,* 1995; 62: 1517S-1520S.

15. Blumberg J: Are antioxidants at an awkward age? *J Am Coll Nutr,* 1994; 13: 218-219.