Antioxidants: what role do they play in physical activity and health?[1,2]

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ABSTRACT Exercise appears to increase reactive oxygen species, which can result in damage to cells. Exercise results in increased amounts of malondialdehyde in blood and pentane in breath; both serve as indirect indicators of lipid peroxidation. However, not all studies report increases; these equivocal results may be due to the large intersubject variability in response or the nonspecificity of the assays. Some studies have reported that supplementation with vitamins C and E, other antioxidants, or antioxidant mixtures can reduce symptoms or indicators of oxidative stress as a result of exercise. However, these supplements appear to have no beneficial effect on performance. Exercise training seems to reduce the oxidative stress of exercise, such that trained athletes show less evidence of lipid peroxidation for a given bout of exercise and an enhanced defense system in relation to untrained subjects. Whether the body's natural antioxidant defense system is sufficient to counteract the increase in reactive oxygen species with exercise or whether additional exogenous supplements are needed is not known, although trained athletes who received antioxidant supplements show evidence of reduced oxidative stress. Until research fully substantiates that the long-term use of antioxidants is safe and effective, the prudent recommendation for physically active individuals is to ingest a diet rich in antioxidants. *Am J Clin Nutr* 2000;72(suppl):637S–46S.

KEY WORDS Vitamin E, vitamin C, reactive oxygen species, supplements

INTRODUCTION

Are antioxidant supplements necessary for individuals who exercise regularly? Should antioxidant supplements be part of the "nutritional game plan" of athletes? These are common questions directed to fitness leaders, athletic trainers, and other health professionals who are consulted about the role of antioxidants in a healthy, active lifestyle.

The reason for this interest in antioxidants is the finding that highly reactive chemical species, called free radicals, may increase during exercise (1–12). A free radical is broadly defined as a molecule containing ≥1 unpaired electrons in its outer orbit. During oxidative metabolism, much of the oxygen consumed is bound to hydrogen during oxidative phosphorylation, thus forming water. However, it has been estimated that 4–5% of the oxygen consumed during respiration is not completely reduced to water, instead forming free radicals. Thus, as oxygen consumption increases during exercise, a concomitant increase occurs in free radical production and lipid peroxidation.

Exercise is postulated to generate free radicals by other means, including *1*) increases in epinephrine and other catecholamines that can produce oxygen radicals when they are metabolically inactivated, *2*) production of lactic acid that can convert a weakly damaging free radical (superoxide) into a strongly damaging one (hydroxyl), and *3*) inflammatory responses to secondary muscle damage incurred with overexertion (1–12).

The body contains an elaborate antioxidant defense system that depends on dietary intake of antioxidant vitamins and minerals and the endogenous production of antioxidant compounds such as glutathione. Vitamins C and E and beta-carotene are the primary vitamin antioxidants. In addition to glutathione, there are numerous enzymes involved in the quenching or removal of free radicals (1–12).

It is not fully known whether the body's natural antioxidant defense system is sufficient to counteract the increase in free radicals with exercise or whether additional supplements are needed. In this article, we briefly focus on the chemistry of free radicals and the measures used to assess oxidative stress in human subjects. We also discuss exercise- and training-induced changes in markers of antioxidant status, the effects of supplementation with antioxidants, and the role of estrogen as a potential antioxidant.

CHEMISTRY OF FREE RADICALS

A free radical is a molecule that contains an unpaired electron in its outer orbit and that can exist independently. Molecular oxygen is a diradical, containing 2 unpaired electrons with parallel spin configurations. Because electrons must have opposite spin to occupy the same orbit, electrons added to molecular oxygen must be transferred one at a time during its reduction (6, 13), resulting in several highly reactive intermediates (13). The complete reduction of oxygen to H_2O requires 4 steps and the generation of several free radicals and H_2O_2, which is not a free radical in itself because it contains no unpaired electrons. H_2O_2 is, however, considered a reactive oxygen species (ROS) because of its ability to generate highly reactive hydroxyl free radicals through interactions with reactive

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transition metals (14, 15). The complete reduction of oxygen is summarized in the following equations:

$$O_2 + e^- \rightarrow O_2^{-\bullet} \text{ superoxide radical} \quad (1)$$

$$O_2^{-\bullet} + H_2O \rightarrow HO_2^{\bullet} + OH^- \text{ hydroperoxyl radical} \quad (2)$$

$$HO_2^{\bullet} + e^- + H \rightarrow H_2O_2 \text{ hydrogen peroxide} \quad (3)$$

$$H_2O_2 + e^- \rightarrow {}^{\bullet}OH + OH^- \text{ hydroxyl radical} \quad (4)$$

Each of these oxygen-derived intermediates is considered highly reactive because its unstable electron configurations allow for the attraction of electrons from other molecules, resulting in another free radical that is capable of reacting with yet another molecule. This chain reaction is thought to contribute to lipid peroxidation (16), DNA damage (17), and protein degradation (18) during oxidatively stressful events. Although all the intermediates are potentially reactive, the intermediates vary in their biological importance. The superoxide radical ($O_2^{-\bullet}$) is the most well-known oxygen-derived free radical (13) and, unlike the other oxygen-derived intermediates, can lead to the formation of additional reactive species (19). In particular, the protonation of $O_2^{-\bullet}$ results in the formation of perhydroxyl radical $HO_2^{\bullet}$, a much stronger radical than $O_2^{-\bullet}$. Additionally, $O_2^{-\bullet}$ acts as a Bronsted base in aqueous solutions to shift the acid-base equilibrium to form a hydroperoxyl radical, thereby forming H_2O_2 in acidic environments (13). Superoxide dismutase catalyzes the dismutation of the superoxide radical at neutral or acidic pH (13).

Hydrogen peroxide (H_2O_2), although not a free radical by definition, is a biologically important oxidant because of its ability to generate the hydroxyl radical, an extremely potent radical (15). Further, because of its nonionized and low charged state, H_2O_2 is able to diffuse through hydrophobic membranes, as seen with the leakage of H_2O_2 from mitochondria (13). The hydroxyl radical (OH^-) is formed not only by the reduction of hydrogen peroxide but also through the interaction of superoxide with hydrogen peroxide as well as the interaction between hydrogen peroxide and the reduced forms of metal ions, ie, copper and iron (20). The ability of the hydroxyl radical to remove or add hydrogen molecules to unsaturated hydrogen bonds of organic lipids makes it potentially one of the most reactive oxidants in biological systems. Its very short half-life (1×10^{-9} at 37°C), however, restricts its diffusion capability and its potency (21).

The most well-described consequence of the generation of free radicals and ROS is lipid peroxidation (16). In vitro, the interaction between free radicals and lipids involves 3 processes: initiation, propagation, and termination. During initiation, conjugated dienes are formed through the abstraction of a hydrogen atom from a backbone methylene group of a lipid (8). This allows for the interaction of molecular oxygen with carbon-centered free radicals to form lipid hydroperoxides, also called propagation. The resultant decomposition of these lipid hydroperoxides produces alkoxyl or peroxyl radicals that continue the process of propagation (20). Polyunsaturated fatty acids (such as those found in biological membranes) are particularly vulnerable to this process of initiation and propagation because of the multiple unsaturation points found along their backbone. Oxidative damage of membranes results in increased membrane fluidity, compromised integrity, and inactivation of membrane-bound receptors and enzymes (22).

Although not as well described, free radicals can damage DNA (23); however, no direct measures are available to quantify such damage in vivo. Additionally, proteins appear to undergo modification when exposed to oxygen-derived free radicals. Alterations in fluorescence have revealed that the oxidation of amino acids results in physical changes, such as fragmentation and aggregation, of proteins themselves (18). These gross conformational changes also make proteins more susceptible to proteolytic degradation via ATP-dependent and ubiquitin pathways (24).

In summary, the univalent reduction of oxygen produces a series of free radicals and ROS that interact with lipids, DNA, and proteins. This interaction degrades proteins and promotes DNA-strand breakage and damage to other genomic structures. These reactive species affect lipids as well, compromising the integrity of polyunsaturated fatty acids, which in turn can affect the homeostatic environment of the cell.

MEASUREMENT OF OXIDATIVE STRESS IN HUMANS

To aid in the interpretation of the studies described in this article, a few in vivo methods of measurement in humans are outlined here. Because the products of peroxidation are affected by both the chemical composition of the tissue being studied and the presence or absence of metal ions, there is no single biomarker that is considered the "gold standard" of lipid or protein oxidation. It is recommended that ≥2 techniques be used for accurate and consistent evaluation of oxidative stress in humans (25). Additionally, Halliwell and Chirico (26) suggested that separation of peroxidation products with high-pressure liquid chromatography before measurement greatly enhances the accuracy of the measure. Further specificity can be obtained by separation through the use of monoclonal antibody techniques (26).

Most investigations in humans have examined byproducts of lipid peroxidation [ie, conjugated dienes, lipid hydrocarbons, thiobarbituric acid–reactive substances (TBARS), such as malondialdehyde (MDA)], or lipid peroxides to infer oxidative stress. Conjugated dienes, which absorb ultraviolet light at 230–235 nm, are one of the first products of the peroxidation of unsaturated fatty acids and are considered accurate and repeatable measurements of lipid peroxidation by some (27). Others do not advocate their use with human body fluids (28). Conjugated dienes may also be apparent in diets, thereby confounding whole-tissue peroxidation. The use of conjugated dienes as a marker of oxidative stress in humans must therefore be interpreted with caution.

An early study (29) and a more recent investigation (30) have used the exhalation of ethane and pentane, the hydrocarbon products of the splitting of lipid peroxides, as indicators of oxidative stress. Pentane is a particularly accurate and repeatable measure because the types of fatty acids from which pentane is derived are found predominantly in cell membranes (30), but it is affected by the oxygen concentration in vivo and in the presence of metal ions (22). Although attractive in its noninvasiveness, this measure must be interpreted with caution and be used as a supplemental technique to other indicators of oxidative stress.

In modern years, electron spin resonance and paramagnetic resonance spectrometry have been used in animals to directly detect superoxide radicals. These techniques are the most powerful available because they both measure transition states of the radicals themselves and do not rely on byproducts of lipid peroxidation (8). Currently, however, they are employed exclusively in animals and few studies describe exercise-related oxidative stress with electron spin resonance and paramagnetic resonance spectrometry. Another relatively new technique employed with

biological samples (blood, serum, and saliva) assays for lipid hydroperoxides by chemiluminescence (31). In this assay, an oxyradical is coupled with the production of light so that the presence of an antioxidant in the sample can be estimated from the decrease in chemiluminescence. This method has been shown to be sensitive, rapid, reproducible, and simple. Moreover, it allows for the measurement of total antioxidant capacity in small volumes of biological fluid (31). Currently, this assay is used in the clinical sciences and is gaining popularity because of its advantages over the determination of peroxidation products (31).

Intracellular glutathione rapidly oxidizes to glutathione disulfide (GSSG) in the presence of H_2O_2 and hydroperoxides, but it is rapidly reduced back to glutathione if the oxidative stress is not severe. If the stress exceeds the capacity of the cell to reduce GSSG to glutathione, the increase in GSSG in the blood may be used as a marker of oxidative stress (6, 32). In a modification of this technique, the consumption of NADPH is used to quantify the peroxide content of particular tissues after the addition of glutathione reductase and NADPH. This technique is often used in the biological sciences (26) but does not appear in the exercise literature.

The most widely used technique to evaluate lipid peroxidation is the use of TBARS that include MDA formed in peroxidizing systems, with results generally expressed as millimoles of MDA equivalents. Although this technique is easy to use and interpret with defined systems such as microsomes and liposomes, the use of the TBARS test with human fluids is problematic for several reasons (33). First, aldehydes other than MDA may react with thiobarbituric acid to produce a compound that absorbs in the same range as MDA (34). Second, the decomposition of lipid peroxides during the test itself may mask the actual MDA content in the fluid before testing (35). Furthermore, the presence or absence of metal ions or other undefined radicals affects the rate of this decomposition, making reliability a problem (35). Finally, when this technique is used on human fluids, other chemicals may produce false-positive results (35). As stated previously, however, although the TBARS test may not reflect the amount of MDA actually found in the sample, accuracy and reliability appear to be greatly enhanced by a series of amplification (with butylated hydroxytoluene) and purification steps (with the use of high-pressure liquid chromatography) described by Halliwell and Chirico (26) before the use of the TBARS test.

In summary, all of the methods to assess lipid peroxidation and oxidative stress in humans should be used with caution because of the lack of accuracy, validity, or both. It is recommended that >1 technique be used to provide a better estimate of oxidative stress.

ANTIOXIDANTS

Antioxidant defenses in the cell can temper the negative influence of free radicals and associated reactions and keep them in check (9, 36–38). Vitamin E is the major lipid-soluble antioxidant in cell membranes. It protects against lipid peroxidation by acting directly with a variety of oxygen radicals, including singlet oxygen, lipid peroxide products, and the superoxide radical, to form a relatively innocuous tocopherol radical (36). Vitamin C can interact with the tocopherol radical to regenerate reduced tocopherol. Vitamin C is water soluble and can directly react with superoxide, hydroxyl radicals, and singlet oxygen (39). Beta-carotene, the major carotenoid precursor of vitamin A, is the most efficient "quencher" of singlet oxygen (40).

Glutathione serves as a substrate for glutathione peroxidase, an enzyme that functions to remove hydrogen peroxide. The reduced form of glutathione (GSH) is acted on by glutathione peroxidase to produce the oxidized form of glutathione, GSSG. The mineral selenium is an essential component of glutathione peroxidase. Glutathione peroxidase and other antioxidant enzymes (eg, superoxide dismutases, catalase, and glutathione reductase) function to reduce lipid peroxidation. For a full description of natural antioxidants and their actions, see Maxwell (5).

Changes induced by exercise

Several studies have examined the effects of acute exercise on changes in amount of antioxidants in the blood and changes in indirect indicators of lipid peroxidation to provide information on oxidative stress induced by exercise (1–12). Because aerobic exercise increases oxygen consumption, many studies have employed prolonged submaximal exercise bouts (1–12). However, exercise that causes muscle damage has also been examined (1–12). These exercises are biased toward eccentric, ie, muscle-lengthening, contractions, which produce damage to muscle fibers and may elicit lipid peroxidation of membranes as a result of either direct damage or the generation of free radicals associated with macrophage or neutrophil invasion.

Changes in blood amounts of vitamins C and E, as well as changes in glutathione in the blood, have been used to indicate increased oxidative reaction. It is thought that these antioxidants may be mobilized from tissue stores to combat oxidative stress elsewhere in the body. Because erythrocytes contain antioxidants and are easy to remove from the body, they have been used most often in in vivo studies of cellular antioxidant response to oxidative stress (1–12).

Changes in ascorbic acid and tocopherol

Gleeson et al (41) reported that the plasma concentration of ascorbic acid increased from 52.7 mmol/L to 67.0 mmol/L immediately after a 21-km running race. However, at 24 h after the race, the ascorbic acid concentrations decreased to 20% below pre-exercise values and remained low for the next 48 h. The increase in ascorbic acid correlated significantly with an increase in cortisol. The authors suggested that the increase in ascorbic acid was a result of concomitant release of cortisol and ascorbic acid from the adrenal glands. Duthie et al (42) also found that plasma ascorbic acid concentrations increased at 5 min after a half-marathon, but values returned to normal at 24 h. In the latter study, some of the change at 5 min after exercise is probably due to the 6% decrease in plasma volume.

Camus et al (43) examined plasma ascorbic acid concentrations after 35 min of treadmill downhill running and uphill walking set to the same intensity (energy cost). Blood samples were taken 20 min into the exercise, immediately after exercise, and 20 min after exercise. With the downhill run, ascorbate concentrations decreased 20 min into the run, decreased further immediately after exercise, and approached resting values at 20 min after exercise. Ascorbic acid concentrations changed little in the walking trial. Because the exercises were similar in intensity, the authors suggested that the increase in cortisol, which reflects the intensity of exercise, would be the same for the 2 exercises and therefore could not be used to explain the difference in ascorbic acid changes between downhill running and uphill walking. They also found that plasma concentration of myeloperoxidase increased, which reflected polymorphonuclear neutrophil

activation, and this increase correlated with the decrease in ascorbic acid concentrations. These authors theorized that ascorbic acid may be used in the process of quenching free radicals produced by polymorphonuclear neutrophils.

Pincemail et al (44) and Camus et al (45) found that plasma tocopherol concentration increased during dynamic exercise. However, they did not account for exercise-induced changes in plasma volume. Vitamin E concentrations increased significantly in long-distance skiers during a graded exercise test (46). Plasma tocopherol concentrations were unchanged after a half-marathon in samples taken immediately after and 120 h after the race (42), but no samples were taken during exercise.

It is not clear why studies examining concentrations of vitamins C and E during and after exercise show various responses. This variability may be due to the differences in the mode of exercise used, the time points examined, the level of training of the subjects, environmental factors (eg, altitude), or lack of control for changes in plasma volume.

Changes in glutathione

Changes in the glutathione system have been assessed because GSSG efflux from cells into the plasma is considered indicative of oxidative stress. GSH is oxidized to GSSG in cells in response to an increase in free radicals (2).

Sastre et al (47) observed changes in glutathione redox status in the blood after exercise. They found that trained men who were exercised to exhaustion on a treadmill had increased blood amounts of GSSG immediately after exercise, but values returned to rest within 1 h. Blood amounts of GSH did not change significantly. Tessier et al (48) also reported that blood GSSG increased in response to a maximal aerobic capacity test, thereby reducing the ratio of GSH to GSSG. Gohil et al (49) found that prolonged submaximal exercise resulted in an increase in GSSG, but they found that GSH decreased. These results were similar to those of Laires et al (50) and Viguie et al (51). It has been suggested that decreased plasma GSH after exercise reflects its consumption by skeletal muscle, which results in a reduced export rate from muscle into plasma (52).

In contrast to the previous studies, Camus et al (43) observed no change in blood GSH or GSSG after uphill walking or downhill running for 35 min, and Marin et al (53) reported no change in blood GSH and GSSG during 30 min of treadmill running. The lower metabolic intensity or the less prolonged exercise used in the latter studies may explain the discrepant results. However, other studies showed that blood GSH increased after progressive-intensity exercise and prolonged exercise (32, 54). Currently, it is difficult to explain these equivocal results.

Duthie et al (42) found that erythrocyte GSH decreased and GSSG was unchanged after a half-marathon. Erythrocytes contain glutathione as an antioxidant to prevent the oxidation of hemoglobin to methemoglobin. Ohno et al (55) and Evelo et al (56) reported that erythrocyte glutathione reductase activity was increased after exercise. Glutathione reductase restores GSH by oxidizing GSSG through the use of NADH. Thus, an increase in glutathione reductase activity suggests a response to oxidative stress induced by exercise.

Changes in lipid peroxidation

Increases in MDA in the blood were found after an 80-km race (57), after a 30-min treadmill test at 60% and 90% maximal oxygen uptake ($\dot{V}O_2max$) (58), after downhill running (59), and after incremental cycling tests to exhaustion in sedentary and moderately trained men (60, 61). In contrast, no increases in MDA were found after a half-marathon (42), after 60 min of bench-stepping exercise (62), after maximal cycle ergometry exercise (63), and after incremental cycle ergometry exercise in elite athletes (64). Sahlin et al (65) also reported no increase in MDA after 1 h of repeated static muscle contractions. MDA amounts were found to decrease immediately after a marathon (66) and immediately after a graded exercise test in long-distance skiers (46). The discrepant results may be due to the high intersubject variability in MDA and the nonspecificity of the assay. Maxwell et al (62) also pointed out that the intensity of the exercise and the training level of the subjects affects the results.

Toskulkao and Glinsukon (67) had sedentary subjects exercise on a cycle ergometer for 60 min at 70% of their maximal heart rates. Erythrocyte activities of superoxide dismutase and glutathione peroxidase decreased 5 min after exercise and remained low for 48 h. Erythrocyte catalase activity decreased 5 min after exercise but returned to baseline by 24 h after exercise. Plasma MDA increased 5 min after exercise and remained elevated at 48 h after exercise. The authors suggested that the decrease in the activity of these enzymes may be due to their inactivation by a large production of free radicals.

Fewer studies have examined other indicators of lipid peroxidation. Expired pentane, the breakdown product of lipid peroxides, was found to increase after exercise (29, 30, 58). Meydani et al (68) reported that 45 min of downhill running resulted in an increase in lipid conjugated dienes in the vastus lateralis muscle immediately after exercise and appeared to be elevated at 5 d after exercise. They also found increases in urinary TBARS in the days after exercise.

The in vitro susceptibility of lipids to peroxidation was decreased in blood samples of 39 individuals within 15 min of completing the Hawaii Ironman Triathalon (69). These results, along with the finding of a reduction in triacylglycerol, cholesterol, and low-density lipoproteins and an increase in high-density lipoproteins, led the authors to speculate that these results may explain why exercise is a factor in reducing the risk of developing cardiovascular disease.

EFFECTS OF SUPPLEMENTATION

Vitamin C

Maxwell et al (62) attempted to determine whether 400 mg of supplemental vitamin C for 3 wk would alter the response of plasma MDA and antioxidants to a bench-stepping exercise. The supplement resulted in an increase in blood amounts of vitamin C. The exercise also produced an elevation in blood vitamin C concentrations, as well as in total antioxidant capacity for the supplement group but not for the control group. Total antioxidant capacity was measured with a chemiluminescent method (62). There was no change in MDA due to exercise. All values were adjusted for changes in plasma volume. The authors suggested that the vitamin C supplement resulted in greater tissue stores that are released into the circulation during exercise.

Using the same subjects, supplementation schedule, and exercise protocol, Jakeman and Maxwell (70) found that the group taking vitamin C showed less strength loss in the triceps surae group after exercise, and a faster recovery. The force response to

tetanic stimulation was less in the vitamin C group as well, indicating a reduction in contractile function. The authors suggested that the vitamin C supplement reduced damage from the eccentrically biased exercise of bench-stepping.

Because delayed-onset muscle soreness is an indicator of muscle damage induced by exercise, studies have examined whether vitamin C supplements would reduce soreness. In 1952, Staton (71) had subjects ingest 200 mg ascorbic acid/d or a placebo for 30 d. Subjects then performed one bout of sit-up exercise to induce soreness and repeated the exercise 24 h later. The vitamin C–supplemented group performed more sit-ups on the second bout than did the placebo group. More recently, Kaminski and Boal (72) had subjects ingest 3 g ascorbic acid/d or a placebo for 3 d before eccentric exercise of the calf muscles. The vitamin C supplement appeared to reduce the intensity of soreness. For approximately one-half of the subjects there was a >33% reduction in soreness compared with the placebo.

Several early studies examined the effects of vitamin C supplementation on exercise performance other than its effect on soreness. The results regarding vitamin C supplementation are equivocal, but most well-controlled studies report no beneficial effect on either endurance or strength performance (73–79). Likewise, studies of vitamin C restriction showed that a marginal vitamin C deficiency did not affect performance. Van der Beek et al (80) placed subjects on a restricted vitamin C intake of 10 mg ascorbic acid/d for 3 wk and added another 15 mg/d for an additional 4 wk. The vitamin C restriction had no harmful effect on health and did not affect $\dot{V}O_2$max. Thus, vitamin C supplementation in those with adequate or even inadequate status does not appear to improve exercise performance. However, vitamin C supplementation may be useful in reducing the effects of exercise-induced muscle damage.

Vitamin E

To test the effects of vitamin E on exercise-induced lipid peroxidation, Dillard et al (29) administered 1200 IU α-tocopherol/d to subjects for 2 wk and observed a significant reduction in expired pentane at rest and during exercise. Sumida et al (61) had subjects perform an incremental exhaustive cycling exercise, ingest 300 mg vitamin E for 4 wk, and repeat the exercise test. They found that MDA and activity of muscle enzymes β-glucuronidase and mitochondrial glutamic-oxalate transaminase in the blood were lower after the supplement and concluded that vitamin E was effective in lessening lipid peroxidation. However, it is well known that repeating an exercise that causes muscle damage results in a rapid adaptation such that less changes in markers indicative of damage occur on a second bout of the same exercise. For example, the increased efflux of muscle enzymes into the blood (an indication of muscle damage) is reduced on the second bout (81, 82). Lower MDA on a repeated bout may also be due to a rapid adaptation effect, but this has not yet been proved. Because there was no placebo group, it is difficult to discern the effect of the supplement from the adaptation effect.

Helgheim et al (83) found that the activity of muscle enzymes [eg, creatine kinase (CK) and lactate dehydrogenase) in the blood after strenuous exercise were similar in subjects who ingested 300 mg vitamin E for 6 wk and in those who ingested a placebo. Furthermore, Francis and Hoobler (84) reported that muscle soreness was not reduced by vitamin E supplementation (600 IU/d) 2 d before and 2 d after a damage-inducing eccentric exercise.

In one of the few studies to investigate long-term supplementation, Rokitzki et al (85) had racing cyclists ingest 300 mg α-tocopherol/d or a placebo for 5 mo. In response to strenuous exercise, the vitamin E–supplemented group showed a lesser increase in serum MDA and CK than did the placebo group. Membrane integrity is thought to be compromised by oxidative stress and is evaluated through the measure of CK in the serum because CK is an intramuscular protein that leaks into the serum after membrane damage (81). The authors suggested that the findings indicate a protective effect of vitamin E on oxidative stress leading to muscle damage induced by exercise.

Several studies have investigated the effects of vitamin E on exercise performance and have found no beneficial effects on measures of endurance or aerobic capacity (3, 86–95), even after 5 mo of supplementation (85). The only studies to find that vitamin E supplementation enhanced performance were done at high altitude, which further increases oxidative stress (96–98). In one study, diets poor in vitamin E were administered to subjects for 13 mo, and there was no effect on workload and no subjective reports of muscle weakness (99). In summary, vitamin E supplementation does not appear to enhance exercise performance but may offer protection from exercise-induced muscle damage, although the study results are equivocal.

Antioxidant mixtures

Kanter et al (58) examined the effect of an antioxidant mixture (148 mg α-tocopherol equivalents, 250 mg ascorbic acid, and 7.5 mg beta-carotene or a placebo) 4 times/d for 6 wk on lipid peroxidation induced by exercise. Both MDA and expired pentane were assessed before and immediately after subjects performed 30 min of treadmill running at 60% $\dot{V}O_2$max, followed by 5 min at 90% $\dot{V}O_2$max. Although the vitamin supplement significantly increased blood amounts of the 3 vitamins at the end of the supplementation period, the increase in expired pentane after exercise was similar before and after supplementation. However, after supplementation, the resting values were lower, and thus the final values were also lower. The same pattern occurred for MDA. These results could suggest that the antioxidant mixture blunted lipid peroxidation.

Dragan et al (100) investigated the effects of a supplement containing selenium, vitamin E, glutathione, and cysteine (concentrations were unspecified) that was ingested by trained cyclists for 3 wk while they were training daily (2 h/d). Although the supplement resulted in significantly less change in MDA than did the placebo, the crossover appeared to affect the results such that there may have been some carryover effect from ingesting the selenium on the first leg of the crossover. Thus, it is difficult to draw any firm conclusions.

A combination of vitamin C (2 g) and glutathione (1 g) were given daily for 7 d before performance of an incremental treadmill test until exhaustion (47). This test was previously found to increase GSSG in the blood, which the author attributed to increased oxidative stress. After the supplementation period, the increase in GSSG after exercise was not seen. Sen et al (63) also noted smaller changes in blood GSSG in subjects who were supplemented with *N*-acetylcysteine, a pro-GSH free radical scavenger.

Rokitzki et al (66) examined the effect of 400 IU vitamin E and 200 mg vitamin C or a placebo for 4.5 wk on the MDA and oxidative stress response to a marathon run. The supplement produced higher plasma amounts of vitamins C and E than did the placebo. The marathon resulted in a lowering of MDA immediately

after and 24 h after the run in both groups. However, at 24 h after the marathon, the supplemented group had significantly lower CK in blood than did the placebo group. This may indicate that the supplement offered some protection against exercise-induced muscle damage.

Other antioxidant supplements

Krotkiewski et al (101) examined whether 4 wk of ingestion of pollen extract, containing superoxide dismutase, or a placebo affected muscle soreness induced by bench-stepping. The supplemented group had lower MDA in plasma and muscle after the exercise than did the placebo group. Selenium supplementation (100 mg/d) for 14 d resulted in a decrease in MDA during exercise (102). This study employed a crossover design in which a repeated bout of exercise was used, and data were presented for each leg of the crossover. The group that received the selenium before the first bout showed lower MDA on bout 1 compared with the placebo trial on bout 2.

Tessier et al (48) examined the effect of 10 wk of selenium supplementation (180 mg/d) or placebo during an endurance training program. There was little difference between supplemented and placebo groups in blood total glutathione or GSSG in response to an aerobic exercise test. The study reported a moderate correlation between erythrocyte glutathione peroxidase activity and $\dot{V}O_2$max in the supplemented group only. In another report from the same study design and subjects, Tessier et al (103) found that selenium supplementation had no effect on resting muscle glutathione peroxidase activity. However, there was an increase in muscle glutathione peroxidase activity in response to acute exercise in the selenium-supplemented group. This finding suggests that the selenium supplement may have enhanced the antioxidant defense during exercise.

TRAINING

There are data to support that chronic exercise increases antioxidant defenses. Yagi (104) reported that blood lipid peroxide decreased in response to exercise with increased time of training (to 9 mo) in a 60-y-old male subject, indicating an adaptation effect. In contrast, Dernbach et al (105) examined blood and muscle MDA over 30 d of training in collegiate rowers. Blood and muscle amounts appeared to remain somewhat constant over the course of the training.

Ohno et al (106) found that erythrocyte catalase and glutathione reductase activity showed significant increases after 10 wk of training. Thus, aerobic training had a positive effect on antioxidant processes in red blood cells. Evelo et al (56) reported increased blood GSH in the first 20 wk of training, but these values returned to initial concentrations in the next 20 wk. Erythrocyte glutathione reductase activity increased over 20 wk and remained elevated to the 40th week.

Robertson et al (107) examined the antioxidant status of highly trained runners (80–147 miles/wk), low-moderate–trained runners (16–43 miles/wk), and sedentary individuals and found that the antioxidant capacity was enhanced in the runners. The runners had the highest erythrocyte vitamin E, GSH, and catalase activity, and there was a significant relationship between weekly training distance and erythrocyte activity of the antioxidant enzymes. Jenkins et al (108) reported that subjects in a high fit group (based on $\dot{V}O_2$max) had significantly greater muscle catalase and superoxide dismutase activities than those of subjects in a low-moderate fit group. Toskulkao and Glinsukon (67) also found that trained runners had higher erythrocyte enzyme activity (eg, superoxide dismutase, glutathione peroxidase, and catalase) than did untrained subjects.

Endurance training lasting 10 wk resulted in a decrease in total glutathione and GSSG in the blood, an increase in erythrocyte glutathione peroxidase activity, and a decrease in glutathione reductase activity (103). The authors suggested that the low GSSG amounts relative to GSH, together with the increase in erythrocyte glutathione peroxidase activity, could be explained by a decrease in peroxides in red blood cells. Robertson et al (107) also reported a decrease in the ratio of GSH to GSSG in trained subjects but in addition found increased GSH. However, in this latter study, subjects with >2 y of training were assessed, whereas in the study by Tessier et al (103), untrained subjects were trained for only 10 wk.

Toskulkao and Glinsukon (67) reported that trained runners did not show the decrease in erythrocyte antioxidant enzyme activity in response to exhaustive cycling exercise, as was displayed in untrained subjects. Furthermore, for the long-distance runners, the increase in plasma MDA in response to exercise was abolished. The short-distance runners showed a more blunted MDA response than did the untrained subjects.

Although some studies suggest that training enhances antioxidant capacity, the results are not clear. Studies have used different markers of antioxidant status and different training levels of subjects.

INFLUENCE OF ESTROGEN AND SEX DIFFERENCES IN EXERCISE-RELATED OXIDATIVE STRESS

At the cellular level, theoretical evidence supports the idea that estrogen itself may serve as an antioxidant, although much of the work examines estrogen's ability to protect membrane integrity rather than oxidative stress directly. The structural similarities between vitamin E and estrogen first prompted the idea that estrogen may interrupt lipid peroxidation, arresting the degradation of the membrane (109). Vitamin E's diterpenoid side chain allows it to insert into the hydrophobic portion of the membrane, whereas the trimethylhydroquinone head portion is responsible for the antioxidant activity. Vitamin E is thought to both stabilize membrane integrity in its insertion in the membrane and quench oxidative activity by accepting electrons from of free radicals species with its trimethylhydroquinone head (109). Estradiol, particularly in the estrone form typically found in tissues, has 2 hydroxyl groups that are potentially capable of arresting lipid peroxidation during exercise (109).

It appears that women taking oral contraceptives exhibited higher erythrocyte glutathione peroxidase activity than either premenopausal or postmenopausal women not taking oral contraceptives (110). This finding suggests that differences in endogenous plasma estrogen may affect lipid peroxidation and antioxidant enzyme status because exogenous steroids are thought to be associated with increased free radicals, lipids, and lipid peroxides in the blood (111). This theory was contradicted by Kanaley and Ji (111), who found that erythrocyte glutathione peroxidase activity was significantly higher in amenorrheic athletes relative to eumenorrheic control subjects. The authors suggest, however, that the observed differences in glutathione peroxidase activity may not be a direct result of estrogen status, but rather estrogen status may influence blood antioxidant activ-

ity through another mechanism, such as plasma iron concentrations (112). In any case, it appears that estrogen status and blood antioxidant capacity are related in some way.

Studies in animals support the theory that estrogen protects membrane integrity during times of oxidative stress (113–116), but the data on the potential prophylactic effect of estrogen in humans are less conclusive. Some authors report sex differences in the increase in CK activity after endurance exercise (117, 118), whereas others report no such difference after high-force eccentric contractions (119).

One study has prospectively investigated the effects of estrogen status on exercise-induced muscle damage after an aerobic activity. This investigation, seeking to elucidate the role of estrogen on muscle damage induced by a bench-stepping exercise, compared women taking oral contraceptives with eumenorrheic control subjects (120). Although there were no differences in peak CK activity, the oral contraceptive users reported significantly less quadriceps soreness than did the control subjects, which suggests a relationship between estrogen and exercise-induced muscle damage in humans.

Sex differences in the response to exercise damage are reported only if the exercise protocol comprised an aerobic, not eccentric, component (117, 118), implying that estrogen protects membrane integrity only in the presence of oxidative stress. This in turn suggests that estrogen itself exhibits or promotes antioxidant activity.

The research suggesting that estrogen may serve as an antioxidant is interesting and provocative. However, no studies have confirmed this effect in humans, especially in response to exercise. Further studies of the effects of estrogen as an antioxidant are warranted.

CONCLUSION

Antioxidant supplements have been touted by manufacturers as a means for athletes to perform better, recover more quickly and fully from vigorous exercise, or allow them to train more strenuously. However, the theoretical basis for why antioxidants should enhance performance is not clear. Studies have generally found that antioxidant supplements do not improve performance. The question remains as to whether athletes need supplements to prevent oxidative damage as a result of exercise or to help them recover from the damage. The results are equivocal in this regard. Furthermore, trained subjects generally show less evidence of damage than do untrained subjects. Thus, physical training may enhance the antioxidant defense system to offset the barrage of ROS generated during exercise. It is not known whether this enhanced defense system sufficiently balances the increase in exercise-induced ROS. However, data showing that trained athletes who ingest antioxidant supplements show evidence of reduced oxidative stress suggest the need for further research to fully document the efficacy and safety of long-term antioxidant supplement use.

It should be noted that most studies that have assessed antioxidant changes after exercise or training have used as endpoints various antioxidants or oxidative byproducts (eg, GSSG) in the blood. Increased or decreased blood concentrations do not necessarily reflect changes at the tissue level and may have minimal physiologic implications. The high degree of variability in resting levels of some of these indicators and in their response to exercise underscores the tenuous nature of our understanding of the relationship among exercise, oxidative stress, and the generation of free radicals.

At present, data are insufficient to recommend antioxidant supplements for athletes or other persons who exercise regularly. Some researchers have suggested that megadoses and long-term use of antioxidants can be harmful (121, 122). Hennekens et al (123), in an editorial published in *The New England Journal of Medicine*, commented on the Finnish study (124) finding that male smokers given beta-carotene supplements developed more cases of lung cancer than did the placebo group. They suggested that the results of the Finnish study should provide support for skepticism and for a moratorium on unsubstantiated health claims for antioxidants. The controversy continues as to whether active individuals should take supplements with antioxidants in amounts that are well in excess of recommended dietary allowance values. However, no one questions the importance of ingesting a diet rich in antioxidants for all who exercise and train regularly.

Since this paper was submitted, several excellent studies were published that examined oxidative stress after exercise, and a sample of these references are provided (125–135).

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