Role of Vitamin E and Oxidative Stress in Exercise

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Reactive oxygen species (ROS) play an important role as mediators of skeletal muscle damage and inflammation after strenuous exercise. These ROS arise largely from increases in mitochondrial oxygen consumption and electron transport flux. Bouts of intense exercise are associated with increases in lipid peroxidation, generating malondialdehyde and $F_{2\alpha}$-isoprostanes, and the release of muscle enzymes like lactate dehydrogenase and creatine kinase. Dietary and enzymatic antioxidant defenses appear to play a protective role in muscle cells by reducing associated oxidative damage to lipids, nucleic acids, and protein. However, studies of the use of dietary antioxidants like vitamin E to reduce exercise-induced muscle injury have met with mixed success. The equivocal nature of these results appear to reflect a diversity of factors including the antioxidant(s) tested, the nature and timing of the exercise, the age and fitness of the subjects, and the methodology for assessing oxidative stress. *Nutrition* 2001;17:809–814. ©Elsevier Science Inc. 2001

INTRODUCTION

Dr. Lawrence J. Machlin did more than contribute directly to our understanding of the role of vitamin E in human health and disease through his own research and reviews. He was among the first scientists to appreciate the role vitamins play beyond that of avoiding deficiency syndromes. Larry was extraordinarily generous in sharing his insights about vitamin research and his vision of the potential preventive nutrition could play in promoting optimal health. As Director of Clinical Nutrition at Hoffmann–LaRoche, Larry helped create a corporate culture supportive of the highest caliber of academic research and obtained substantial funding for young and established nutrition investigators around the world. This review is dedicated to his memory.

EXERCISE-INDUCED PRODUCTION OF FREE REACTIVE OXYGEN SPECIES

As a result of greater oxygen consumption during exercise, there is an increased flux of oxygen through the mitochondria, 2% to 5% of which is not completely reduced to water and thus forms reactive oxygen species (ROS).[1] During exercise of higher intensity, ROS generation can be generated by an influx of neutrophils and macrophages into muscle and an activation of cytokines secondary to muscle damage. Further, exercise stimulates a redistribution of blood flow, provoking hypoxia and reoxygenation in some tissues, which can increase the production of superoxide by xanthine oxidase in muscle. When skeletal muscle is subjected to excessive contractile activity, prostanoids and their free-radical intermediates are also released. Moreover, disruption of calcium homeostasis or damage to iron-containing proteins within the muscle can activate ROS production. Of potential relevance to older adults, skeletal muscle and several other tissues in senescent rats present with higher basal levels of ROS and appear more susceptible to exercise-induced oxidative stress.[2,3]

The generation of ROS during endurance exercise can be measured directly in muscle with electron spin-resonance spectroscopy and indirectly with spin-trapping agents. For example, Davies et al.[4] found an intensification of signals with electron spin-resonance spectroscopy from rat hindlimb muscle and liver after an acute bout of exhaustive running. Similarly, Jackson et al.[5] found a 70% increase in free-radical signals from electrically stimulated rat muscle compared with controls. However, limitations associated with electron spin-resonance spectroscopy and spin-trapping methods have promoted the examination of oxidatively modified lipids, nucleic acids, and proteins in muscle and other tissues after exercise.

EXERCISE, MUSCLE DAMAGE, AND BIOMARKERS OF OXIDATIVE STRESS

Exercise-Induced Muscle Damage

Elevations of muscle enzymes such as lactate dehydrogenase and creatine kinase (CK) in plasma are characteristic responses to strenuous exercise and often used as indicators of muscle damage. However, because CK in the circulation reflects its release from muscle fibers and its clearance from the circulation, interpretation of this parameter should be made with caution. Nonetheless, Apple and Rhodes[6] found significantly elevated CK levels in marathon runners 24 to 60 h after a marathon race. Schwane et al.[7] tested subjects running downhill for 45 min and found a significant increase in plasma CK levels at 24 and 48 h postexercise compared with subjects running on a level surface.

Clarkson and Tremblay[8] proposed that the CK release after a high-force eccentric exercise might be due to cellular necrosis. However, changes in blood levels of myofiber proteins have been weakly associated with decreases in muscle function and histologic signs of injury.[9] Manfredi et al.[10] found a four-fold greater number of muscle fibers with ultrastructural damage in older men than in younger men, even though there were no differences in CK after eccentric-cycle ergometry. Cannon et al.[11,12] reported that eccentric exercises increased CKs and neutrophils 24 h postexercise in young and older men and noted that this response was smaller in the older men.

Muscle Damage and Lipid Peroxidation

Lipid peroxidation appears to be an important mechanism underlying exercise-induced muscle damage. An early study by Dillard

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et al.[13] measured graded exercise on a cycle ergometer and found no change in pentane exhalation after low-intensity bouts but did find a two-fold increase relative to resting values after high-intensity cycling. Similarly, Lovlin et al.[14] measured serum thiobarbituric-acid–reactive substances (TBARS) after graded-cycle ergometry to exhaustion and found no difference in serum TBARS during the lower intensities (40% to 70% maximum oxygen consumption, or $V_{O_{2max}}$) but found them significantly elevated at the end of the exercise (100% $V_{O_{2max}}$).

Maughan et al.[15] had subjects perform a 45-min bout of downhill running and found that neither plasma TBARS nor CK rose immediately postexercise but that TBARS were significantly elevated at 6 h and CK peaked at 24 h postexercise. It is worth noting that those subjects with the greatest increase in CK also had the most TBARS. This relationship between muscle-enzyme release and biomarkers of oxidative stress might result from an increase in membrane permeability due to lipid peroxidation. Kanter et al.[16] showed that the increase in plasma malondialdehyde (MDA) was significantly correlated with an increase in serum CK after an 80-km running race. Krotkiewski and Brzezinska[17] also found that the increases in plasma MDA correlated with the increase in CK at 24 h after 90 min of strenuous exercise. Furthermore, the content of MDA in the middle portion of the vastus-lateralis muscle was positively correlated with the percentage and the relative cross-sectional area of the type I muscle fibers but negatively with type II muscle fibers. Thus, the production of lipid peroxides might parallel the exercise-induced increase of oxygen uptake in the muscle, being higher in those muscle fibers that consume the most oxygen.

However, not all studies have shown an increase in lipid peroxidation after strenuous exercise.[18–21] Viinikka et al.[20] examined lipid peroxidation from runners after 10 to 14 min of exhaustive cycle-ergometer exercise and found no change serum TBARS immediately or 30 min after the exercise. Dernbach et al.[22] investigated men and women rowers participating in high-intensity training for 4 wk and found no significant changes in measures of oxidative stress. Saxton et al.[23] had men perform bouts of 70 maximum eccentric and 70 concentric muscle actions of the forearm flexors or 80 maximum eccentric and 80 concentric muscle actions of the knee extensors and found no changes in plasma TBARS or conjugated dienes after either exercise protocol.

$F_{2\alpha}$-isoprostanes derived from arachidonic acid have been associated with oxidant injury and appear to a much more specific biomarker of lipid peroxidation than TBARS.[24,25] A few exercise studies have investigated the relationship between exercise and isoprostanes. Hinchcliff et al.[26] found an increase in plasma isoprostanes of sled dogs that was correlated positively with plasma CK and inversely with serum vitamin E. Kirschvink et al.[27] reported an increase in plasma isoprostanes of horses after a treadmill test, although the results were not controlled for postexercise plasma-volume shifts. In contrast, Mori et al.[28] examined for 8 wk patients with type 2 diabetes who underwent moderate (5565% of $V_{O_{2max}}$) or low-intensity exercise training (heart rate $<$ 100 beats/min) and found that neither training regimen affected urinary isoprostane excretion. Wang et al.[29] compared trained (10 wk of treadmill exercise) with untrained rats and found that the trained group had lower urinary excretion rates of isoprostanes.

Exercise and Measures of Antioxidant Capacity

Liu et al.[30] found the total peroxyl-radical trapping, antioxidant capacity of plasma and the resistance of low-density lipoprotein to oxidation increased among men immediately after a marathon run and 4 d later. Similarly, after a half-marathon, Child et al.[31] found that trained men showed increases in total antioxidant capacity and serum MDA, CK, and uric acid.[31] Child et al.[21] also examined eight men and women performing 70 maximal voluntary eccentric contractions with the knee extensors. Biopsies were taken 7 d before and 4 and 7 d after exercise in addition to blood samples through 12 d postexercise. Muscle soreness increased during the first couple of days after the eccentric exercise and, although no changes in serum total antioxidant capacity, CK, or MDA were noted at that time, total antioxidant capacity of muscle increased 7 d postexercise. Another observation of an exercise-induced increase in antioxidant capacity associated with an increase in biomarkers of oxidative stress was provided by Alessio et al.[32] who noted increased lipid peroxides in serum after isometric contractions and increases in protein carbonyls and oxygen radical-absorbance capacity after aerobic exercise.

Exercise and DNA Damage

The excretion of oxidized DNA-repair products in urine might reflect the overall rate of DNA damage in the body, and those products in tissue might reflect the steady-state balance between damage and repair. After a 9-wk regimen of swim training in rats, Radak et al.[33] reported lower levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) in skeletal muscle but no change in TBARS or 4-hydroxynonenal compared with levels in sedentary controls. Results from human studies are mixed, with several showing no effect of exercise on indices of DNA damage.[33–46]

Physical exercise in some human studies has been associated with lower concentrations of 8-OHdG in leukocytes and urine,[42,43] whereas other studies found no association between frequency of physical exercise and increased leukocyte 8-OHdG.[44] Niess et al.[36] examined young trained and untrained men after an incremental exercise test to exhaustion and found that the untrained men had higher DNA-strand breakage in white blood cells postexercise than did the trained men. The trained men also had lower MDA responses 15 min and 24 h postexercise. Thus, training might evoke increases in cellular antioxidant defenses (increases in total peroxyl-radical trapping, total antioxidant capacity, and oxygen radical-absorbance capacity were discussed) to decrease oxidative damage to DNA. Interestingly, Inoue et al.[45] studied trained swimmers and runners and found decreases in lymphocyte 8-OHdG and increases in urinary 8-OHdG after a training session. Those results suggested an enhanced clearance mechanism for oxidatively damaged DNA in young, trained individuals.

Okamura et al.[37] investigated the urinary excretion of 8-OHdG in well-trained runners before and after an 8-d training camp. Increases in urinary 8-OHdG were reported during the training but no change was found in lymphocyte levels. Hartmann et al.[38] tested young, untrained men with an incremental treadmill exercise to exhaustion and found that DNA-strand breaks from white blood cells began to increase 6 h postexercise and were significantly higher 24 h postexercise. Because the effect on DNA was so delayed, the investigators postulated that the damage was not directly induced by ROS but rather mediated by a secondary factor. Radak et al.[46] assigned women to an eccentric exercise or a control group and found higher muscle 8-OHdG 24 h postexercise; however, biopsies were not obtained before exercise so intrasubject comparisons were not made.

Exercise and Glutathione Status

Reduced glutathione (GSH) is the major intracellular non-protein thiol compound affecting DNA and protein synthesis, protecting against ROS-induced damage, and sparing vitamin E via GSH peroxidase and GSH reductase. The ratio of GSH to oxidized glutathione (GSSG) has been correlated with oxidative stress and physical exercise.[47] Moderate to intense exercise can decrease GSH and increase GSSG through 3 d postexercise in young, trained individuals.[18,34,48] The activity of the GSH-containing enzymes is localized to the mitochondria and cytosol, depends on fiber type, with the highest concentrations in the slow, oxidative muscle fibers, and, with catalase, increases in response to acute exercise and exercise training.[49,50] That latter response suggests that those enzymes, in particular GSH peroxidase is a compensatory response to and a sensitive marker of oxidative stress induced

by exercise.[49–52] Recent studies in distance running and sprint or distance swimming have shown decreases in GSH:GSSG and increases in GSH peroxidase immediately postexercise, which return to baseline levels by 1 h postexercise.[53,54]

Animal models have suggested a complex relationship between exercise, GSH, and oxidative stress. For example, Liu et al.[55] found that acute exercise in rats reduced GSH and cystine and increased MDA in liver. However, chronic exercise was associated with an increase of MDA in fast and slow muscles, whereas brain tissue had a reduced concentration of MDA in association with elevated levels of vitamin C. Protein carbonyls in the liver were decreased with chronic exercise, but neither acute nor chronic exercise affected oxidative damage to DNA as reflected by 8-OHdG. Ohkuwa et al.[3] reported an age-related decrease in GSH synthesis in rats associated with an apparent compensatory increase in regenerating capacity. Although Leeuwenburgh et al.[56] found an age-related increase in rat muscle GSH, exercise training did not offer additional protection against oxidative stress in the muscles of old rats in the manner it did in young rats.

EXERCISE-INDUCED INFLAMMATORY RESPONSES

The local inflammatory response in skeletal muscle to strenuous exercise is followed by an influx of leukocytes and a systemic acute-phase response. The various elements of the acute-phase response are mediated by cytokines, including the proinflammatory interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), and IL-6. These cytokines facilitate the influx of lymphocytes, neutrophils, monocytes, and other cells into the tissue after injury. Neutrophilia is one of the most consistent changes during and after strenuous exercise.[57–62] Circulating neutrophil counts increase during exercise and continue to increase postexercise.[63] Exercise activates those phagocytes to generate an oxidative burst of ROS such as superoxide and hypochlorous acid, which aid in proteolysis.[59,61,64] Neutrophilia is greater for eccentric than for concentric exercise of similar metabolic loads[65] and corresponds to the extent of myocellular disruption after eccentric exercise.[9,10,66]

After examining the effects of prior submaximal cycle ergometry on changes in circulating neutrophils, neutrophil activation, and myocellular enzymes after 10 sets of 10 repetitions of eccentric maximum contractions of the quadriceps, Fielding et al.[67] found a close association between neutrophilia and CK release but no association between *Z*-band damage and CK release. Others have found close associations between myocellular-enzyme release, suggesting that systemic neutrophil hyperactivity affects muscle inflammation after exercise.[11,60] Neutrophil mobilization appears to depend on exercise intensity and be mediated by the secretion of stress hormones such as catecholamines, cortisol, and growth hormone.[64,68] Myeloperoxidase-catalyzed production of ROS is significantly enhanced after exhaustive exercise of high intensity[64] and endurance exercise.[60] Belcastro et al.[69] examined exercise-induced neutrophil activation that occurred with prolonged running in several tissues from rats and found elevated myeloperoxidase activity not only in muscle but also in liver and heart tissue.

Circulating IL-6 has been associated with the rise in neutrophils after exercise and might prime these cells for phagocytosis and oxidative-burst activity.[60,62,70] Eccentric exercise or exercise for long durations (e.g., a marathon) significantly increases plasma IL-6 during and/or soon after exercise, and that change has been associated with indicators of muscle damage.[71–74] Ostrowski et al.[71] obtained muscle biopsies before and after a marathon and found elevated levels of IL-6 mRNA postmarathon.

Elevated levels of circulating IL-6 after exercise have been found consistently in exercise studies,[57,58,71,73–76] but changes in IL-1β are more difficult to detect,[11,58,62,71,72,77–80] especially with immunoassays.[81] IL-1β has been shown in some studies to increase in plasma and muscle after eccentric exercise and marathon running,[11,77,79,80,82] whereas other studies have shown a decrease in the production of IL-1β by peripheral blood mononuclear cells immediately postexercise, with a return to baseline levels after 24 h.[58,83] This drop in cytokine production of peripheral blood mononuclear cells also has been found for TNF-α and IL-6 after an exhaustive exercise test, even though plasma IL-6 increased.[83] After a marathon, Ostrowski et al.[80] found maximal elevations in plasma IL-1β and IL-1 receptor antagonist by 1 h postrace, whereas peak increases in IL-6 and TNF-α occurred immediately postexercise. Suzuki et al.[62] also tested athletes after a marathon and found no changes in circulating IL-1β or TNF-α, but IL-1 receptor antagonist and IL-6 increased over 100-fold. Production of IL-1β and TNF-α by peripheral blood mononuclear cells also has been reported to increase 24 h after downhill running.[77] These apparently contrasting results are due in part to the difference between determining cytokines in circulation and examining the ability of cells to respond to an endotoxin. Further, strenuous exercise involving eccentric contractions resulting in muscle damage most likely would result in inflammation mediated by those cytokines.

VITAMIN E AND EXERCISE

Vitamin E and Exercise-Induced CK Responses

Several studies have shown that antioxidant enzymes increase with oxidative stress and exercise training.[18,48,52,84] However, that increase in antioxidant defenses might not be physiologically proportionate to the needs created by the increase in prooxidant events and thus might affect the requirement for dietary antioxidants such as vitamin E.[85–87] Such a change in requirements likely would depend on several factors, including the duration and intensity of the exercise or training program and the age, diet, and health status of the individual.

Rokitzki et al.[88] supplemented 30 trained male cyclists with 330 mg/d of vitamin E for 5 mo and found no change in lactate threshold, although there was a significant reduction in serum CK and MDA. Similarly, vitamin E supplementation did not affect lactate threshold (or speed) in swimmers.[89,90] Compared with a placebo group, Rokitzki et al.[91] found diminished CK responses with 400 mg of vitamin E plus 200 mg of vitamin C administered daily to trained long-distance runners for 4.5 wk before a marathon. Consistent with those results, Sumida et al.[92] found that 300 mg/d of vitamin E but not of placebo reduced serum CK activity amd MDA in healthy young men after an exhaustive exercise bout. After administering 1200 IU/d of vitamin E 4 wk before 6 d of running training, Itoh et al.[93] found that the supplementation lowered pre-exercise TBARS and CK and reduced the rise in postexercise lactate dehydrogenase compared with subjects taking a placebo. Also using 1200 IU/d of vitamin E but with a heavy resistance exercise, McBride et al.[94] observed lower CK 24 h postexercise compared with the placebo group but found no differences in MDA.

In contrast to those reports, several studies have not confirmed an effect of vitamin E on exercise-induced changes in CK or measures of lipid peroxidation. Helgheim et al.[90] found no effect with 450 IU/d of vitamin E over 6 wk on antioxidant-enzyme activity in serum after exercise in trained and untrained subjects. Similarly, Jakeman and Maxwell[65] found no effect of vitamin E supplementation on serum CK up to 7 d after eccentric exercise. In a double-blind, cross-over study, Niess et al.[95] found no effect of supplemention with vitamin E for 28 d on CK levels in response to exhaustive treadmill running in men. Kaikkonen et al.[96] supplemented men with 13.5 mg/d of vitamin E and 90 mg/d of coenzyme Q10 for 3 wk before a marathon and found no change in lipid peroxidation or CK response after the race when compared with control subjects. Those findings are consistent with those of a study on sled dogs where supplementation with vitamin E plus other antioxidants for 3 wk had no impact on CK after 3 d of endurance running.[97]

Cannon et al.[12] supplemented young (<30 y) and older (>55 y) men with 800 IU/d of vitamin E for 48 d and found that the older subjects had increased CK and neutrophil responses corresponding to those observed in the young subjects after an acute and intense bout of downhill running. Those investigators suggested that CK is a manifestation of increased muscle-protein turnover to clear partly damaged proteins, and their results associated aging with an accumulation of damaged proteins caused by reduced clearance mechanisms. Vitamin E also might promote neutrophil accumulation at specific sites of muscle damage to increase clearance of cellular debris and promote protein turnover.[77]

Vitamin E and Exercise-Induced Lipid Peroxidation

Vitamin E deficiency can increase free-radical–induced tissue injury to levels comparable to those found after exercise, so an adequate status of vitamin E is important for maintaining membrane integrity during exercise.[2,84] However, several studies with vitamin E supplementation have suggested no effect[90,98] or small but significant reductions in lipid peroxidation before or after exercise, even with an exercise-induced increase in this biomarker.[12,88,92,99–101]

Maxwell et al.[98] investigated the effect of supplementation with vitamins E and C (each at 400 mg/d for 3 wk) in a 1-h box-stepping exercise and found increases in antioxidant capacity and plasma MDA in the placebo and treatment groups. Simon-Schnass and Pabst[101] found vitamin E (400 mg/d for 10 wk) effective in preventing increases in pentane exhalation after a high-altitude mountain climb. Similarly, Chao et al.[102] found breath that pentane was reduced by an antioxidant supplement (440 mg of vitamin E, 2000 μg retinol equivalents (RE), 500 mg of vitamin C, or a combination thereof with added selenium and zinc) administered 2 wk before and during 2 wk of field training by U.S. Marines at a moderate mountain altitude. Serum lipid peroxides, MDA, oxygen radical-absorbance capacity, and urinary 8-OHdG increased after the training, but the supplement had no effect on those biomarkers.

An attenuation of exercise-induced oxidative stress during submaximal (60% $V_{O_{2max}}$) and near-maximal (90% $V_{O_{2max}}$) running exercises was shown by Kanter et al.[100] with the use of an antioxidant mixture (800 IU of vitamin E, 1000 mg of vitamin C, and 30 mg of β-carotene daily). The supplement did not abolish the exercise-induced increase in lipid peroxidation but did lower the levels of breath pentane and serum MDA at rest and after both exercise bouts. Kaikkonen et al.[96] supplemented runners with vitamin E and coenzyme Q10 before a marathon and reported decreases in the susceptibility of their very–low-density and low-density lipoproteins to copper-induced oxidation and increases in total peroxyl-radical trapping. Meydani et al.[99] found that vitamin E (800 IU/d for 48 d) significantly increased the concentration of α-tocopherol in skeletal muscle and reduced oxidative injury after eccentric exercise, as indicated by the sparing of fatty acids and diminished production of conjugated dienes in muscle and decreased urinary excretion of TBARS.

Vitamin E and DNA Damage

The effect of vitamin E on DNA damage has not been well explored in human studies.[103–105] Huang et al.[106] examined the effect of supplementation with vitamins E and C for 2 mo on urinary 8-OHdG in non-smoking adults; even though this measure of oxidative damage tended to be higher in subjects who exercised, no treatment effect was noted. In contrast, with the use of DNA-strand breaks in white blood cells as a biomarker, Hartman et al.[38] found that supplementation with vitamin E for 2 wk was effective in reducing DNA damage after an incremental exercise test to exhaustion. After 3 d of endurance training in sled dogs, Baskin et al.[107] associated supplementation with vitamin E, β-carotene, and lutein for 1 mo with a decrease in 8-OHdG, whereas the non-treated dogs showed an increase in 8-OHdG.

VITAMIN E AND CYTOKINE RESPONSES

There have been few studies that examined the effects of vitamin E supplementation on cytokine responses during exercise. Cannon et al.[77] tested 21 men supplemented with 800 IU of vitamin E or placebo daily for 48 d before a downhill run. Endotoxin-induced secretion of IL-1β increased 24 h postexercise in the placebo but not in the supplemented group. Baseline levels of IL-6 were reduced by vitamin E, which also blunted the exercise-induced increase of this cytokine. TNF-α secretion also increased in response to exercise but was not significantly affected by vitamin E.

Singh et al.[108] examined the effects of acute supplementation with 400 mg of vitamin E in women before their running at 65% to 70% $V_{O_{2max}}$ until exhaustion. Plasma IL-6 rose linearly throughout exercise and peaked immediately postexercise, but vitamin E had no affect on this cytokine. Similarly, Niess et al.[95] found no effect with 500 mg/d of vitamin E supplementation for 8 d on the postexercise elevation of plasma IL-6 after an acute treadmill exercise test in young men. In contrast, preliminary data from Sacheck et al.[109] suggested that supplementation for 3 mo with 1000 mg/d of vitamin E decreases peak IL-6 production at 6 h after downhill running in young men and promotes mitogen-stimulated production of IL-1β by peripheral blood mononuclear cells in older men 24 h postexercise, although the exercise did not significantly affect IL-1β secretion.

CONCLUSION

Optimal physical-performance capacity is unlikely to be achieved without optimum cellular function. Strenuous exercise creates situations that subject cell metabolism and structure to significant stress, including those of oxidative stress and muscle damage. However, studies of the use of dietary antioxidants such as vitamin E to reduce exercise-induced muscle injury have met with mixed success. The equivocal nature of the results to date reflect a diversity of factors including the antioxidant tested, the nature of the exercise, and the methodology for assessing oxidative stress. It is important to appreciate that the impact of antioxidants or other nutritional interventions depend on the duration, intensity, and type of exercise and the status of the subjects participating in the test. For example, age increases the susceptibility of cells to prooxidant insults and reduces the adaptability of their endogenous antioxidant defenses, so older adults appear more vulnerable to exercise-induced stress.

The design of human studies to assess the value of nutrition interventions oftentimes is limited for practical reasons such as cost and time. However, advances in our understanding of the relation between vitamin E and other antioxidant interventions and exercise will be reached as investigators more rigorously employ the full criteria of clinical trials, e.g., the use of randomized (placebo-controlled) trials with cross-over or parallel designs in studies with subjects carefully matched for physical fitness, age, sex, health, and lifestyle. Because of the marked interindividual responses to antioxidants and exercise, outcome parameters must be paired with appropriate pretest evaluations, e.g., of oxidative-stress status, rather determining those parameters only at the end of the study. Further, protocols should recognize the dose and duration of the intervention necessary to significantly increase antioxidant concentrations in muscle and other target tissues. Importantly, the time points at which samples are collected for measurement will significantly influence the results because muscle injury and repair, acute-phase immune responses, changes in antioxidant status, and other events affecting ROS generation can take place in distinct periods over an extended duration depending on the nature of the exercise.

Few studies have combined antioxidants despite the recognition of a dynamic interrelationship between the many classes of those compounds, so more attention to that matter is required. Although different lipid, nucleic acid, and protein biomarkers of

oxidative stress are available, antioxidant interventions during exercise also should include assessment of other antioxidant enzymes and nutrients that affect the total balance between ROS generation and antioxidant defenses. In addition, evaluations of the susceptibility of relevant molecules to resist oxidation ex vivo and measures of transcription-factor activation or signal-transduction events would better inform our understanding of the relation between antioxidants and exercise. Although the capacity for adequately assessing relevant genomic factors is limited, adding that facet to research approaches will become increasingly important for determining which individuals are most likely to benefit from antioxidant interventions.

REFERENCES

1. Sjodin T, Westing Y, Apple F. Biochemical mechanisms for oxygen free radical formation during exercise. Sports Med 1990;10:236
2. Bejma J, Ji L. Aging and acute exercise enhance free radical generation in rat skeletal muscle. J Appl Physiol 1999;87:465
3. Ohkuwa T, Sato Y, Naoi M. Glutathione status and reactive oxygen generation in tissues of young and old exercised rats. Acta Physiol Scand 1997;159:237
4. Davies K, Quintanilha A, Brooks A, Packer L. Free radical tissue damage produced by exercise. Biochem Res Commun 1982;107:1178
5. Jackson M, Edward R, Symons M. Electron spin resonance studies of intact mammalian skeletal muscle. Biochim Biophys Acta 1985;847:185
6. Apple FS, Rhodes M. Enzymatic estimation of skeletal muscle damage by analysis of changes in serum creatine kinase. J Appl Physiol 1988;65:2598
7. Schwane J, Johnson S, Vandenakker C, Armstrong R. Delayed-onset muscle soreness and plasma CPK and LDH activities after downhill running. Med Sci Sports Exerc 1983;15:51
8. Clarkson PM, Tremblay I. Exercise induced muscle damage, repair, and adaptation in humans. J Appl Physiol 1988;65:1
9. Fielding R, Manfredi T, Ding W, et al. Acute phase response in exercise III. Neutrophil and IL-1beta accumulation in skeletal muscle. Am J Physiol 1993; 265:R166
10. Manfredi T, Fielding R, O'Reilly K, et al. Plasma creatine kinase activity and exercise-induced muscle damage in older men. Med Sci Sports Exerc 1991;23:1028
11. Cannon J, Fiatarone M, Fielding R, Evans W. Aging and stress-induced changes in complement activation and neutrophil mobilization. J Appl Physiol 1994;76: 2616
12. Cannon J, Orencole S, Fielding R, et al. Acute phase response in exercise: interaction of age and vitamin E on neutrophils and muscle enzyme release. Am J Physiol 1990;259:R1214
13. Dillard C, Litov R, Savin W, Dumelin E, Tappel A. Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. J Appl Physiol 1978;45:927
14. Lovlin R, Cottle W, Pyke I, Kavanagh M, Belcastro A. Are indices of free radical damage related to exercise intensity? Eur J Appl Physiol 1987;56:313
15. Maughan RJ, Donnelly A, Gleeson M, Whitting P, Walker K. Delayed-onset muscle damage and lipid peroxidation in man after a downhill run. Muscle Nerve 1989;12:332
16. Kanter M, Lesmes G, Kaminsky L, Ham-Saeger J, Nequin N. Serum creatine kinase and lactate dehydrogenase changes following an eighty kilometer race. Eur J Appl Physiol 1988;57:60
17. Krotkiewski M, Brzezinska Z. Lipid peroxides production after strenuous exercise and in relation to muscle morphology and capillarization. Muscle Nerve 1996;19:1530
18. Duthie GG, Robertson JD, Maughan RJ, Morrice PC. Blood antioxidant status and erythrocyte lipid peroxidation following distance running. Arch Biochem Biophys 1990;282:78
19. Sahlin K, Cizinsky S, Warholm M, Hoberg J. Repetitive static muscle contractions in humans—a trigger of metabolic and oxidative stress. Eur J Appl Physiol 1992;64:228
20. Viinikka L, Vuori J, Ylikorkala O. Lipid peroxides, prostacyclin, and thromboxane A2 in runners during acute exercise. Med Sci Sports Exerc 1984;16:275
21. Child R, Brown S, Day S, Donnelly A, Roper H, Saxton J. Changes in indices of antioxidant status, lipid peroxidation and inflammation in human skeletal muscle after eccentric muscle actions. Clin Sci 1999;96:105
22. Dernbach AR, Sherman WM, Simonsen JC, Flowers KM, Lamb DR. No evidence of oxidant stress during high-intensity rowing training. J Appl Physiol 1993;74:2140
23. Saxton J, Donnelly A, Roper H. Indices of free radical mediated damage following maximum voluntary eccentric and concentric muscular work. Eur J Appl Physiol 1994;68:189
24. Meydani M. Isoprostanes as oxidant stress markers in coronary reperfusion. Nutr Rev 1997;55:404
25. Reckelhoff JF, Kanji V, Racusen LC, et al. Vitamin E ameliorates enhanced renal lipid peroxidation and accumulation of F2-isoprostanes in aging kidneys. Am J Physiol 1998;274:R767
26. Hinchcliff K, Reinhart G, DiSilvestro R, et al. Oxidant stress in sled dogs subjected to repetitive endurance exercise. Am J Vet Res 2000;61:512
27. Kirschvink N, Art T, Smith N, Lekeux P. Effect of exercise and COPD crisis on isoprostane concentration in plasma and bronchoalveolar lavage fluid in horses. Equine Vet J 1999;30(suppl):88
28. Mori TA, Dunstan DW, Burke V, et al. Effect of dietary fish and exercise training on urinary F2-isoprostane excretion in non-insulin-dependent diabetic patients. Metabolism 1999;48:1402
29. Wang J, Lin C, Chen J, Wong M. Role of chronic exercise in decreasing oxidized LDL-potentiated platelet activation by enhancing platelet-derived NO release and bioactivity in rats. Life Sci 2000;66:1937
30. Liu M-L, Bergholm R, Makimattila S, et al. A marathon run increases the susceptibility of LDL to oxidation in vitro and modifies plasma antioxidants. Am J Physiol 1999;276:E1083
31. Child R, Wilkinson D, Fallowfield J, Donnelly A. Elevated serum antioxidant capacity and plasma malondialdehyde concentration in response to a simulated half-marathon run. Med Sci Sports Exerc 1998;30:1603
32. Alessio H, Hagerman A, Fulkerson B, et al. Generation of reactive oxygen species after exhaustive aerobic and isometric exercise. Med Sci Sports Exerc 2000;32:1576
33. Radak Z, Kaneko T, Tahara S, et al. The effect of exercise training on oxidative damage of lipids, proteins, and DNA in rat skeletal muscle: evidence for beneficial outcomes. Free Radic Biol Med 1999;27:69
34. Viguie C, Frei B, Shinenaga M, et al. Antioxidant status and indices of oxidative stress during consecutive days of exercise. J Appl Physiol 1993;75:566
35. Poulsen HE, Loft S, Vistisen K. Extreme exercise and oxidative DNA modification. J Sports Sci 1996;14:343
36. Niess A, Hartmann A, Grunert-Fuchs M, Poch B, Speit G. DNA damage after exhaustive treadmill running in trained and untrained men. Int J Sports Med 1996;17:397
37. Okamura K, Doi T, Hamada K, et al. Effect of repeated exercise on urinary 8-hydroxy-deoxyguanosine excretion in humans. Free Radic Res 1997:26
38. Hartmann A, Nieb A, Grunert-Fuchs M, Poch B, Speit G. Vitamin E prevents exercise-induced DNA damage. Mutat Res 1995;346:195
39. Sumida S, Okamura K, Doi T, et al. No influence of a single bout of exercise on urinary excretion of 8-hydroxy-deoxyguanosine in humans. Biochem Mol Biol Int 1997;42:601
40. Pilger A, Germadnik D, Formanek D, et al. Habitual long-distance running does not enhance urinary excretion of 8-hydroxydeoxyguanosine. Eur J Appl Physiol 1997;75:467
41. Hartmann A, Pfuhler S, Dennog C, et al. Exercise-induced DNA effects in human leukocytes are not accompanied by increased formation of 8-hydroxy-2′-deoxyguanosine or induction of micronuclei. Free Radic Biol Med 1998;24:245
42. Asami S, Hirano T, Yamaguchi R, Itoh H, Kasai H. Reduction of 8-hydroxyguanine in human leukocyte DNA by physical exercise. Free Radic Res 1998;29:581
43. Kasai H, Iwamoto-Tanaka N, Miyamoto T, et al. Life style and urinary 8-hydroxyldeoxyguanosine, a marker of oxidative DNA damage: effects of exercise, working conditions, meat intake, body mass index, and smoking. Jpn J Cancer Res 2001;92:9
44. Lodovici M, Casalini C, Cariaggi R, Michelucci L, Dolara P. Levels of 8-hydroxyguanosine as a marker of DNA damage in human leukocytes. Free Radic Biol Med 2000;28:13
45. Inoue T, Mu Z, Sumikawa K, Adachi K, Okochi T. Effect of physical exercise on the content of 8-hydroxydeoxyguanosine in nuclear DNA prepared from human lymphocytes. Jpn J Cancer Res 1993;84:720
46. Radak Z, Pucsok J, Mecseki S, Csont T, Ferdinandy P. Muscle soreness-induced reduction in force generation is accompanied by increased nitric oxide content and DNA damage in human skeletal muscle. Free Radic Biol Med 1999;26:1059
47. Ji LL. Exercise and oxidative stress: role of the cellular antioxidant systems. In: Holloszy JO, ed. *Exercise and sport sciences reviews.* Baltimore: Williams & Wilkins, 1995:135
48. Gohil K, Viguie C, Stanley WC, Brooks GA, Packer L. Blood glutathione oxidation during human exercise. J Appl Physiol 1988;164:115
49. Ji LL. Antioxidant enzyme response to exercise and aging. Med Sci Sports Exerc 1993;25:225
50. Tessier F, Margaritis I, Richard MJ, Moynot C, Marconnet P. Selenium and training effects on the glutathione system and aerobic performance. Med Sci Sports Exerc 1995;27:390
51. Ortenblad N, Madsen K, Djurhuus MS. Antioxidant status and lipid peroxidation after short-term maximal exercise in trained and untrained humans. Am J Physiol 1997;272:R1258

52. Robertson JD, Maughan RJ, Duthie GG, Morrice PC. Increased blood antioxidant systems of runners in response to training load. Clin Sci 1991;80:611
53. Dufaux B, Heine O, Kothe A, Prinz U, Rost R. Blood glutathione status following distance running. Int J Sports Med 1997;18:89
54. Inal M, Akyuz F, Turgut A, Getsfrid W. Effect of aerobic and anaerobic metabolism on free radical generation in swimmers. Med Sci Sports Exerc 2001;33:564
55. Liu J, Yeo H, Overvik-Douki E, et al. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. J Appl Physiol 2000;89:21
56. Leeuwenburgh C, Fiebig R, Chandwaney R, Ji LL. Aging and exercise training in skeletal muscle: responses of glutathione and antioxidant enzyme systems. Am J Physiol 1994;267:R439
57. Sprenger H, Jacobs C, Nain M, et al. Enhanced release of cytokines, interleukin-2 receptors, and neopterin after long-distance running. Clin Immunol Immunopathol 1992;63:188
58. Drenth J, VanUum S, VanDeuren M, et al. Endurance run increases circulating IL-6 and IL-1ra but downregulates ex vivo TNF-alpha and IL-1 beta production. J Appl Physiol 1995;79:1497
59. Nieman D, Nehlsen-Cannarella S, Fagoaga O, et al. Effects of mode and carbohydrate on the granulocyte and monocyte response to intensive, prolonged exercise. J Appl Physiol 1998;84:1252
60. Suzuki K, Totsuka M, Nakaji S, et al. Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. J Appl Physiol 1999;87:1360
61. Suzuki K, Naganuma S, Totsuka M, et al. Effects of exhaustive endurance exercise and its one-week daily repetition on neutrophil count and functional status in untrained men. Int J Sports Med 1996;17:205
62. Suzuki K, Yamada M, Kurakake S, et al. Circulating cytokines and hormones with immunosuppressive but neutrophil-priming potentials rise after endurance exercise in humans. Eur J Appl Physiol 2000;81:281
63. McCarthy D, Dale M. The leucocytosis of exercise. A review and model. Sports Med 1988;6:333
64. Suzuki K, Sato H, Kikuchi T, et al. Capacity of circulating neutrophils to produce reactive oxygen species after exhaustive exercise. J Appl Physiol 1996;81:1213
65. Jakeman P, Maxwell S. Effect of antioxidant vitamin supplementation on muscle function after eccentric exercise. Eur J Appl Physiol 1993;67:426
66. Van der Meulen JH, Kuipers H, Drukker J. Relationship between exercise-induced muscle damage and enzyme release in rats. J Appl Physiol 1991;71:999
67. Fielding R, Violin M, Svetkey L, et al. Effects of prior exercise on eccentric exercise-induced neutrophilia and enzyme release. Med Sci Sports Exerc 2000; 32:359
68. Yamada M, Suzuki K, Kudo S, et al. Effect of exhaustive exercise on human neutrophils in athletes. Luminescence 2000;15:15
69. Belcastro A, Arthur G, Albisser T, Raj D. Heart, liver and skeletal muscle myeloperoxidase activity during exercise. J Appl Physiol 1996;80:1331
70. Mullen P, Windsor A, Walsh C, Fowler A, Sugerman H. Tumor necrosis factor-alpha and interleukin-6 selectively regulate neutrophil function in vitro. J Surg Res 1995;58:124
71. Ostrowski K, Rohde T, Zacho M, Asp S, Pedersen B. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. J Physiol 1998;508:949
72. Ostrowski K, Hermann C, Bangash A, et al. A trauma-like elevation of plasma cytokines in humans in response to treadmill running. J Physiol 1998;513:889
73. Bruunsgaard H, Galbo H, Halkjaer K, et al. Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. J Physiol 1997;499:833
74. Rohde T, Maclean D, Richter E, Kiens B, Pedersen B. Prolonged submaximal eccentric exercise is associated with increased levels of plasma IL-6. Am J Physiol 1997;273:E85
75. Nehlsen-Cannarella SL, Fagoaga OR, Nieman DC, et al. Carbohydrate and the cytokine response to 2.5 h of running. J Appl Physiol 1997;82:1662
76. Ullum H, Haahr P, Diamant M, et al. Bicycle exercise enhances plasma IL-6 but does not change IL-1alpha, IL-1beta, IL-6, or TNF-alpha pre-mRNA in BMNC. J Appl Physiol 1994;77:93
77. Cannon J, Meydani S, Fielding R, et al. Acute phase response in exercise. II. Associations between vitamin E, cytokines, and muscle proteolysis. Am J Physiol 1991;260:R1235
78. Evans W, Meredith C, Cannon J, et al. Metabolic changes following eccentric exercise in trained and untrained men. J Appl Physiol 1986;61:1864
79. Cannon J, Fielding R, Fiatarone M, et al. Increased interleukin 1beta in human skeletal muscle after exercise. Am J Physiol 1989;257:R451
80. Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen B. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol 1999;515:287
81. Cannon J, Nerad J, Poutsiaka D, Dinarello C. Measuring circulating cytokines. J Appl Physiol 1993;75:1897
82. Deschenes M, Brewer R, Bush J, et al. Neuromuscular disturbance outlasts other symptoms of exercise-induced muscle damage. J Neurol Sci 2000;174:92
83. Weinstock C, Konig D, Harnischmacher R, et al. Effect of exhaustive exercise stress on the cytokine response. Med Sci Sports Exerc 1997;29:345
84. Quintanilha AT. Effects of physical exercise and/or vitamin E in tissue oxidative metabolism. Biochem Soc Trans 1984;12:403
85. Clarkson P. Micronutrients and exercise: antioxidants and minerals. J Sports Sci 1995;13:S11–S24
86. Kanter M. Free radicals, exercise, and antioxidant supplementation. Int J Sport Nutr 1994;4:205
87. Bendich A. Exercise and free radicals: effects of antioxidant vitamins. Med Sport Sci 1991;32:59
88. Rokitzki L, Logemann E, Huber G, Keck E, Keul J. Alpha-tocopherol supplementation in racing cyclists during extreme endurance training. Int J Sport Nutr 1994;4:253
89. Lawrence J, Bower R, Riehl W, Smith J. Effects of alpha-tocopherol acetate on the swimming endurance of trained swimmers. Am J Clin Nutr 1979;28:205
90. Helgheim I, Hetland O, Nilsson S, Ingjer F, Stromme S. The effects of vitamin E on serum enzyme levels following heavy exercise. Eur J Appl Physiol 1979;40:283
91. Rokitzki L, Logemann E, Sagredos A, et al. Lipid peroxidation and antioxidative vitamins under extreme endurance stress. Acta Physiol Scand 1994;151:149
92. Sumida S, Tanaka K, Kitao H, Nakadomo F. Exercise-induced lipid peroxidation and leakage of enzymes before and after vitamin E supplementation. Int J Biochem 1989;21:835
93. Itoh H, Ohkuwa T, Yamazaki Y, et al. Vitamin E supplementation attenuates leakage of enzymes following 6 successive days of running training. Int J Sports Med 2000;21:369
94. McBride J, Kraemer W, Triplett-McBride T, Sebastianelli W. Effect of resistance exercise on free radical production. Med Sci Sports Exerc 1998;30:67
95. Niess A, Sommer M, Schneider M, et al. Physical exercise-induced expression of inducible nitric oxide synthase and heme oxygenase-1 in human leukocytes: effects of RRR-alpha-tocopherol supplementation. Antiox Redox Signal 2000;2:113
96. Kaikkonen J, Kosonen L, Nyyssonen K, et al. Effect of combined coenzyme Q10 and D-alpha-tocopheryl acetate supplementation on exercise-induced lipid peroxidation and muscular damage: a placebo-controlled double-blind study in marathon runners. Free Radic Res 1998;29:85
97. Piercy R, Hinchcliff K, DiSilvestro R, et al. Effect of dietary supplements containing antioxidants on attenuation of muscle damage in exercising sled dogs. Am J Vet Res 2000;61:1438
98. Maxwell S, Jakeman P, Thomason H, Leguen C, Thorpe G. Changes in plasma antioxidant status during eccentric exercise and the effect of vitamin supplementation. Free Radic Res Commun 1993;19:191
99. Meydani M, Evans WJ, Handelman G, et al. Protective effect of vitamin E on exercise-induced oxidative damage in young and older adults. Am J Physiol 1993;264:R992
100. Kanter M, Nolte L, Holloszy J. Effects of an antioxidant mixture on lipid peroxidation at rest and postexercise. J Appl Physiol 1993;74:965
101. Simon-Schnass I, Pabst H. Influence of vitamin E on physical performance. Int J Vitam Nutr Res 1988;58:49
102. Chao WH, Askew EW, Roberts DE, Wood SM, Perkins JB. Oxidative stress in humans during work at moderate altitude. J Nutr 1999;129:2009
103. Fenech M, Dreosti I, Aitken C. Vitamin-E supplements and their effect on vitamin-E status in blood and genetic damage rate in peripheral blood lymphocytes. Carcinogenesis 1997;18:359
104. Poulsen H, Weimann A, Loft S, et al. Antioxidants as inhibitors of oxidative damage to DNA. Pharmacol Toxicol 1999;85:8
105. Prieme H, Loft S, Nyyssonen K, Salonen J, Poulsen H. No effect of supplementation with vitamin E, ascorbic acid, or coenzyme Q10 on oxidative DNA damage estimated by 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion in smokers. Am J Clin Nutr 1997;65:503
106. Huang H, Helzlsouer K, Appel L. The effects of vitamin C and vitamin E on oxidative DNA damage: results from a randomized controlled trial. Cancer Epidemiol Biomarkers Prev 2000;9:647
107. Baskin C, Hinchcliff K, DiSilvestro R, et al. Effects of dietary antioxidant supplementation on oxidative damage and resistance to oxidative damage during prolonged exercise in sled dogs. Am J Vet Res 2000;61:886
108. Singh A, Papanicolaou D, Lawrence L, et al. Neuroendocrine responses to running in women after zinc and vitamin E supplmentation. Med Sci Sports Exerc 1999;31:536
109. Sacheck JM, Milbury P, Walter MF, Blumberg JB, Roubenoff R. The effect of eccentric exercise on acute phase response oxidative stress and cytokines in young and elderly men. FASEB J 2001;15;A417