brief review

Oxidants and antioxidants in exercise

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Sen, Chandan K. Oxidants and antioxidants in exercise. *J. Appl. Physiol.* 79(3): 675–686, 1995.—There is consistent evidence from human and animal studies that strenuous physical exercise may induce a state wherein the antioxidant defenses of several tissues are overwhelmed by excess reactive oxygen. A wide variety of physiological and dietary antioxidants act in concert to evade such a stress. Submaximal long-duration exercise training may augment the physiological antioxidant defenses in several tissues; however, this enhanced protection may not be sufficient to completely protect highly fit individuals from exhaustive exercise-induced oxidative stress. Regular physical activity in association with dietary habits that ensure adequate supply of a combination of appropriate antioxidants may be expected to yield desirable results. The significance of this area of research, current state of information, and possibilities of further investigation are briefly reviewed.

oxygen free radicals; therapy; pathophysiology; performance; fatigue; training; deoxyribonucleic acid; glutathione; tissue damage

INCREASED ENERGY DEMAND during physical exercise, especially of the aerobic type, necessitates a multifold increase in oxygen supply to active tissues. The rate of oxygen uptake by the body during exercise may increase by 10- to 15-fold. Oxygen flux in the active peripheral skeletal muscle tissue may, however, increase by ~100-fold with an ~30-fold increase in blood flow and ~3-fold increase in arteriovenous oxygen difference. Delivery of increased amounts of oxygen to tissues active during exercise fuels oxidative metabolism, maximizing energy yield per unit substrate and avoiding lactate accumulation. However, aerobic organisms have to pay a price for such a metabolic advantage (38). Recent advances in the biochemistry of oxygen toxicity have attracted considerable interest in the effects on various biological structures of the increased oxygen consumption that accompanies exercise (86). The initial report in this field, published in 1978, indicated that strenuous physical exercise induces oxidative damage to lipids in various tissues (27). Since then, a considerable body of research has accumulated concerning the effects of exercise and training on indexes of oxygen toxicity and defenses against such challenge. The widely accepted hypothesis that oxygen toxicity may be implicated in the etiology of a wide variety of pathophysiological conditions (e.g., atherosclerosis, retinopathies, muscular dystrophy, some cancers, diabetes, rheumatoid arthritis, aging, ischemia-reperfusion injury, Alzheimer's disease, and Parkinson's disease) makes the study of exercise-induced oxygen toxicity even more significant. A vivid understanding of the possible mechanisms that may contribute to exercise-induced oxygen toxicity, associated physiological response, and the design of appropriate measures to circumvent or minimize such toxicity are fundamental to *1*) enhance the effectiveness of physical exercise as a preventive and therapeutic tool in clinical practice; *2*) control exercise-induced oxygen toxicity-dependent tissue damage and possible augmentation of other possible health risks; and *3*) perhaps enhance endurance performance capacity in sports as well.

OXYGEN FREE RADICALS AND REACTIVE OXYGEN SPECIES

Free radicals may be broadly defined as molecules or ions containing an unpaired electron (i.e., radical) and capable of existing independently (i.e., free). Molecular O_2 contains two unpaired electrons with parallel spins and is therefore a radical itself. Ground-state molecular O_2 is often, therefore, referred to as a diradical, triplet O_2, or simply dioxygen. In biological systems, diradical oxygen is reduced to produce energy and water. Because the oxygen molecule has two unpaired electrons, the reduction (i.e., electron acceptance) process is not simple. This is especially so because, according to the Pauli exclusion principle, no more than two electrons may occupy the same atomic orbital, and to do so these electrons must have opposite spins. A discussion of the theoretical possibilities for oxygen reduction is beyond the scope of this article. Univalent reduction of oxygen, a process with significant biologi-

cal relevance to energy production, refers to the reduction of molecular oxygen by one electron at a time. Intermediates produced in this process are oxygen-derived free radicals that are highly reactive. Some of these reactive intermediates may escape from the process of complete tetravalent reduction and react with biological structures and other components, inciting oxidative damage and producing other reactive oxygen species (ROS). "ROS" and "oxygen free radical" are not synonymous terms. Unlike the latter, ROS represent a broader spectrum of species including nonradical derivatives of oxygen (e.g., H_2O_2, peroxides, singlet oxygen, hydroperoxides, epoxides, etc.) that are capable of inciting and propagating oxidative tissue damage.

In certain cells, strategic use of ROS, produced by physiologically controlled mechanisms, may contribute to our well-being. In normal phagocytosis, ROS destroy invading microorganisms by a process commonly referred to as respiratory burst (60, 89). ROS are capable of inducing genes, e.g., c-*fos*, c-*myc*, c-*jun*, and β-actin, which encode transcription factors involved in the induction of cell growth, differentiation, and development (7). Through this mechanism in early inflammation, ROS may contribute to wound healing. ROS are also known to activate nuclear factor-κB (NF-κB), the cytosolic multisubunit transcription factor (79). NF-κB controls the transcription of cytokine genes including interleukin-2 and tumor necrosis factor-α. Tumor necrosis factor-α promotes angiogenesis, a process critical to wound healing. The physiological role of $O_2^-\cdot$ includes its possible involvement in vasoregulation (36) and fibroblast proliferation. Lower concentrations of ROS have been hypothesized to serve as true second messengers in plants (51).

ANTIOXIDANTS

Evolution of organisms in an oxygen-rich atmosphere has led to the development of endogenous physiological defense systems that cooperate to scavenge and detoxify ROS. In addition, a second line of defense is provided by exogenous antioxidants primarily obtained as nutrients or nutritional supplements. An antioxidant may be defined as any substance that, when present in low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (38). In this definition, the term "oxidizable substrate" embraces almost everything found in living cells, including proteins, lipids, carbohydrates, and DNA. Broadly, the possible mechanisms by which antioxidants may protect against oxygen toxicity are as follows: *1*) prevention of ROS formation, *2*) interception of ROS attack by scavenging the reactive metabolites and converting them to less reactive molecules and/or by enhancing the resistance of sensitive biological targets to ROS attack, *3*) avoiding the transformation of less reactive ROS (e.g., $O_2^-\cdot$) to more deleterious forms (e.g., $\cdot OH$), *4*) facilitating the repair of damage caused by ROS and triggering the expression of genes that encode antioxidant proteins, and *5*) providing a favorable environment for the effective functioning of other antioxidants (e.g., as a cofactor or by acting to maintain a suitable redox status). A large number of enzymatic and nonenzymatic physiological substances are known to have "antioxidant-like" functions (82). However, the primary contributors are the enzymes superoxide dismutase (EC 1.15.1.1) and catalase (EC 1.11.1.6), and the glutathione system (Table 1).

TABLE 1. *Primary components of the physiological antioxidant defense*

SOD	Dismutates superoxide anion (one electron reduction product of diradical O_2) to H_2O_2 $O_2 + e \rightarrow O_2^-\cdot$, $2O_2^-\cdot + 2H^+ - SOD \rightarrow H_2O_2 + {}^1O_2$
Cat	Decomposes H_2O_2, $2H_2O_2 - Cat \rightarrow 2H_2O + {}^1O_2$ Utilizes peroxide to oxidize a range of H donors (AH_2) such as methanol, ethanol, and formate $AH_2 + H_2O_2 - Cat \rightarrow A + 2H_2O$
Glutathione	Cellular reservoir of cysteine (water soluble) Most prominent contributor to intracellular -SH pool Spontaneously scavenges a wide variety of ROS Acts as a substrate for enzyme family of glutathione peroxidases (GPX) that catalyze the decomposition of a wide range of substrates extending from H_2O_2 to organic hydroperoxides $2H_2O_2 - GPX \rightarrow 2H_2O + {}^1O_2$ During course of its antioxidant action, reduced glutathione (GSH) gets oxidized to glutathione disulfide (GSSG) 2 GSH − ROS → GSSG In presence of NADPH, GSSG may be reduced to GSH by glutathione reductase (GRD) GSSG + NADPH − GRD → 2 GSH A major cellular electrophile conjugator, detoxifies xenobiotics. Glutathione S-transferases (GST) catalyze reaction between -SH group of GSH and potential alkylating agents, thereby neutralizing their electrophilic sites and rendering them more water soluble GST also has some GPX-like function Suggested to be implicated in facilitating antioxidant actions of vitamins E and C Modulates activity of redox sensitive transcription factors in mammalian cells

ROS, reactive oxygen species; SOD, superoxide dismutase; Cat, catalase. For further details see Ref. 82.

Antioxidant chain reaction. Antioxidants like vitamins E, C, dihydrolipoic acid, and glutathione are known to act synergistically in the form of an antioxidant chain reaction (80, 82, 88; Fig. 1). As can be observed from the scheme, the -SH pool contributed by reduced glutathione (GSH) and dihydrolipoate plays a central role in regenerating vitamins C and E from their radical forms. In this context, it should be noted that the antioxidant activities of selenium and vitamin B_6 are also glutathione dependent. Selenium functions as a cofactor of glutathione peroxidase, an enzyme family that requires GSH as its substrate for the scavenging of hydrogen- and other peroxides (18). Vitamin B_6 facilitates the availability of selenium for glutathione peroxidase (100). To obtain best therapeutic results, antioxidant supplementation protocols should consider the requirement of all components of the chain (Fig.

1). For example, excess vitamin E in the absence of adequate amounts of regenerating agents will fail to provide full-strength antioxidant protection, and accumulation of oxidized vitamin E (α-tocopheroxyl radical) may even lead to the initiation of pathophysiological processes (14, 57). Pascoe and Reed (72) suggest that glutathione and vitamin E are interdependent on each other with respect to the circumvention of oxidative stress-induced cytotoxicity. Bast and Haenen (13) hypothesized that lipoic acid is capable of performing "glutathione-like" (donation of -SH reducing power) regeneration of vitamin E and prevention of lipid peroxidation. Recently, dihydrolipoate has been shown to upregulate glutathione level in human lymphocytes (39, 71). The mechanism of such upregulation is, however, presently unclear. Apart from its antioxidant functions, lipoate has been observed to remarkably improve glucose uptake by skeletal muscle cells, an effect comparable with insulin (97). Lipoate may thus be expected to be of crucial therapeutic value in the treatment of diabetes-associated oxidative stress (63). Ascorbic acid has been suggested to be the most effective antioxidant in human blood plasma (29). However, ascorbate can only function as an antioxidant when transition metal ions are absent (37). In the presence of transition metal ions, e.g., Fe^{3+} or Cu^{2+}, high concentration (~1 mM) of ascorbic acid may actually act as a pro-oxidant (10, 17).

EXERCISE AND OXIDATIVE STRESS MARKERS

Oxidative stress refers to an imbalance in the pro- and antioxidant status in favor of the former. In humans, skeletal muscle and heart antioxidant defenses are poor, thus rendering these organs highly susceptible to oxidative stress. Basal metabolism in the heart being almost 100% higher than that in the liver, the heart stands a high risk of oxidative damage. At rest, a kilogram of the human heart and liver has been estimated to consume oxygen at a rate of 94 and 44 ml/min, respectively (26). In adult men, the activities of superoxide dismutase and catalase have been estimated to be 40 times (for catalase) and 16 times (for superoxide dismutase) less in the gastrocnemius muscle compared with that in the liver (44). In 1978, Dillard et al. (27) reported that in humans physical exercise [at 75% maximum oxygen consumption ($\dot{V}O_{2\,max}$)] increased the level of pentane (a possible by-product of oxidative lipid damage or lipid peroxidation) by 1.8-fold in the expired air compared with resting subjects. This finding was later confirmed (11). Electron spin resonance or electron paramagnetic resonance (EPR) spectroscopy is the most powerful device to directly detect and characterize oxygen free radicals. EPR is sensitive to transitions involving unpaired electrons (76). Results of studies examining exercise-induced oxidative stress using the EPR approach to directly detect and characterize oxygen free radicals are scanty. Most of the evidence in this area has been derived from studies that have monitored the by-products of exercise-induced oxidative tissue damage. The early work of Dillard et al. (27) was followed by Davies et al. (24), who tested the hypothesis that exhaustive exercise increases free radical concentration in the liver and muscle and induces oxidative tissue damage. The first EPR ($g \simeq 2.004$) data showed that exhaustive treadmill ex-

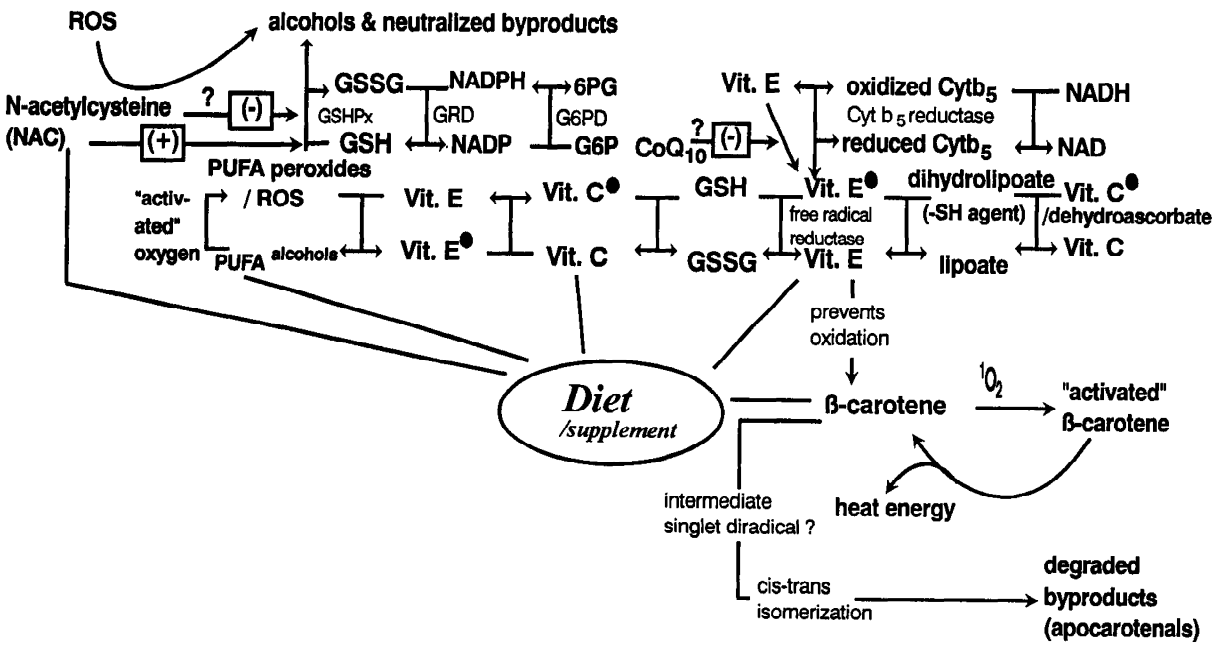

FIG. 1. Antioxidant chain reaction representing possible cooperation between physiological and exogenous antioxidants. PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species; superscripted •, radical form of respective compounds; (+), NAC is a pro-glutathione drug; ? (-), NAC and ubiquinone are suggested to "spare" oxidation of glutathione (GSH) to glutathione disulfide (GSSG) and vitamin E to tocopheroxyl radical, respectively; GSHPx, glutathione peroxidase; GRD, GSSG reductase; G6PD, glucose-6-phosphate dehydrogenase; G6P, glucose 6-phosphate; 6PG, 6-phosphogluconate; Cyt b_5, cytochrome-b_5; CoQ_{10}, coenzyme Q_{10}. [From Sen and Hanninen (82) with permission from Elsevier Science Publishers B. V., Amsterdam, The Netherlands.]

TABLE 2. *Lipid peroxidation by-products after physical exercise*

Experimental Material	Work	Assay	Sample and Effect[1]	Ref.
		Animal studies		
Male rats (6 mo) UT	Submaximal treadmill run to exhaustion	MDA	Mu[2] ↑81% Li ↑135%	24
Male rats (100–125 g) UT and Tr	Submaximal treadmill run to exhaustion	MDA	RMu, WMu & Li (Tr)[3] → RMu (UT) → WMu (UT) ↑34% Li (UT) ↑22%	3
Male rats (6 mo) UT	Brief (1 min) high-intensity run	MDA	Mu[4] ↑83% (soleus), ↑157–167% (RMu and WMu)	5
		LHP	Mu[5] →	
	20-min medium-intensity run	MDA	Mu[5] ↑62% (RMu), ↑90% (WMu)	
Male rats (6 mo) UT	Submaximal treadmill run to exhaustion	MDA	Mu[6] → Li ↑43%	47
Male rats (2 mo) UT	Submaximal treadmill run to exhaustion	MDA	Mu[7] ↑~50% (RMu), ↑~130% (vastus Mu) Heart → Plasma ↑~75%	80
Male rats (200–225 g) UT and Tr	Medium-intensity treadmill run for 1 h (acute) or until exhaustion	MDA	Mu[8] (UT and Tr) →[9] Li (Tr) →[9] Li (UT) →[9], ↑~100%[10]	50
Male rats (~200 g) UT and Tr	Submaximal treadmill run to exhaustion	MDA	Urine (UT and Tr) ↑~70%[11]	45
	Perfused gastrocnemius Mu electric stimulation (20 min)	MDA	Perfusate (UT and Tr) ↑~500%[12]	
Female rats (3 mo) Tr	Exhaustive swim exercise	MDA	Heart ↑17%	56
Male mice (6.5 mo) UT	9-h treadmill run, 6° uphill grade, speed 13.5 m/min[13] (low intensity)	Lipofuscin MDA	Mu[14] → Mu[14] →[15], ↑~180%[16]	77
Male mice (4.5 mo) UT[17]	Treadmill run; the following run program was repeated thrice: 25 m/min for 10 min, followed by 28 m/min for 5 min, followed by 18 m/min for 15 min[18]	Lipofuscin MDA	Mu[19] → Mu[19] →[20], ↑~27–72%[21]	77
Male mice (4.5 mo) UT and Tr	Treadmill run; the following run program was repeated thrice: 25 m/min for 15 min, followed by 31 m/min for 10 min. After the 3 repetitions, mice were exhausted by gradual increase of speed; ~3 h[22]	MDA	Mu[19] ↑43% (UT), → (Tr)	77
Quarterhorses (adult; 510 kg)	Soft sand track run, 2.2 km in 10 min	MDA	Venous erythrocytes ↑96%[15], →[23]	16
		Human studies		
Men (21 yr) mean $\dot{V}O_{2\,max}$ 47 ml·kg^{-1}·min^{-1}	5-min bicycling, 40% $\dot{V}O_{2\,max}$ 5 min	MDA	Plasma ↓12%	61
	Above protocol followed by 5-min rest, followed by 5-min bicycling, 70% $\dot{V}O_{2\,max}$, 5 min		Plasma →	
	Above protocol followed by 5-min rest, followed by intermittent exercise to voluntary exhaustion (60 rpm, increasing load 30 W/min)		Plasma ↑26%	
Men (22 yr) healthy, normally physically active but not trained athletes	First 5 min horizontal treadmill, running speed adjusted such that the heart rate was 75% of each subject's age-adjusted maximum. This was followed by a 45-min downhill (12° gradient) run	MDA	Serum →[24], ↑~100%[25]	62
Men (35 yr) mean $\dot{V}O_{2\,max}$ 67 ml·kg^{-1}·min^{-1}	Half-marathon race (21.1 km) Run time 81 min	MDA CD	Plasma →[26] Plasma →[26]	28

TABLE 2—*Continued*

Experimental Material	Work	Assay	Sample and Effect[1]	Ref.
		Human studies		
Men, fasted (20–29 yr)	Treadmill exercise at 60% $\dot{V}O_{2\,max}$ 30 min. Rest, 1 min.	MDA	Serum ↑~20%[27]	53
Tr ($\dot{V}O_{2\,max}$ > 50 ml·kg^{-1}·min^{-1})	Followed by 5-min run; speed	Pentane	Expired air ↑100+%[27]	
UT ($\dot{V}O_{2\,max}$ < 50 ml·kg^{-1}·min^{-1})	and inclination gradually increased such that subjects exercised at 90% $\dot{V}O_{2\,max}$ for ≥ 2.5 min			
Men (young, 22–29 yr; old 55–74 yr)	Three 15-min periods of downhill (−16%) treadmill run with 5-min rest between bouts. Work intensity corresponded to 75% of respective heart rate maxima	MDA	Urine →[28], ↑60–70%[29] Mu[30] ↑~100%[31]	66
		CD		
Men (30 yr) mean $\dot{V}O_{2\,max}$ 38.5 ml·kg^{-1}·min^{-1}	Graded bicycle ergometric test to maximum, 14 min	MDA	Plasma →	88
	Submaximal (work intensity corresponding to respective aerobic threshold) bicycle exercise for 30 min.	MDA	Plasma ↑~50%[32]	
	Submaximal (work intensity corresponding to the respective anaerobic threshold) bicycle exercise for 30 min	MDA	Plasma ↑~100%[32]	
Rheumatoid arthritis and knee of effusion patients (25–78 yr)				
Men and women	Isometric quadriceps contraction for 2 min	MDA	Synovial fluid ↑[33]	65
		CD	Synovial fluid →[33]	
	300 yard walking on the flat	MDA	Synovial fluid ↑[34]	
Men and women (Tr); long-distance runners	Exhaustive bicycle ergometer test; 10–14 min	MDA	Venous serum →[35]	96

Age (for humans, mean age or range is indicated) or body weight (g) indicated in parentheses. ↑, Increased; ↓, decreased; →, change statistically not significant. MDA, malondialdehyde (mostly studied as thiobarbituric acid reactive substance); LHP, lipid hydroperoxide; CD, conjugated dienes; Mu, skeletal muscle; RMu, red Mu; WMu, white Mu, Li, liver; Tr, trained; UT, untrained; $\dot{V}O_{2\,max}$; maximal oxygen consumption capacity. [1] Compared with corresponding controls; [2] homogenate of gastrocnemius, plantaris and soleus; [3] endurance training appeared to protect against exercise-induced lipid peroxidation in WMu and Li; [4] slow-twitch oxidative tissue from soleus muscle and red and white vastus muscles; [5] red and white vastus muscles; [6] deep portion of vastus lateralis muscle; [7] red gastrocnemius and mixed vastus lateralis muscles; [8] deep vastus lateralis muscle; [9] after acute exercise, exhaustion, and 24 and 48 h postexhaustion compared with rested; [10] 24 and 48 h after exhaustive exercise only when compared with rats immediately after exhaustion but not vs. rested rats; [11] results normalized against urinary creatinine clearance; [12] poststimulation increased MDA was cleared by liver; this effect was more pronounced in Tr vs. UT; [13] after 3- and 6-h run, there was a 10 to 15-min pause allowing access to drink water. Samples were collected immediately after exercise and after 2, 4, and 6 days postexercise; [14] red quadriceps muscle; [15] immediately after exercise; [16] 2, 4, and 6 days postexercise; [17] just before actual exercise program familiarized with the running situation at speed 18 m/min for 30 min; [18] some mice were not able to follow highest speed and were allowed to rest during these high-speed-run periods. After intermittent run program, these mice were exhausted (in 0.5–2 h) by gradually increasing speed for 18 to 28 m/min. Samples were collected immediately after exercise and after 1, 2, 5, and 10 days postexercise; [19] proximal vastus medialis muscle; [20] immediately after, and 10 days postexercise; [21] 1, 2 (effect most pronounced), and 5 day postexercise samples; [22] samples collected 3 days postexercise; [23] 1 and 24 h postexercise; [24] immediately after exercise regimen and in the 72-h postexercise recovery samples; [25] 6-, 24-, and 48-h postexercise recovery samples; MDA levels in recovery samples were as follows: 6 h > 24 h > 48 h indicating clearance with time; [26] data from 5 min, 24 h, 48 h, 72 h, and 120 h postrace were compared with 1 h prerace results; [27] both after the 60% $\dot{V}O_{2\,max}$ bout as well as after the 90% $\dot{V}O_{2\,max}$ bout; [28] observed (in both age groups) in postexercise samples on the day of exercise, and 1, 2, and 5 days after day of exercise; [29] postexercise samples on the 12th day after day of exercise (in both age groups) compared with results from day before exercise; [30] vastus lateralis muscle needle biopsies; [31] >100% increase was observed in immediate postexercise samples, effect was even more pronounced (↑~175%) in samples collected 5 days after exercise; [32] increase in 2-min postexercise samples was no more seen in 24-h recovery samples; [33] samples were collected immediately after contractions and after 2, 4, and 6 min, of postexercise recovery. Most remarkable effect in MDA was observed in the 2- and 4-min recovery samples; [34] greatest increase at 6–8 min after exercise; [35] samples were collected after 7 min of exercise, immediately after exercise, and 30 min postexercise.

ercise may increase muscle and liver free radical concentration by two- to threefold. A bout of exhaustive exercise also decreased mitochondrial respiratory control and resulted in a loss of sarcoplasmic (and endoplasmic) reticulum integrity. The authors proposed that exercise-induced free radical damage may be implicated in mitochondrial biogenesis (24). The effect of 30 min of excessive contractile activity on muscle free radical concentration was studied by Jackson et al. (42). EPR (g = 2.0036–2.004) signals revealed that there was a a 70% increase in the concentration of the major free radical in the active intact muscle compared with muscle at rest. In a separate study, exhaustive swim exercise was observed to increase EPR free radi-

cal signals ($g = 2.004$) in the rat myocardium. Higher levels of lipid peroxidation by-product were also detected in the postexercise myocardium, indicating that the bout of exercise did indeed induce oxidative stress (56). The contention that physical exercise may increase free radical generation in the heart is supported by a recent investigation studying α-phenyl-*N*-terbutyl nitrone (a spin trap)-ROS adduct and the ascorbyl EPR signal (92).

Oxidative stress is suggested to be implicated in the generation of oxidative skeletal muscle fatigue (12) and muscular atrophy (54). With the use of pump-perfused mouse soleus muscle and canine gastrocnemius-plantaris muscle preparations it was shown that xanthine oxidase-generated superoxides ($O_2^- \cdot$) may attenuate the function and enhance the fatigue rate of contracting muscles. Such effects of $O_2^- \cdot$ were not observed in the presence of a hydroxyl radical scavenger, a xanthine oxidase activity blocker, or a Fe^{2+} chelator. It was thus concluded that free radicals may be one of the factors that contribute to oxidative skeletal muscle fatigue (12). The primary factor triggering muscle contraction is the action potential, an electrical signal based on Na^+ influx followed by K^+ efflux. Human and animal studies have revealed that venous plasma K^+ concentration is markedly elevated following muscular contraction. It has been demonstrated that the major source of this K^+ is the contracting muscle (90). Because of the remarkable increase in K^+ efflux and water influx, intense muscular contraction has been shown to result in a 6–20% decrease in intracellular K^+ concentration in the tissue. Considerable efflux of K^+ from the cell may impair excitation of sarcolemmal and T-tubular membranes and finally contribute to the onset of fatigue (90). In a recent study, we investigated the effect of oxidant exposure on the K^+ transport systems of cultured skeletal muscle cells. Even very low doses of hydroperoxide had remarkable specific effects on the different components of K^+ influx in the skeletal muscle-derived cells (83). The mechanisms and physiological significance of such effects remain to be elucidated.

Multiple unsaturation points in polyunsaturated fatty acids (PUFA) make them highly susceptible to ROS attack and oxidative damage. The process of lipid peroxidation is initiated when a ROS having sufficient energy to abstract an H atom of a methylene ($-CH_2$) group (of the PUFA backbone) reacts with a PUFA (1). Peroxyl radicals, thus formed, are particularly dangerous because they are capable of propagating oxidative damage. These ROS are carried by the blood to distant targets where fresh oxidative damage may be initiated. Membrane lipid peroxidation may alter fluidity and permeability, and compromise the integrity of the barrier. Hence, the study of lipid peroxidation to estimate oxidative stress is a popular practice. Results describing the effect of exercise on lipid peroxidation by-products are inconsistent. Much of this inconsistency may be explained by the wide variety of methods employed and the different exercise conditions (e.g., intensity, type, duration, training protocols, etc.) used in different studies (Table 2). Malondialdehyde (MDA), a lipid peroxidation by-product, has been the most common parameter that has been studied with respect to exercise. In most cases, the thiobarbituric acid reactivity approach has been used for MDA assay. In addition, degradation of lipid peroxidation by-products produces the hydrocarbons ethane and pentane. Expired air containing these hydrocarbons may be collected in inert-gas bags and measured using the gas chromatograph and hydrogen flame detectors. However, expired pentane-dependent estimations of oxidative stress may have certain drawbacks (43). Unfortunately, to date there appears to be no single assay that would satisfactorily estimate the actual extent of lipid peroxidation in biological samples. The assessment of oxidative stress on the basis of lipid peroxidation data is an indirect approach that may be rendered valid only if other defined oxidative stress indexes are also studied simultaneously.

The oxidation of GSH in various tissues has proven to be a more consistent index of exercise-induced oxidative stress. When challenged with oxidative stress, intracellular glutathione appears to rapidly oxidize to glutathione disulfide (GSSG). GSSG produced intracellularly may be reduced back to glutathione in the presence of glutathione reductase activity that requires NADPH as a cofactor. However, if the oxidative stress challenge is severe, the rate of GSSG formation may exceed the capacity of the cell to reduce the disulfide. In such a situation, the heart and skeletal muscle cell have been observed to expel GSSG from the cell by a putative energy-linked mechanism (40, 87). Exhaustive exercise has been observed to increase the level of GSSG and decrease the level of total glutathione (GSH + GSSG) in tissues such as the skeletal muscle, heart, and liver (46–48, 58, 59, 80, 84). Plasma levels of GSSG are remarkably higher in exhaustively exercised experimental animals (58, 59, 80). Such results justify the hypothesis that during strenuous exercise glutathione is oxidized to GSSG in the active tissues such as the skeletal muscle and heart. Generation of GSSG at a rate that exceeds the capacity of glutathione reductase to reduce the disulfide leads to the efflux of GSSG from the tissue and its appearance in the plasma. The capacity for glutathione synthesis in the liver is very high, and exercise-induced decrease of hepatic glutathione has been viewed as more of a protective response (84). Because of increased hepatic efflux of glutathione during exercise, additional glutathione is thus made available to the peripheral tissues under need. Data from totally hepatectomized rats reveal that the glutathione store of the heart is highly dependent on hepatic supply. However, no remarkable effect on the glutathione status of skeletal muscle was observed in the hepatectomized animals (55). Previously, we observed that skeletal muscle-derived cells are highly active in glutathione synthesis (87). Based on the estimated intracellular water content of the cells (81), we calculated that the muscle cells were able to maintain an intracellular concentration of ~3 mM glutathione. Given the volume of the tissue in animals and humans, skeletal muscle glutathione metabolism may be considered to play a crucial role in whole body glutathione homeostasis.

Exercise-induced glutathione oxidation in humans

has been studied in the blood (31, 49, 88, 94). Mammalian erythrocytes are rich in glutathione (~2 mM) and account for most of the blood glutathione. The primary article on human blood glutathione oxidation during exercise by Gohil et al. (31) reported that submaximal exercise influences blood glutathione status. A sharp increase (100%) in blood GSSG was observed within the first 15 min of exercising at 65% peak oxygen consumption (31, 94). Ji et al. (49) studied eight healthy cyclists who exercised at 70% $\dot{V}O_{2\,max}$ for a mean duration of 134 min. Samples collected after 60 and 120 min and at completion of exercise showed no significant changes in GSSG levels. In our study with healthy young men, blood GSSG level was significantly elevated after maximal bicycle ergometeric test (14 min), after 30-min bicycling at aerobic threshold, or 30-min bicycling at the anaerobic threshold of the respective individuals (88). Our results were consistent with the reports of Gohil et al. (31) and Viguie et al. (94). We observed that 24 h of recovery was sufficient to restore blood GSSG levels to preexercise values (88).

Ames et al. (6) estimated that in humans the number of oxidative hits to the DNA per cell per day is 10,000. Oxidative lesions of DNA accumulate with age. A 2-yr-old rat is estimated to have 2 million oxidative DNA lesions per cell, which is about twice of that in a young rat (6). Enzymatic excision of oxidative DNA lesions and excretion of such damaged by-products through the urine constitute a crucial step of antioxidant defense. In mammals, oxidative DNA damage appears to be roughly related to the metabolic rate (6). Such a trend develops a strong rationale to study the effect of exercise on oxidative DNA modifications. However, information regarding exercise-induced oxidative DNA damage is scanty. Ten hours after marathon running, the ratio of urinary oxidized nucleosides per creatinine increased 1.3-fold above rest (2). Myeloperoxidase-dependent superoxide production in neutrophils is a physiological process having microbicidal activity. Neutrophils represent 50–60% of the total circulating leukocytes, and Smith et al. (91) have shown that a single bout of exercise may remarkably increase ROS production by the neutrophils. We were therefore interested to see how different intensities of exercise may affect leukocyte DNA in humans. Results obtained in our study (88) indicate the possibility that exercise-associated oxidative stress may initiate DNA damage in the leukocytes. However, out of the 36 measurements carried out with nine subjects during four exercise tests, DNA damage was not detected in 11 cases. In another recent study, Viguie et al. (94) observed no significant change in the urinary level of the RNA adduct 8-hydroxyguanosine after 90-min bicycle exercise of young healthy men. The vital parameter of DNA damage, which is so intricately associated with cancer and aging, demands further attention.

Oxidants such as the hydroxyl radical and the peroxyl radical (a lipid peroxidation intermediate) can fragment and cross-link proteins as well. Oxidatively modified proteins are rapidly degraded to thier constituent amino acids by ATP- or ubiquitin-independent pathways (23). Components of protein, e.g., methionine, tryptophan, histidine, and sulfhydryl residues, that are crucial for protein function are highly sensitive to oxidative damage. The physiological significance of protein oxidation is broad, including receptor modification, altered signal transduction, and other important processes. Although physical exercise is known to induce lipid peroxidation in human blood plasma, the effect of such oxidative challenge on low-density lipoprotein apoB protein oxidation has not yet been tested. Such experiments are of critical clinical significance because apoB oxidation will alter the immunologic identity of low-density lipoprotein and contribute to the development of atherogenesis. Despite the enormous significance of the subject, exercise-induced protein oxidation has been very poorly studied. Reznick et al. (75) has reported the first evidence that exhaustive exercise does increase skeletal muscle protein oxidation in rats. In another recent study carried out with rats, 10- to 15-min swim exercise resulted in oxidation of erythrocyte membrane protein. After exercise, skeletal muscle microsomes contained decreased sulfhydryls, and protein cross-linking was extensive (74). Recently we observed that in skeletal muscle-derived cells certain membrane K^+ transport proteins are highly sensitive to oxidant exposure (83).

PHYSICAL TRAINING

In organs such as skeletal muscle, heart, and liver, antioxidant defenses appear to be modulated by the state of physical training. In 1973, Caldarera et al. (19) were the first to show that acute exercise increases catalase activity in rat liver, heart, and skeletal muscle. Since then, a relatively large number of studies have tested the effect of a variety of endurance exercise training regimens on antioxidant defenses (46, 70, 82). Jenkins et al. (44) studied the antioxidant enzymes of the muscle. Needle biopsy samples were collected from the vastus lateralis muscle of healthy men. The subjects were split into high-fit ($\dot{V}O_{2\,max}$ >60 $ml \cdot kg^{-1} \cdot min^{-1}$) and low-fit ($\dot{V}O_{2\,max}$ <60 $ml \cdot kg^{-1} \cdot min^{-1}$) groups. The high-aerobic-capacity group had significantly greater activities of catalase and superoxide dismutase in their muscle. A strong positive correlation ($r = 0.72$, $P < 0.01$) between the subject's $\dot{V}O_{2\,max}$ and muscle catalase was noted. A similar correlation was also observed between the subject's $\dot{V}O_{2\,max}$ and muscle superoxide dismutase ($r = 0.60$, $P < 0.05$). The study also found that there was a rank order relationship in both the tissue oxygen consumption and antioxidant enzyme activity (44). In a recent study on exercise-induced oxidative stress in diabetic young men, we observed that resting plasma MDA and exercise-induced increase in plasma MDA strongly correlated ($r = -0.82$ and 0.81, respectively) with the respective aerobic capacity of the individuals, suggesting a protective effect of physical fitness (unpublished observation). Information on the effect of sprint training on tissue antioxidant defenses is scanty. Criswell et al. (22) studied the effect of 12-wk interval training and observed favorable changes in the skeletal muscle of rats. The authors proposed that 5-min interval high-

intensity training was superior to moderate-intensity continuous exercise in upregulating muscle antioxidant defenses. In another study, sprint training of rats was observed to significantly increase the total glutathione pool of skeletal muscles, and glutathione peroxidase activity of the heart and skeletal muscle. Skeletal muscle or heart superoxide dismutase activity was not influenced (our unpublished observation). The contention that moderate-intensity endurance exercise training may enhance the physiological antioxidant defenses has received substantial support. On the other hand, activity restriction has been observed to significantly compromise such defenses, rendering the tissues more susceptible to oxidative damage (54, 84). Thus habitual physical exercise is crucial to maintain and promote our natural capacity to defend against the ravages of reactive oxygen.

Exercise training is involved in a chronic and intermittent increase in the exposure of active tissues such as the skeletal muscle and heart to oxygen flux. In prokaryotes, some mechanisms of ROS-dependent induction of antioxidant defense proteins have been unraveled (79, 82). The superoxide-sensitive *sox* and *mar* systems, and the hydrogen-peroxide sensitive *oxyR* system have been described (35, 79, 93). Although it is suspected that similar regulation of antioxidant protein expression does take place in mammalian cells, little is known about the details of such processes in eukaryotic cells. Recently, the transcripion factors activator protein-1 and NF-κB have been identified as redox sensitive in mammalian (including human) cells (7, 25, 78, 79). The thiol redox status in different compartments of such cells appears to have a remarkable influence in the modulation of these transcription factors. For example, high cytosolic GSSG induces the activation of NF-κB, but high nucleic GSSG inhibits the binding of the activated dimer to the target oligonucleotide sequence (67). We observed a remarkable inhibitory effect of glutathione and *N*-acetyl-L-cysteine (NAC) on the activation of these transcription factors in challenged primate cells that were either controls or transfected to overexpress catalase and cytosolic superoxide dismutase (unpublished observations). Exercise-induced changes in the thiol redox status of tissues may influence intracellular signal transduction processes that trigger antioxidant defense protein expression.

MANIPULATION OF ANTIOXIDANT DEFENSES

To develop an understanding of how different physiological and dietary antioxidants may, individually or in combination, contribute to defense against exercise-induced oxidative stress, several studies with antioxidant supplementation and deficiency have been carried out (32, 34, 46, 82, 99). After such modulation of antioxidant defenses, biochemical indexes of tissue damage and exercise performance have been monitored.

Studies attempting to modulate the glutathione status of tissues have been performed only recently (21, 46, 69, 82). Two small studies have shown that exogenously administered glutathione remarkably (100–140%) increased the endurance to physical exercise in mice in a dose-dependent manner (21, 69). However, no biochemical parameter was considered in either study, and the exercise-protocol involved was perhaps too brief (~5 min) to induce oxidative stress. We revealed the fate of intraperitoneally administered glutathione and the effects of glutathione supplementation on long-duration exhaustive exercise-induced oxidative stress and endurance to such exercise. The effect of repeated glutathione administration was also compared with that of a single injection (80). This biochemical investigation was necessary before any supposition regarding the role of the thiols in endurance enhancement, an area of obvious popular interest, could be formulated. Injection of glutathione remarkably increased plasma glutathione and was followed by a rapid clearance (faster during exercise) of the thiol. The excess postinjection plasma glutathione was rapidly oxidized to GSSG. Exogenous glutathione per se was an ineffective delivery agent of glutathione to tissues. After repeated administration of glutathione (1 time/day for 3 days), blood and kidney total glutathione levels were increased. Supplementation of glutathione did not influence endurance to exhaustive treadmill run that lasted for ~2 h (80). However, results from mitochondrial superoxide dismutase immunoreactivity indicate that glutathione supplementation may marginally decrease the exercise-induced release of the protein to the plasma (85).

NAC (2-mercapto-propionyl glycine) is known to have strong antioxidant and nucleophilic properties with little or no side effect in humans. Exogenous NAC may act to rescue glutathione as follows: promoting glutathione synthesis in vivo by providing cysteine, a precursor of glutathione the availability of which limits glutathione synthesis, and/or directly scavenging ROS (8). NAC is therefore often referred to as a proglutathione drug. In a recent study with young healthy men, we observed that NAC supplementation can remarkably attenuate exercise-induced blood glutathione oxidation (88). Oral NAC did not elevate blood total glutathione level; however, plasma peroxyl radical scavenging capacity was significantly higher in postsupplementation samples. Such results indicate that oral NAC may have had a sparing effect on exercise-induced blood glutathione oxidation (88). A similar trend was also noticed in our experiment with rats. Intraperitoneally administered NAC decreased exhaustive exercise-induced glutathione oxidation in the blood and lung (80).

Recently, we evaluated the relative role of endogenous glutathione in the attenuation of exercise-induced oxidative stress. For the first time in exercise-related studies, glutathione-deficient rats prepared by blocking glutathione synthesis in vivo were used as a model (80). Treatment of the rats with glutathione synthesis inhibitor decreased total glutathione pool in *1*) the liver, lung, blood, and plasma by ~50% and *2*) in skeletal muscle and heart by 80–90%. Glutathione deficiency caused a profound increase in tissue lipid peroxidation and compromised long-duration treadmill run performance (80). Such results suggest a critical role of endogenous glutathione in the circumvention of exer-

cise-induced oxidative stress and as a determinant of exercise performance. A similar study with swim exercise of glutathione-deficient mice was more recently carried out by Leeuwenburgh and others (C. Leeuwenburgh, personal communication), who concluded that glutathione homeostasis is essential for the prooxidant-antioxidant balance during physical exercise. In this model, involving low-intensity swim exercise for a longer duration, glutathione deficiency did not influence swim performance in the mice.

The trend in results obtained from studies investigating the effect of dietary antioxidants on exercise-induced oxidative stress is encouraging (32, 34, 99). In rats, vitamin E supplements have been observed to protect against exercise-induced lipid peroxidation in the liver, heart, blood, and skeletal muscle. A protective effect of vitamin E on run-induced protein oxidation in skeletal muscle has also been evident (75). Vitamin E deficiency is known to be followed by enhanced tissue lipid peroxidation, increased susceptibility of skeletal muscle to damage, greater fragility of lysosomal membranes, compromised respiratory control ratio in skeletal muscle, increased sarcoplasmic and endoplasmic reticulum lesions, increased erythrocyte hemolysis, decreased oxidative phosphorylation in liver, and decreased exercise performance (34). In humans, vitamin E supplementation for 1.5 mo accelerated recovery from downhill run-induced muscle damage (20). Urinary and muscle lipid peroxidation by-products were also lower in vitamin E-supplemented subjects (66). Studies that have examined the effect of vitamin E alone with respect to human exercise are limited and not all of them reveal a favorable response (34).

There is a remarkable lack of information regarding the possible protective effect of vitamin C supplementation on exercise-induced oxidative stress. Vitamin C supplementation did not alter the run time to exhaustion in vitamin E-deficient rats (30). Ten young men were given either a placebo or a vitamin C supplement in a double-blind cross-over design. The subjects ran at 80% $\dot{V}O_{2\,max}$ for 30 min. Vitamin C was able to attenuate the exercise-induced increase in lipid peroxidation as indicated by thiobarbituric acid-reactive substances and lipid hydroperoxides after 2 wk of supplementation (34). In another human study, Alessio et al. (4) reported that vitamin C supplementation appeared to exhibit a mild protective effect based on the total oxyradical scavenging capacity of the plasma.

Selenium and carotene supplementation studies have been done in association with either vitamin E deficiency or in combination with other antioxidants. Hepatic and skeletal muscle glutathione peroxidase activity was increased as a result of selenium supplementation. In contrast, when selenium was deficient in the diet, the enzyme activity was markedly attenuated (15). Selenium deficiency had little effect when vitamin E was present (16).

Design of antioxidant supplementation protocols should be guided by the requirements of the antioxidant chain reaction; such an approach will maintain a favorable redox status of each of the constituent antioxidants and avoid the accumulation of reactive oxidized antioxidant by-products (e.g., chromanoxyl and ascorbyl radicals). The effect of a combination of several antioxidants as supplements have only been examined in a relatively few articles (52, 53, 95). Supplementation of individuals with a vitamin mixture containing β-carotene, vitamin C, and vitamin E for 5 wk resulted in decreased serum MDA and breath pentane, decreased lipid peroxidation at rest and that induced by exercise at both 60 and 90% $\dot{V}O_{2\,max}$ (53). In contrast, a similar mixture of antioxidants did not reveal any beneficial effect on muscle damage of subjects who exercised at 65% of maximal heart rate (52). In another study, antioxidant mixture given to human appeared to protect the erythrocytes and skeletal muscle from exercise challenge (99). There is an appalling lack of information on the possible beneficial effects of antioxidant therapy in the circumvention of exercise-induced oxidative stress. The current state of information builds a strong rationale for further research and calls for policies encouraging investigations that would test the effects of different combinations of antioxidant supplements and promote the use and availability of nutritional antioxidants. The long-term effects of taking regular antioxidant supplements on health-related factors need to be examined.

SUMMARY

A delicate balance between the virtues and vices of oxygen metabolism is expected to determine our state of health and longevity. Physical exercise is protective in a multitude of ways; however, strenuous long-duration exercise overwhelms our capacity to detoxify reactive oxygen. The result is oxidative stress. Physiological antioxidant defense may be expected to remarkably vary from one individual to another. Assessment of one's susceptibility to oxidative stress is therefore desired. Regular physical activity in association with dietary habits that ensure adequate supply of a combination of appropriate antioxidants may be expected to be a prudent course.

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REFERENCES

1. **Alessio, H. M.** Lipid peroxidation processes in healthy and diseased models. In: *Exercise and Oxygen Toxicity*, edited by C. K. Sen, L. Packer, and O. Hanninen. Amsterdam: Elsevier Science, 1994, p. 269–295.
2. **Alessio, H. M., and R. G. Cutler.** Evidence that DNA damage and repair cycle activity increases following a marathon race (Abstract). *Med. Sci. Sports Exercise* 22: S126, 1990.
3. **Alessio, H. M., and A. H. Goldfarb.** Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. *J. Appl. Physiol.* 64: 1333–1336, 1988.

4. **Alessio, H. M., A. H. Goldfarb, G. Cao, and R. G. Cutler.** Short and long term vitamin C supplementation, exercise and oxygen radical absorption capacity (Abstract). *Med. Sci. Sports Exercise* 25: S79, 1993.
5. **Alessio, H. M., A. H. Goldfarb, and R. G. Cutler.** MDA content increases in fast- and slow-twitch skeletal muscle with intensity of exercise in a rat. *Am. J. Physiol.* 255 (*Cell Physiol.* 24): C874–C877, 1988.
6. **Ames, B. N., M. K. Shigenaga, and T. M. Hagen.** Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA* 90: 7915–7922, 1993.
7. **Amstad, P. A., G. Krupitza, and P. A. Cerutti.** Mechanism of c-*fos* induction by active oxygen. *Cancer Res.* 52: 3952–3960, 1992.
8. **Aruoma, O. I., B. Halliwell, B. M. Hoey, and J. Butler.** The antioxidant action of *N*-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radical Biol. Med.* 6: 593–597, 1989.
9. **Åstrand, P.-O., and C. K. Sen.** The significance of exercise science in the maintenance of general health and enhancement of performance. *Indian J. Physiol. Allied Sci.* 48: 56–68, 1994.
10. **Aust, S. D., L. A. Morehouse, and C. E. Thomas.** Role of metals in oxygen radical reactions. *Free Radical Biol. Med.* 1: 3–25, 1985
11. **Balke, P. O., M. T. Snider, and A. P. Bull.** Evidence for lipid peroxidation during moderate exercise. *Med. Sci. Sports Exercise* 16: 181, 1984.
12. **Barclay, J. K., and M. Hansel.** Free radicals may contribute to oxidative skeletal muscle fatigue. *Can. J. Physiol. Pharmacol.* 69: 279–284, 1991.
13. **Bast, A., and G. R. M. M. Haenen.** Regulation of lipid peroxidation by glutathione and lipoic acid: involvement of liver microsomal vitamin E free radical reductase. In: *Antioxidants in Therapy and Preventive Medicine*, edited by I. Emerit, L. Packer, and C. Auclair. New York: Plenum, 1990, vol. 264, p. 111–116.
14. **Bowry, V. W., K. U. Ingold, and R. Stocker.** Vitamin E in human low-density lipoprotein. When and how this becomes a pro-oxidant. *Biochem. J.* 288: 341–344, 1992.
15. **Brady, P. S., L. J. Brady, and D. E. Ullrey.** Selenium, vitamin E and the response to swimming stress in the rat. *J. Nutr.* 109: 1103–1109, 1979.
16. **Brady, P. S., P. K. Ku, and D. E. Ullrey.** Lack of effect of selenium supplementation on the response of the equine erythrocyte glutathione system and plasma enzymes to exercise. *J. Anim. Sci.* 47: 493–496, 1978.
17. **Buettner, G. R.** Ascorbate autoxidation in the presence of iron and copper chelates. *Free Radical Res. Commun.* 1: 349–352, 1986.
18. **Burk, R. F.** Biological activity of selenium. *Annu. Rev. Nutr.* 3: 53–70, 1983.
19. **Caldarera, C. M., C. Guarnieri, and F. Lazzari.** Catalase and peroxidase activity of cardiac muscle. *Boll. Soc. Ital. Biol. Sper.* 49: 72–77, 1973.
20. **Cannon, J. G., S. F. Orencole, R. A. Fielding, M. Meydani, S. N. Meydani, M. A. Fiatarone, J. B. Blumberg, and W. J. Evans.** Acute phase response in exercise: interaction of age and vitamin E on neutrophils and muscle enzyme release. *Am. J. Physiol.* 259 (*Regulatory Integrative Comp. Physiol.* 28): R1214–R1219, 1990.
21. **Cazzulani, P., M. Cassin, and R. Ceserani.** Increased endurance to physical exercise in mice given oral reduced glutathione (GSH). *Med. Sci. Res.* 19: 543–544, 1991.
22. **Criswell, D., S. Powers, S. Dodd, J. Lawler, W. Edwards, K. Renshler, and S. Grinton.** High intensity training-induced changes in skeletal muscle antioxidant enzyme activity. *Med. Sci. Sports Exercise* 25: 1135–1140, 1993.
23. **Davies, K. J. A.** Protein modification by oxidants and the role of proteolytic enzymes. *Biochem. Soc. Trans.* 21: 346–353, 1993.
24. **Davies, K. J. A., A. T. Quintanilha, G. A. Brooks, and L. Packer.** Free radicals and tissue damage produced by exercise. *Biochem. Biophys. Res. Commun.* 107: 1198–1205, 1982.
25. **Devary, Y., R. A. Gottlieb, T. Smeal, and M. Karin.** The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. *Cell* 71: 1081–1091, 1992.
26. **Diem, K., and C. Lentner** (Editors). *Scientific Tables. Documenta Geigy* (7th ed.). Basel, Switzerland: Geigy, 1970.
27. **Dillard, C. J., R. E. Litov, W. M. Savin, E. E. Dumelin, and A. L. Tappel.** Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. *J. Appl. Physiol.* 45: 927–932, 1978.
28. **Duthie, G. G., J. D. Robertson, R. J. Maughan, and P. C. Morrice.** Blood antioxidant status and erythrocyte lipid peroxidation following distance running. *Arch. Biochem. Biophys.* 282: 78–83, 1990.
29. **Frei, B., L. England, and B. N. Ames.** Ascorbate is an outstanding antioxidant in human blood plasma. *Proc. Natl. Acad. Sci. USA* 86: 6377–6391, 1989.
30. **Gohil, K., L. Packer, B. De Lumen, G. A. Brooks, and S. E. Terblanche.** Vitamin E deficiency and vitamin C supplements: exercise and mitochondrial oxidation. *J. Appl. Physiol.* 60: 1986–1991, 1986.
31. **Gohil, K., C. Viguie, W. C. Stanley, G. A. Brooks, and L. Packer.** Blood glutathione oxidation during human exercise. *J. Appl. Physiol.* 64: 115–119, 1988.
32. **Goldfarb, A. H.** Antioxidants: role of supplementation to prevent exercise-induced oxidative stress. *Med. Sci. Sports Exercise* 25: 232–236, 1993.
33. **Goldfarb, A. H., M. K. McIntosh, B. T. Boyer, and J. Fatouros.** Vitamin E effects on indexes of lipid peroxidation in muscle from dehydroepiandrosterone-treated and exercised rats. *J. Appl. Physiol.* 76: 1630–1635, 1994.
34. **Goldfarb, A., and C. K. Sen.** Antioxidant supplementation and the control of oxygen toxicity during exercise. In: *Exercise and Oxygen Toxicity*, edited by C. K. Sen, L. Packer, and O. Hanninen. Amsterdam: Elsevier Science, 1994, p. 163–189.
35. **Greenberg, J. T., J. H. Chou, P. A. Monach, and B. Demple.** Activation of oxidative stress genes by mutations at the soxQ/cfxB/marA locus of *Escherichia coli*. *J. Bacteriol.* 173: 4433–4439, 1991.
36. **Halliwell, B.** Superoxide, iron, vascular endothelium and reperfusion injury. *Free Radical Res. Commun.* 5: 315–318, 1989.
37. **Halliwell, B.** How to characterize a biological antioxidant? *Free Radical Res. Commun.* 9: 569–571, 1990.
38. **Halliwell, B., and J. M. C. Gutteridge.** *Free Radicals in Biology and Medicine.* Oxford, UK: Clarendon Press, 1989.
39. **Han, D., H. J. Tritschler, and L. Packer.** Alpha-lipoic acid increases intracellular glutathione in a human T-lymphocyte Jurkat cell line. *Biochem. Biophys. Res. Commun.* 207: 258–264, 1995.
40. **Ishikawa, T., M. Zimmer, and H. Sies.** Energy-linked cardiac transport system for glutathione disulfide. *FEBS Lett.* 200: 128–132, 1986.
41. **Jackson, M. J., D. A. Jones, and R. H. T. Edwards.** Vitamin E and skeletal muscle. In: *Biology of Vitamin E*, edited by R. Porter and J. Wheelan. London: Pitman Medical, 1983, p. 224–239. (Proc. Ciba Found. Symp.)
42. **Jackson, M. J., R. H. T. Edwards, M. C. R. Symons.** Electron spin resonance studies of intact mammalian skeletal muscle. *Biochem. Biophys. Acta* 847: 185–190, 1985.
43. **Jenkins, R. R.** Free radical chemistry, relationship to exercise. *Sports Med.* 5: 156–170, 1988.
44. **Jenkins, R. R., R. Friedland, and H. Howald.** The relationship of oxygen uptake to superoxide dismutase and catalase activity in human skeletal muscle. *Int. J. Sports Med.* 5: 11–14, 1984.
45. **Jenkins, R. R., and A. Goldfarb.** Introduction: oxidant stress, aging, and exercise. *Med. Sci. Sports Exercise* 25: 210–212, 1993.
46. **Ji, L. L.** Exercise-induced oxidative stress in the heart. In: *Exercise and Oxygen Toxicity*, edited by C. K. Sen, L. Packer, and O. Hanninen. Amsterdam: Elsevier Science, 1994, p. 249–267.
47. **Ji, L. L., and R. Fu.** Responses of glutathione system and antioxidant enzymes to exhaustive exercise and hydroperoxide. *J. Appl. Physiol.* 72: 549–554, 1992.
48. **Ji, L. L., R. Fu, and E. W. Mitchell.** Glutathione and antioxidant enzymes in skeletal muscle: effects of fiber type and exercise intensity. *J. Appl. Physiol.* 73: 1854–1859, 1992.
49. **Ji, L. L., A. Katz, R. Fu, M. Griffiths, and M. Spencer.** Blood glutathione status during exercise: effect of carbohydrate supplementation. *J. Appl. Physiol.* 74: 788–792, 1993.

50. **Ji, L. L., F. W. Stratman, and H. A. Lardy.** Enzymatic down regulation with exercise in rat skeletal muscle. *Arch. Biochem. Biophys.* 263: 137–149, 1988.
51. **Jones, A. M.** Surprising signals in plant cells. *Science Wash. DC* 263: 183–184, 1994.
52. **Kanter, M. M., and D. E. Eddy.** Effect of antioxidant supplementation on serum markers of lipid peroxidation and skeletal muscle damage following eccentric exercise (Abstract). *Med. Sci. Sports Exercise* 24: S17, 1992.
53. **Kanter, M. M., L. A. Nolte, and J. O. Holloszy.** Effects of an antioxidant vitamin mixture on lipid peroxidation at rest and postexercise. *J. Appl. Physiol.* 74: 965–969, 1993.
54. **Kondo, H., and Y. Itokawa.** Oxidative stress in muscular atrophy. In: *Exercise and Oxygen Toxicity*, edited by C. K. Sen, L. Packer, and O. Hanninen. Amsterdam: Elsevier Science, 1994, p. 319–342.
55. **Kretzschmar, M., U. Pfeifer, G. Machnik, and W. Klinger.** Glutathione homeostasis and turnover in the totally hepatectomized rat: evidence for a high glutathione export capacity of extrahepatic tissues. *Exp. Toxicol. Pathol.* 44: 273–281, 1992.
56. **Kumar, C. T., V. K. Reddy, M. Prasad, K. Thyagaraju, and P. Reddanna.** Dietary supplementation of vitamin E protects heart tissue from exercise-induced oxidant stress. *Mol. Cell. Biochem.* 111: 109–115, 1992.
57. **Kuzuya, M., M. Naito, C. Funaki, T. Hayashi, K. Asai, and F. Kuzuya.** Protective role of intracellular glutathione against oxidized low density lipoprotein in cultured endothelial cells. *Biochem. Biophys. Res. Commun.* 163: 1466–1472, 1989.
58. **Lew, H., S. Pyke, and A. Quintanilha.** Changes in the glutathione status of plasma, liver and muscle following exhaustive exercise in rats. *FEBS Lett.* 185: 262–266, 1985.
59. **Lew, H., and A. Quintanilha.** Effects of endurance training and exercise on tissue antioxidative capacity and acetaminophen detoxification. *Eur. J. Drug Metab. Pharmacokinet.* 16: 59–68, 1991.
60. **Lilius, E. M., and P. Marnila.** Photon emission of phagocytes in relation to stress and disease. *Experientia Basel* 48: 1082–1091, 1992.
61. **Lovlin, R., W. Cottle, I. Pyke, M. Kananagh, and A. N. Belcastro.** Are indices of free radical damage related to exercise intensity. *Eur. J. Appl. Physiol. Occup. Physiol.* 56: 313–316, 1987.
62. **Maughan, R. J., A. E. Dennelly, M. Gleeson, P. H. Whiting, M. R. C. Path, K. A. Walker, and P. J. Clough.** Delayed-onset muscle damage and lipid peroxidation in man after a downhill run. *Muscle Nerve* 12: 332–336, 1989.
63. **McCance, D. R. D. G. Dyer, J. A. Dunn, K. E. Bailie, S. R. Thorpe, J. W. Baynes, and T. J. Lyons.** Maillard reaction products and their relation to complications in insulin-dependent diabetes mellitus. *J. Clin. Invest.* 91: 2470–2478, 1993.
64. **McIntosh, M., A. H. Goldfarb, L. Curtis, and P. Cote.** Vitamin E alters hepatic antioxidant enzymes in rats treated with dehydroepiandrosterone (DHEA). *J. Nutr.* 123: 216–224, 1993.
65. **Merry, P., M. Grootveld, J. Lunec, and D. R. Blake.** Oxidative damage to lipids within the inflamed human joint provides evidence of radical-mediated hypoxic-reperfusion injury. *Am. J. Clin. Nutr.* 53, *Suppl.*: 362S–369S.
66. **Meydani, M., W. J. Evans, G. Handelman, L. Biddle, R. A. Fielding, S. N. Meydani, J. Burrill, M. A. Fiatarone, J. B. Blumberg, and J. G. Cannon.** Protective effect of vitamin E on exercise-induced oxidative damage in young and older adults. *Am. J. Physiol.* 264 (*Regulatory Integrative Comp. Physiol.* 33): R992–R998, 1993.
67. **Mihm, S., D. Galter, and W. Droge.** Modulation of transcription factor NFκB activity by intracellular glutathione levels and by variations of extracellular cysteine supply. *FASEB J.* 9: 246–252, 1995.
68. **Novelli, G. P., G. Braccitiotti, and S. Falsini.** Spintrappers and vitamin E prolong endurance to muscle fatigue in mice. *Free Radical Biol. Med.* 8: 9–13, 1990.
69. **Novelli, G. P., S. Falsini, and G. Bracciotti.** Exogenous glutathione increases endurance to muscle effort in mice. *Pharmacol. Res.* 23: 149–155, 1991.
70. **Ohno, H., K. Suzuki, J. Fujii, H. Yamashita, T. Kizaki, S. Oh-Ishi, and N. Taniguchi.** Superoxide dismutases in exercise and disease. In: *Exercise and Oxygen Toxicity*, edited by C. K. Sen, L. Packer, and O. Hanninen. Amsterdam: Elsevier Science, 1994, p. 127–161.
71. **Packer, L., E. H. Witt, H. J. Tritschler.** Alpha-lipoic acid as a biological antioxidant. *Free Radical Biol. Med.* In press.
72. **Pascoe, G. A., and D. J. Reed.** Cell calcium, vitamin E, and the thiol redox system in cytotoxicity. *Free Radical Biol. Med.* 6: 209–224, 1989.
73. **Quintanilha, A. T., and L. Packer.** Vitamin E, physical exercise and tissue oxidative damage. In: *Biology of Vitamin E*, edited by R. Porter and J. Wheelan. London: Pitman Medical, 1983, p. 56–69. (Proc. Ciba Found. Symp.)
74. **Rajguru, S. U., G. S. Yeargans, and N. W. Seidler.** Exercise causes oxidative damage to rat skeletal muscle microsomes while increasing cellular sylfhydryls. *Life Sci.* 54: 149–157, 1994.
75. **Reznick, A. Z., E. Witt, M. Matsumoto, and L. Packer.** Vitamin E inhibits protein oxidation in skeletal muscle of resting and exercise rats. *Biochem. Biophys. Res. Commun.* 189: 801–806, 1992.
76. **Rice-Evans, C. A., A. T. Diplock, and M. C. R. Symons.** *Techniques in Free Radical Research.* Amsterdam: Elsevier, 1991, vol. 22, chapt. 3, p. 291.
77. **Salminen, A., and V. Vihko.** Lipid peroxidation in exercise myopathy. *Exp. Mol. Pathol.* 38: 380–388, 1983.
78. **Schreck, R., P. Rieber, and P. A. Baeuerle.** Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-κB transcription factor and HIV-1. *EMBO J.* 10: 2247–2258, 1991.
79. **Sen, C. K.** Detoxification of reactive oxygen species: molecular strategies. In: *Pharmacology in Exercise and Sports*, edited by S. M. Somani. Boca Raton, FL: CRC Press, 1995, chapt. 5. In press.
80. **Sen, C. K., M. Atalay, and O. Hänninen.** Exercise-induced oxidative stress: glutathione supplementation and deficiency. *J. Appl. Physiol.* 77: 2177–2187, 1994.
81. **Sen, C. K., O. Hänninen, and S. N. Orlov.** Unidirectional sodium and potassium flux in myogenic L6 cells: mechanisms and volume-dependent regulation. *J. Appl. Physiol.* 78: 272–281, 1995.
82. **Sen, C. K., and O. Hanninen.** Physiological antioxidants. In: *Exercise and Oxygen Toxicity*, edited by C. K. Sen, L. Packer, and O. Hanninen. Amsterdam: Elsevier Science, 1994, p. 89–126.
83. **Sen, C. K., I. Kolosova, O. Hänninen, and S. N. Orlov.** Inward potassium transport systems in skeletal muscle derived cells are highly sensitive to oxidant exposure. *Free Radical Biol. Med.* 18: 795–800, 1995.
84. **Sen, C. K., E. Marin, M. Kretzschmar, and O. Hänninen.** Skeletal muscle and liver glutathione homeostasis in response to training, exercise, and immobilization. *J. Appl. Physiol.* 73: 1265–1272, 1992.
85. **Sen, C. K., T. Ookawara, K. Suzuki, N. Taniguchi, O. Hanninen, and H. Ohno.** Immunoreactivity and activity of mitochondrial superoxide dismutase following training and exercise. *Pathophysiology* 1: 165–168, 1994.
86. **Sen, C. K., L. Packer, and O. Hanninen** (Editors). *Exercise and Oxygen Toxicity*, Amsterdam: Elsevier Science, 1994, p. 1–536.
87. **Sen, C. K., P. Rahkila, and O. Hanninen.** Glutathione metabolism in skeletal muscle derived cells of the L6 line. *Acta Physiol. Scand.* 148: 21–26, 1993.
88. **Sen, C. K., T. Rankinen, S. Väisänen, and R. Rauramaa.** Oxidative stress after human exercise: effect of *N*-acetylcysteine supplementation. *J. Appl. Physiol.* 76: 2570–2577, 1994.
89. **Sha'Afi, R. I., and T. F. P. Molski.** Activation of the neutrophil. *Prog. Allergy* 42: 1–64, 1988.
90. **Sjogaard, G.** Exercise-induced muscle fatigue; the significance of potassium. *Acta Physiol. Scand.* 140, *Suppl.* 593: 1–63, 1990.
91. **Smith, J. A., R. D. Telford, I. B. Mason, and M. J. Weidemann.** Exercise, training and neutrophil microbicidal activity. *Int. J. Sports Med.* 11: 179–187, 1990.
92. **Somani, S. M.** Influence of exercise-induced oxidative stress on the central nervous system. In: *Exercise and Oxygen Toxicity*, edited by C. K. Sen, L. Packer, and O. Hanninen. Amsterdam: Elsevier Science, 1994, p. 463–479.
93. **Tsaneva, I. R., and B. Weiss.** *soxR*, a locus governing a super-

oxide response regulation in *Escherichia coli* K-12. *J. Bacteriol.* 172: 4197–4205, 1990.
94. **Viguie, C. A., B. Frei, M. K. Shigenaga, B. N. Ames, L. Packer, and G. A. Brooks.** Antioxidant status and indexes of oxidative stress during consecutive days of exercise. *J. Appl. Physiol.* 75: 566–572, 1993.
95. **Viguie, C. A., L. Packer, and G. A. Brooks.** Antioxidant supplementation affects indices of muscle trauma and oxidant stress in human blood during exercise (Abstract). *Med. Sci. Sports Exercise* 21: S16, 1989.
96. **Viinikka, L., J. Vuori, and O. Ylikorkala.** Lipid peroxides, prostacyclin, and thromboxane A_2 in runners during acute exercise. *Med. Sci. Sports Exercise* 16: 275–277, 1984.
97. **Volchuk, A., D. E. Estrada, T. Ramlal, H. Tritschler, and A. Klip.** Stimulation of glucose uptake and translocation of glucose transporters in muscle and fat cells by lipoic acid (Abstract). In: *Oxygen Club of California Annual Meeting 1995*, p. 40.
98. **Warren, J. A., R. R. Jenkins, L. Packer, E. H. Witt, and R. B. Armstrong.** Elevated muscle vitamin E does not attenuate eccentric exercise-induced muscle injury. *J. Appl. Physiol.* 72: 2168–2175, 1992.
99. **Witt, E. H., A. Z. Reznick, C. A. Viguie, P. Stark-Reed, and L. Packer.** Exercise, oxidative damage and effects of antioxidant manipulation. *J. Nutr.* 122: 766–773, 1992.
100. **Yin, S., I. Sato, Y. Hosokawa, S. Niizeki, H. Tojo, and K. Yamaguchi.** The necessity of dietary vitamin B_6 to selenium biopotency for tissue selenium glutathione peroxidase in rats. *J. Nutr. Sci. Vitaminol.* 37: 509–516, 1991.