Antioxidants and Oxidative Stress in Exercise (44453)

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Abstract. **Strenuous exercise increases oxygen consumption and causes disturbance of intracellular pro-oxidant–antioxidant homeostasis. The mitochondrial electron transport chain, polymorphoneutrophil, and xanthine oxidase have been identified as major sources of intracellular free radical generation during exercise. Reactive oxygen species pose a serious threat to the cellular antioxidant defense system, such as diminished reserve of antioxidant vitamins and glutathione, and increased tissue susceptibility to oxidative damage. However, enzymatic and nonenzymatic antioxidants have demonstrated great adaptation to acute and chronic exercise. The delicate balance between pro-oxidants and antioxidants suggests that supplementation of antioxidants may be desirable for physically active individuals under certain physiological conditions by providing a larger protective margin.** [P.S.E.B.M. 1999, Vol 222]

Oxygen is a universal electron acceptor that allows aerobic organisms to use energy stored in foodstuffs, such as carbohydrates, fats, and protein. It is widely accepted and experimentally proven that this catabolic process can generate oxygen free radicals and other reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot -}$), hydroxyl radical (·OH), and hydrogen peroxide (H_2O_2)(1). Under normal physiological conditions, the majority of ROS is produced in the mitochondrial electron transport chain (ETC) since 90% of the oxygen consumption by the body is reduced to water in the mitochondria (2). However, ROS are also generated in other biochemical pathways in the cell. For example, ROS are produced during respiratory burst by neutrophils to kill bacteria, viruses, and other xenobiotics (3). Peroxisomal production of H_2O_2 may increase substantially when a large amount of fat is metabolized *via* β-oxidation (4). Many other one-electron transfer pathways in the presence of oxygen can potentially generate $O_2^{\cdot -}$ and H_2O_2 such as the oxidation of D-amino acids, activation of cytochrome P_{450}, degradation of xanthine to uric acid, and catecholamine autoxidation (5, 6). It is estimated that a normal cell produces 2×10^{10} $O_2^{\cdot -}$ and H_2O_2 per day, which amounts to 3.3×10^{-14} moles per day (2). Although all these processes are part of normal cell life, ROS have a strong tendency to extract electrons to reach a chemically more stable structure; therefore, they are capable of eliciting oxidative damage to the various cellular components (2, 5).

For most animals, mobility is essential for survival. In humans, exercise is no longer a means of survival, but becomes a lifestyle, recreation, and sometimes, a means of therapeutic treatment. An elevated metabolic rate as a result of exercise can dramatically increase oxygen consumption in the locomotive muscles and heart as well as other tissues. In the past decade, evidence has accumulated that unaccustomed and strenuous exercise may manifest an imbalance between ROS and antioxidant defense, resulting in an oxidatively stressful environment in the body (7–11). In this chapter, the author intends to answer the following questions based on the current literature: 1) What is the evidence that ROS generation is increased during and/or after exercise? 2) Where are the cellular sources of exercise-induced ROS? 3) Is there sufficient antioxidant defense capacity to protect the cell from oxidative stress during acute exercise? 4) Can cellular antioxidant defense systems be induced after chronic exercise training?

Does Exercise Increase ROS Generation?

A small but rather creditable amount of data indicates that exercise at high intensity can cause increased free radical production in the skeletal muscle and myocardium. In 1982, Davies *et al.* (12) reported that electron paramagnetic resonance (EPR) signals were intensified in the muscle homogenate of rat after an acute bout of treadmill running to

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exhaustion. The increased free radical species were identified as semiquinone. Vitamin E deficiency alone or in conjunction with exercise also increased free radical production, accompanied by a series of cellular disorders, such as lipid peroxidation, loss of sarcoplasmic reticulum latency, and mitochondrial uncoupling. Kumur *et al.* (13) showed that ascorbical free radical signals were increased in the rat heart homogenate after several consecutive days of exercise. Using dichlorofluorescin (DCFH) as an intracellular probe, Bejma and Ji (14) recently demonstrated that the ROS production rate was significantly increased in the homogenate of vastus lateralis muscle from exhaustively exercised young and old rats (Fig. 1A). Furthermore, heart ROS production was also increased due to exercise, but only in the old rats (Fig. 1B). Under more controlled experimental conditions, several investigators have demonstrated enhanced ROS generation during muscular contraction *in vitro*. With an electrically stimulated muscle model, Jackson *et al.* (15) found a 70% increase in the EPR signals in working vs. resting muscles. Reid *et al.* (16), using the DCFH method in isolated diaphragm muscle, showed that ROS not only were increased during muscle contraction, but might also contribute to low-frequency fatigue. In an *in situ* model, O'Neill *et al.* (17) reported increased ·OH production in contracting feline triceps muscle in proportion to maximal tension development.

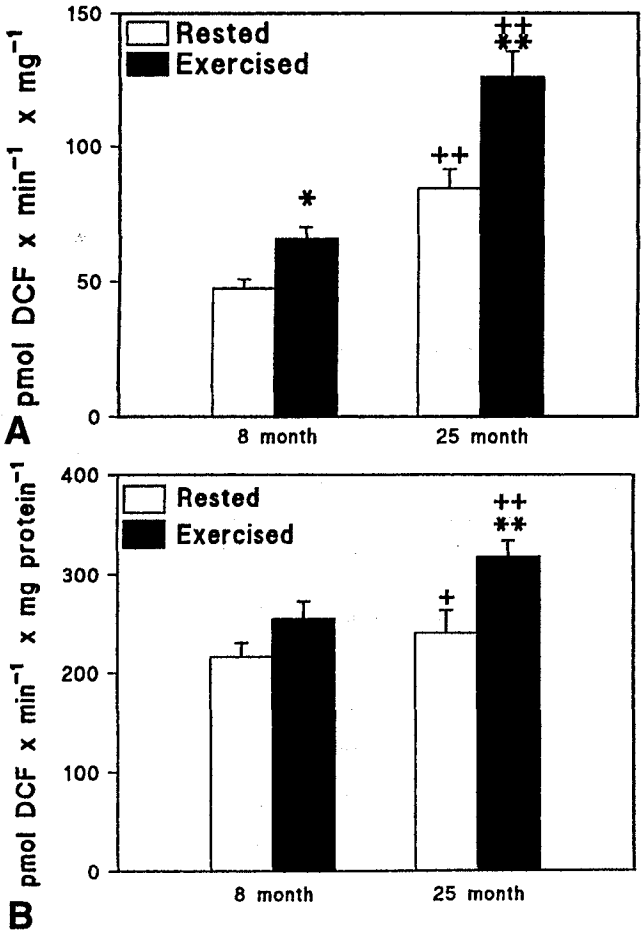

Figure 1. Oxidation rate of dichlorofluorescin (DCFH) to dichlorofluorescein (DCF) in the homogenate of rat vastus lateralis muscle (A) and cardiac muscle (B). The assay buffer contained 130 m*M* KCl, 5 m*M* $MgCl_2$, 20 m*M* NaH_2PO_4, 20 m*M* Tris-HCl, and 30 m*M* glucose (pH 7.4), plus 2 m*M* malate and 2 m*M* pyruvate. Exercise time: 55.4 ± 2.7 min for young rats at 25 m/min and 5% grade; 58.0 ± 2.7 min for old rats at 15 m/min and 5% grade. Each bar represents mean ± SEM (n =12).*P < 0.09; **P < 0.01, Exercised vs Rested. †P < 0.05; ‡P < 0.01, 25 month vs 8 month old rats.

The above studies have provided reasonable confidence that strenuous *in vivo* and *in vitro* exercise indeed can enhance ROS production in skeletal muscle and, to a lesser degree of certainty, in the heart. However, all the above studies have some limitations methodologically, and some critical questions remain to be answered. First, in the *in vivo* exercise studies cited above (12–14), ROS generation was detected in tissue homogenate collected immediately *after* exercise, whereas the true rate of ROS production *during* exercise remained unknown. ROS production has been shown to decrease rapidly within the first 1–2 min after muscle contraction ceases (17). Therefore, these methods likely underestimated the real-time oxidant production during exercise. In contrast, the *in vitro* studies measured ROS production in a somewhat artificial environment, and may have limited relevance to whole-body exercise. Secondly, in all the experiments mentioned, the cellular antioxidant system was left intact and might compete with the ROS probes. Thus, the results may be greatly influenced by changes in antioxidant function. Finally, we now still have minimal knowledge about the source and species of the ROS produced under the various experimental conditions.

Sources of Exercise-Induced ROS Production

ROS can be produced during exercise from several potential cellular sources. Some sources may be more important than others in a certain organ, at a specific time, or with a specific exercise mode. However, these sources are not mutually exclusive and can be activated simultaneously.

Mitochondrial Electron Transport Chain. NADH-ubiquinone reductase (complex 1) and ubiquinone-cytochrome-*c* reductase (complex 3) of the ETC are well-known sites of $O_2^{\cdot-}$ and H_2O_2 generation (6). The latter is caused by the transition from two-electron (NADH and $FADH_2$) to one-electron (ubiquinone) transfer, involving semiubiquinone ($QH^{\cdot}$). Electron leakage to molecular oxygen at this segment of the ETC produces $O_2^{\cdot-}$ (6). $O_2^{\cdot-}$ is readily reduced to H_2O_2 by mitochondrial SOD (Mn containing). A metal-catalyzed Fenton reaction or Haber-Weiss reaction between $O_2^{\cdot-}$ and H_2O_2 may give rise to $^{\cdot}OH$ (1). It is estimated that liver mitochondria produce 24 nmol $O_2^{\cdot-}$/min/g of tissue, and heart mitochondria generate 0.3–0.6 nmol/min/mg protein representing 2% of the tissue's total oxygen consumption (6).

Despite the theoretical soundness, there is little direct evidence that mitochondrial $O_2^{\cdot-}$ production is increased during exercise. The premise that exercise increases mitochondrial ROS production lies heavily on the well-known

fact that tissue and whole-body oxygen consumption increase dramatically during strenuous exercise. During maximal exercise, whole-body oxygen consumption (VO_2) increases up to 20-fold, whereas VO_2 at the muscle fiber level is estimated to be elevated by as much as 100-fold (7). We often assume that the percentage of O_2 to become $O_2^{\cdot -}$ remains the same (i.e., ETC efficiency maintains the same); therefore, ROS production will increase proportionally. However, *in vitro* data challenged this hypothesis demonstrating that the ROS production rate in state 3 (ADP stimulate) respiration is actually lower than state 4 (basal) (6). In our own study using the DCFH method, we found no significant increase in ROS production rate in either isolated muscle, heart, or liver mitochondria from exercised versus rested rats (14). However, these findings shall not be used as conclusive evidence against the mitochondrial theory because heavy exercise is not equivalent to state 3 respiration and could induce mitochondrial uncoupling due to inner membrane damage and hyperthermia (12, 18). Further, isolation of mitochondria took almost an hour, and damaging exercise effects might have disappeared during this time period.

The hypothesis that mitochondria are a primary site of ROS generation during exercise has been supported by indirect data mainly showing mitochondrial oxidative damage. State 4 respiration was shown to increase in muscle and liver mitochondria (12) and heart mitochondria (19) after exhaustive exercise, indicating a possible inner membrane leakage. In these studies, the respiratory control index was decreased as a result of the augmented state 4 respiration with no change or a proportionally smaller increase in state 3 respiration in response to exercise. Mitochondrial lipid peroxidation is enhanced after exercise, accompanied by loss of protein thiol content and inactivation of oxidative enzymes (20). Further, both muscle and heart mitochondria from severely exercised animals demonstrated lower coupling and decreased GSH redox status (21, 22). The mitochondrial theory of ROS production is also indirectly supported by the training adaptation of mitochondrial antioxidant enzymes, such as MnSOD and GPX (23, 24).

Xanthine Oxidase. Xanthine oxidase (XO)-catalyzed reactions have been well established as one of the major sources of free radical generation in the ischemia and reperfused (I-R) heart (25, 26). During ischemia, ATP is degraded to ADP and AMP due to the energy demand of contracting myocardium. If oxygen supply is insufficient, AMP is continuously degraded to hypoxanthine, which may be converted to xanthine and uric acid by XO coupled with one-electron reduction of O_2, giving rise to $O_2^{\cdot -}$. To activate this pathway, sufficient amounts of hypoxanthine and xanthine must be present in the tissue. The enzyme XO must be converted from its reduced form (xanthine dehydrogenase) to the oxidized form by intracellular protease that may be activated by Ca^{2+}. Also, O_2 must be available as the electron acceptor.

High-intensity exercise may produce a cellular environment in favor of activating the XO pathway (27). Hypoxanthine was reported to accumulate after intense muscular contraction, and uric acid concentration was shown to increase in both contracting arm muscle and in the plasma, suggesting that XO was activated (28, 29). Blood hypoxanthine and xanthine concentrations increased dramatically in human subjects after intense exercise (30). Furthermore, XO activity was increased 10-fold in the plasma of rats after repeated high-intensity running to exhaustion (31). It was proposed that the enzyme came from the endothelial cells of the contracting muscle. Rasanen *et al.* (32) showed that strenuous exercise increased peroxyl radical production and XO activity in the plasma of horses. Uric acid concentration increased exponentially with workload indicating a rapid degradation of purine products. Hellsten *et al.* (33) recently reported an increase in XO-immunoreactive cells, presumably capillary endothelial and leukocyte cells, in human subjects after 7 days of intense exercise training. The authors suggested that XO activation might be etiologically related to muscular oxidative damage during exercise.

Whether or not XO plays a significant role in free radical production during exercise remains to be clarified. Under aerobic conditions, sufficient oxygen supply ensures that ATP is replenished primarily *via* mitochondrial oxidative phosphorylation and that hypoxanthine/xanthine are converted to uric acid by xanthine dehydrogenase rather than XO (30). Furthermore, skeletal muscle has low XO activity. Nevertheless, XO may become an important pathway when skeletal muscle undergoes a significant deficit of adenine nucleotides. This situation theoretically may apply to ischemic muscle contraction, isometric exercise, sprinting, exercising in a hypoxic environment, and exercise with impaired blood flow due to vascular diseases.

Neutrophils and the Inflammatory Response. Polymorphoneutrophils (PMN) are blood-borne cells that play a critical role in defending tissues from viral and bacterial invasion (34). Activation of PMN typically starts with muscle or soft tissue damage, caused by either ROS-induced oxidative processes or simply stretching or other mechanical forces (7). In acute phase response, PMN migrate to the injury site attracted by chemotactic factors produced by the damaged cells and release two primary factors during phagocytosis, lysozymes and $O_2^{\cdot -}$. Lysozymes facilitate the breakdown of damaged protein and cell debris, whereas $O_2^{\cdot -}$ is produced by myeloperoxidase and NADPH oxidase (35). Cytoplasmic SOD may convert $O_2^{\cdot -}$ to H_2O_2 that is further converted to $^{\cdot}OH$ by metal ions or to hydrochloric acid (HOCl). Although this inflammatory response is considered critical in removing damaged proteins and preventing bacterial and viral infection, ROS and other oxidants released from neutrophils can also cause secondary damage such as lipid peroxidation (7, 36). Thus, the body's immune system responds to an acute bout of intense exercise much the same as to sepsis wherein they share a common mediator, ROS (37).

Strenuous exercise can elicit muscle injury accompa-

nied by an inflammatory response, characterized by increased protease and lysozymal enzyme activities in working muscle (38). Furthermore, biomarkers of the inflammatory responses often coincided with elevation of antioxidant enzyme activities such as GPX and catalase (38). Hack *et al.* (39) showed that an acute bout of exhaustive exercise in humans significantly increased cell counts of leukocytes, lymphocytes, and neutrophils. Phagocytosis assays revealed that ingestion capacity was elevated immediately after exercise up to 24 hr postexercise, whereas a significant increase in $O_2^{\cdot -}$ production was noticed only at 24 hr postexercise. Meydani *et al.* (36) showed that following an acute bout of eccentric exercise in sedentary men, circulating cytokine (interleukin-1) levels were significantly elevated, possibly released from activated monocytes. Vitamin E administration attenuated urinary markers of lipid peroxidation found during the postexercise period. Smith *et al.* (40) reported that 1 hr of moderate exercise increased neutrophil H_2O_2 generation three-fold under *in vitro* challenge as well as receptor expression. Recently, Best *et al.* (41) showed in an *in situ* rabbit muscle model that stretching injury caused by maximal isokinetic contraction was associated with significant ROS generation 24 hr post-treatment. In the injured leg, there was an accumulation of PMN leukocytes. GSH level, GPX and GR activities were found to be higher in the injured versus contralateral control leg. However, current data do not elucidate a clear role for neutrophils in enhancing ROS production during normal dynamic exercise. Given the time required for neutrophil infiltration, this pathway probably is not the primary source of free radical production during short-term exercise. However, it may serve as an important secondary source of free radical production during the recovery period following heavy exercise. In addition, it may contribute to the oxidative tissue damage during ultra endurance exertion, such as marathon running, or to injury observed after eccentric exercise.

Other ROS-Generating Pathways. Under physiological conditions, liver microsomes generate oxygen free radicals primarily *via* the cytochrome P_{450} system (5). NADPH is oxidized with the catalysis of the mixed function oxidase, giving rise to $O_2^{\cdot -}$ that can be dismuted to H_2O_2 (6). The rate of H_2O_2 production is increased at elevated oxygen consumption in the microsome (1). In the presence of ADP and Fe^{+3}, NADPH oxidase catalyzes a one-electron transfer from NADPH to O_2 to produce $O_2^{\cdot -}$. This enzyme, once thought to be located only on the plasma membrane, has now been found to be present in many cellular components including the mitochondria. Kim *et al.* (42) reported that ROS production was increased in the liver microsomes from old rats, and that chronically active animals produced less ROS than their sedentary counterparts. Bejma and Ji (14) found that oxidant production through NADPH oxidase was doubled in aged muscle mitochondria. Further, the muscle mitochondrial ROS production rate *via* this pathway was significantly increased in young rats, but not in aged rats, exercised to exhaustion.

Circulating catecholamine levels are elevated during prolonged exercise. Catecholamines enhance myocardial and skeletal muscle oxidative metabolism *via* activation of β-adrenergic receptors thereby potentially increasing ROS production in the mitochondria. Furthermore, autoxidation of epinephrine to adrenochrome is associated with $O_2^{\cdot -}$ formation that is considered as a possible source of ROS production in heart I-R injury (43). β-Blockade has been shown to reduce oxidative stress markers in plasma of human subjects working at high intensity (44). However, quantitative significance of catecholamine as a source of ROS production during exercise has not been investigated and remains unclear.

Peroxisomes are organelles in the cell involved in nonmitochondrial oxidation of fatty acids and D-amino acids. Under physiological conditions, peroxisomes contribute to the steady state production of H_2O_2 but not $O_2^{\cdot -}$ (6). Prolonged starvation has been shown to increase H_2O_2 generation mainly because of the increased fatty acid oxidation in this organelle (4). Since fatty acids are the primary energy substrate for the myocardium and skeletal muscle during prolonged exercise, peroxisomes may be potential sites for ROS production. The findings that catalase activity is increased after an acute bout of exercise in muscle seem to support this hypothesis (45, 46). However, direct evidence that exercise increases peroxisomal ROS production is lacking.

Antioxidant Protection Against ROS During Acute Exercise

Higher organisms have developed a remarkably efficient antioxidant system over the course of evolution (1). The extent of oxidative damage during physical exercise is determined not only by the level of free radical generation, but also by the defense capacity of antioxidants. In recently years, a general awareness has developed of the importance of antioxidants in the diseased state. However, we still have insufficient knowledge about the interaction of each antioxidant and exercise, which is important in assessing the adequacy of protection against oxidative damage and the necessity of dietary manipulation and/or supplementation.

Antioxidant Vitamins. Vitamin E (α-tocopherol), vitamin C (ascorbic acid), and β-carotene are important antioxidants that cannot be synthesized by most mammals and humans and therefore are required from the diet. Vitamin E is a lipophilic compound that is located in the cell membrane and is particularly efficient at quenching free radicals originating from the mitochondrial inner membrane and other biomembranes (47). Vitamin E is essential for normal cell function during exercise. Rat with vitamin E deficiency demonstrated exacerbated muscle and liver free radical production and excessive lipid peroxidation and mitochondrial dysfunction after an acute bout of exhaustive exercise compared with vitamin E–adequate rats (12). Endurance performance has also been reported to decrease in rats fed vitamin E-deficient diets (12, 48). Vitamin E defi-

ciency has also been shown to enhance lipid peroxidation, disturb GSH/GSSG redox status, and cause early fatigue in the diaphragm muscle during resistance breathing in rats (49). An acute bout of exercise does not seem to affect vitamin E content significantly in tissues, suggesting that physiological levels of tissue vitamin E are adequate protection against exercise-induced ROS generation (50, 51). However, the protective margin may be relatively small since its concentration has been shown to decrease in rat skeletal muscle, liver, and heart after chronic exercise (51–53). Dietary supplementation of vitamin E has been shown to increase tissue resistance to exercise-induced lipid peroxidation in humans (54) and rats (13, 55, 56). These findings support the recommendation that humans involved in an active lifestyle consider increasing daily dietary vitamin E intake (47). However, dosage of the supplementation should be carefully considered since no study has either confirmed or denied a potential effect of overdose and side effects.

Vitamin C is a water-soluble antioxidant in the cytosol and extracellular fluid. Its chemical properties allow it to interact directly with $O_2^{\cdot -}$ and $^{\cdot}OH$ in the aqueous phase such as plasma thus preventing damage to erythrocyte membranes (57). Vitamin C also reduces vitamin E radicals, and the oxidized semidihydroascorbate is reduced by a GSH and/or dihydrolipoic acid redox cycle (1, 58). Vitamin C deficiency has been shown to affect heart mitochondrial respiratory function in guinea pigs (which cannot synthesize vitamin C) and attenuate running time to exhaustion (59). However, dietary vitamin C supplementation did not prevent tissue-specific disorders of mitochondrial function caused by vitamin E deficiency during training in rats (48).

The effect of dietary supplementation of vitamin C has also been studied in human subjects involved in physical exercise. Although it was claimed that large doses of vitamin C intake reduced fatigue and muscle damage in several studies, no specific oxidative stress markers were measured. Therefore, it is difficult to determine whether the observed benefits were related to the antioxidant functions of vitamin C (60, 61). Furthermore, overdose of vitamin C may cause metabolic defects in the heart and early fatigue during prolonged exercise, possibly due to the pro-oxidant properties of vitamin C that react with transition metal ions to form ROS (1, 4).

Glutathione and Other Low-Molecular-Weight Antioxidants. GSH (γ-glutamylcysteinylglycine) is the most abundant nonprotein thiol source in the cell and serves multiple functions in protecting tissues from oxidative damage and keeping the intracellular environment in the reduced state (62). GSH reduces hydrogen- and organic-peroxides *via* a reaction catalyzed by GSH peroxidase (GPX); it serves as a scavenger of $^{\cdot}OH$ and singlet oxygen ($^1O_2$); GSH also reduces tocopherol radicals, either directly, or indirectly by reducing semidihydroascorbate thereby preventing free radical chain reaction and lipid peroxidation (58).

The important role of GSH in protecting against exercise-induced oxidative stress has been reviewed in detail in several previous articles (10, 63, 64). The following are the highlights of GSH function as an antioxidant: 1) GSH concentrations in most tissues are in the millimolar range, far exceeding the levels of most other antioxidants; however, clear differences exist among various tissues and types of muscle fibers depending on their metabolic rate, potential to generate ROS, and enzyme activities in the γ-glutamyl cycle (63). Therefore, exercise responses of GSH antioxidant system are tissue and fiber-specific (65). 2) Although a substantial amount of GSH is oxidized to GSSG in skeletal muscle and heart during exercise due to increased ROS production, GSH redox status (i.e., GSH:GSSG) is not altered significantly because GSSG can be reduced back to GSH by glutathione reductase (GR) using NADPH as the reducing power. Furthermore, exercising skeletal muscles appear to increase GSH import from plasma via the γ-glutamyl cycle (46, 66–68). 3) Liver synthesizes GSH from endogenous or dietary amino acids *de novo* and supplies most of the circulating GSH (62). During prolonged exercise, hepatic GSH efflux is increased due to the stimulation of elevated plasma glucagon and vasopressin levels (69). This ensures plasma GSH homeostasis despite enhanced tissue GSH use (66, 68, 70). However, plasma and muscle GSH content may be decreased eventually during prolonged exhaustive exercise when hepatic GSH reserve is diminished and GSH use exceeds GSH uptake (70–72). 4) Similar to the skeletal muscle, the heart actively uses GSH to cope with increased ROS production during exercise (73). However, myocardial GSH is decreased during an acute bout of exercise, possibly due to a lower γ-glutamyl transpeptidase (GGT) activity (65, 73). 5) GSH deficiency is associated with a wide range of physiological and biochemical disorders during exercise, such as decreased GSH:GSSG ratio and increased lipid peroxidation in skeletal muscle and heart (74), and attenuated contractile properties in rat diaphragm muscle (75). Severe GSH depletion resulted in a significant downregulation of liver GPX, muscle GGT, and mitochondrial citrate synthase activities (66). Exhaustive swimming in GSH-depleted mice enhanced liver and muscle MDA formation, but no loss of endurance was found (66). 6) Although several studies have demonstrated increased endurance with GSH supplementation, no clear benefit of GSH supplementation during acute exercise has been established (76, 77). Plasma GSH was elevated 20-fold as a result of intraperitoneal injection of GSH, whereas GSH concentration in the liver and other tissues showed little change (74, 77). GSH ethy ester supplementation also showed little promise in improving tissue GSH status (77). However, supplementation of cysteine analogs such as N-acetylcysteine (NAC) has been reported to decrease exercise-induced GSSG and blood lipid peroxidation in rats (78, 79), to improve muscle contractile functions, and to reduce low-frequency fatigue in diaphragm muscle (80) and human leg muscle (16). Other cysteine analogs such as OTC supple-

mentation have been shown to protect against oxidative stress, but their merit has not been tested on exercise (81).

In addition to GSH, several low-molecular-weight compounds such as ubiquinone (Q_{10}), uric acid, and α-lipoic acid have exhibited potent antioxidant function *in vitro* and *in vivo*. Shimomura *et al.* (82) reported that Q_{10} administration attenuated muscle creatine kinase and lactate dehydrogenase release in rats caused by downhill running. Whether or not the observed effect was due to ubiquinone's antioxidant function is not clear. Gohil *et al.* (50) showed that training could increase ubiquinone content significantly in skeletal muscle and adipose tissues. However, data in this area are scarce, and it is difficult to draw a sound conclusion concerning the merit of these compounds in protection against exercise-induced oxidative damage.

In recent years there has been increasing recognition of the antioxidant property of certain phytochemicals such as green teas, ginseng, garlic, and oats. Many of the compounds found in rich existance in these plant products, such as tocopherol, polyphenols, carotenoids, isopretol, and lycopene have long been established as antioxidants. However, few studies have examined their effects during exercise.

Antioxidant Enzymes. Antioxidant enzymes may be activated selectively during an acute bout of strenuous exercise depending on the oxidative stress imposed on the specific tissues as well as the intrinsic antioxidant defense capacity. Skeletal muscle may be subjected to a greater level of oxidative stress during exercise than liver and heart due to increased ROS production. Therefore, the muscle needs greater antioxidant protection against potential oxidative damage occurring during and/or after exercise. SOD, CAT, and GPX provide the primary defense against ROS generated during exercise, and activities of these enzymes are known to increase in response to exercise in both animal and human studies (8, 10, 11).

An acute bout of exercise has been shown to increase SOD activity in a number of tissues including liver (26, 45, 46, 67, 83, 84), skeletal muscle (46, 67, 83, 85–87), heart (19, 86), and red blood cells (88, 89). Most of the studies, with a few exceptions (86), also indicate that acute exercise increases CuZnSOD rather than MnSOD activity. This activation of SOD was proposed to result from increased $O_2^{\cdot -}$ production during exercise (90). Since we now know that CuZnSOD has a quick turnover rate and a short $t_{1/2}$ in the range of minutes (see previous section), *de novo* synthesis of new enzyme protein cannot be ruled out in explaining the SOD responses to acute exercise lasting a few hours. Recently, Radak *et al.* (31) showed that enzyme activities and immunoreactive enzyme contents of both CuZn and MnSOD in rat soleus and tibialis muscles were significantly elevated after a single bout of exhaustive treadmill running lasting 60–70 min. Interestingly, CuZnSOD activity and content gradually returned to the resting levels 1–3 days later, whereas MnSOD activity and protein content continued to increase during the postexercise period. This finding indicates that the stimulating effects of exercise on CuZn SOD and Mn SOD gene expression may be different in terms of threshold required and time course of induction.

GPX activity has demonstrated variable responses to an acute bout of exercise in the various types of skeletal muscle. Some studies showed no change in this enzyme in skeletal muscle after acute exercise (66, 83, 91, 92), whereas others reported significant elevation of GPX activity (46, 67, 93–95). Furthermore, heart GPX activities have been shown to increase after exercise (95). Muscle fiber–specific responses of GPX have also been noticed. For example, GPX activity was found to increase as a function of treadmill speed in DVL and SVL, but not soleus (46). GPX activity was increased a day after an acute bout of treadmill running to exhaustion in rat soleus, but not tibialis muscle (31). Most of the previous studies have revealed no significant alteration in CAT activity with acute exercise (7, 10). However, there are exceptions; CAT activity was found to increase significantly after an acute bout of exercise to exhaustion or at high intensity in rat DVL muscle (46, 67).

To examine whether increased SOD and GPX activities in response to acute exercise were caused by an activation or an alteration of gene expression, some researchers measured the relative mRNA abundance for the various enzymes in the skeletal muscles. Oh-ishi *et al.* (96) reported a significant downregulation of mRNA levels for both CuZn- and MnSOD isozymes in soleus muscle of untrained rats, but no exercise downregulation was observed in the trained rats. We have recently investigated the effects of a single bout of prolonged exercise on the mRNA abundance of muscle antioxidant enzymes in rats (97). mRNA abundance of CuZnSOD, MnSOD, and CAT was not altered by exercise, but exercise decreased GPX mRNA levels by 21.6% and 60.8% ($P < 0.05$) in DVL and SVL, respectively. These data demonstrate that despite increased enzyme activity, an acute bout of exhaustive exercise may decrease the mRNA abundance of GPX, MnSOD, and CuZnSOD.

Understanding the mechanisms involved in the increased antioxidant enzyme activity during exercise remains a challenge. The findings that acute exercise decreases mRNA levels for GPX and SOD appear to be paradoxical. Current data suggest that exercise may cause post-translational modulation of the enzyme protein. The role of ROS in this activation remains to be elucidated.

Adaptation of Antioxidant Systems to Training

The benefit of exercise in promoting good health and preventing various diseases is well known. However, chronic exercise also represents a form of oxidative stress to the organisms and therefore can alter the balance between pro-oxidants and antioxidants. In this section concerning the effects of chronic exercise on cellular antioxidant systems, the following questions will be brought into consideration: 1) Do tissues involved in long-term exercise show a deficit of their antioxidant reserve? 2) Can the antioxidant defense system adapt to meet the increased challenge? 3) Is it ben-

eficial to supplement exogenous antioxidants during chronic exercise?

Nonenzymatic Antioxidants. An acute bout of exercise does not seem to reduce vitamin E content in various tissues. However, vitamin E concentration has been shown to decrease in skeletal muscle, liver, and heart after endurance training (51–53). More dramatic changes were observed when tissue vitamin E levels were expressed per unit of mitochondrial protein content (50). On the other hand, dietary supplementation of vitamin E has been shown to increase tissue resistance to exercise-induced lipid peroxidation during chronic exercise (7, 13, 54). Thus, physically active individuals should consider increasing daily dietary vitamin E intake. Since vitamin E in the cell membrane is regenerated *via* a vitamin C-GSH redox cycle (see previous discussion), levels of the latter two antioxidants are expected to have major impact on tissue vitamin E response to training.

Endurance training has been shown to cause a significant decrease of GSH content in rat soleus muscle (65, 98). Rigorous swimming training in rats was reported to cause a similar level of reduction of myocardial GSH content (22). These findings were in contrast with skeletal muscle such as DVL and gastrocnemius that showed an increase in GSH with training (65, 70). As highly oxidative muscles, soleus and myocardium share many metabolic and biochemical characteristics such as mitochondrial enzyme activities and GSH content. Despite a 4–5-fold higher GPX activity, these two tissues have 60%–70% and 32% lower GGT and GCS activity, respectively, than DVL. It is conceivable that the oxidation of GSH may far exceed their capacity to import GSH from an extracellular source, resulting in a net deficit after training. In contrast, DVL muscle, which has the highest GGT activity among the various muscle types, showed no change or an increase in GSH content (65, 70, 98, 99). Endurance training has also been shown to increase GSH content in several hindlimb muscles in dogs along with increased γ-glutamyl enzymes such as GGT, GCS, and GS (64, 70, 100). The rate of GSH oxidation versus the capacity of GSH import within each fiber type controlled by the γ-glutamyl cycle seems to determine the fiber-specific training response and adaptation. Physically trained human subjects and animals generally demonstrate a greater tolerance of exercise-induced disturbance of blood GSH (68, 101, 102). Furthermore, plasma and erythrocyte GSH contents have been shown to increase significantly after physical training (101, 103, 104).

Antioxidant Enzyme Adaptation. Although an acute bout of exercise may activate certain antioxidant enzymes without synthesizing new protein, the protective margin could be quite limited depending on individual enzymes and the tissues involved. As a long-term strategy, cells may activate *de novo* synthesis of antioxidant enzymes to cope with the encountered oxidative stress. The availability of monoclonal antibodies to certain antioxidant enzymes such as SOD has provided a powerful tool to investigate their gene regulation.

SOD activity in skeletal muscle has been reported to increase significantly after training (23, 65, 70, 96, 99, 105, 106). However, many studies failed to detect SOD training adaptation even though similar animal training models were used (90, 107–109). The discrepancies may be explained by the different SOD isozymes studied, the different SOD assays used, the different training intensity and frequency used that imposed different oxidative stress to the muscle, and different muscle fiber types tested. Higuchi *et al.* (23) and Ji *et al.* (24) demonstrated that MnSOD is primarily responsible for the increased SOD activity with training, whereas CuZnSOD activity was unaffected. Oh-ishi *et al.* (96) recently studied the relationship among SOD isozyme activity, protein content, and mRNA abundance in rat soleus muscle after endurance training. Resting CuZnSOD activity was significantly increased with training, but the enzyme protein and mRNA level were not altered. In contrast, MnSOD showed both increased activity and protein content. Again, mRNA levels were not affected. These data suggest that training induction of both SOD isozymes is caused by post-transcriptional mechanisms and that post-translational modulation may play a role in CuZnSOD. In general agreement with the above study, a recent study in our laboratory (110) showed that MnSOD training induction was muscle fiber–specific. Increases in both Mn SOD activity and enzyme protein content were found in rat DVL, but not in other muscles, after 10 weeks of training. CuZnSOD activity and protein content showed no effect in all muscles examined, and resting mRNA levels for both Mn- and CuZnSOD were unaltered with training. Both of these studies pointed to the importance of MnSOD training adaptation, which seems to support the notion that mitochondrial inner membrane is a major source of ROS production during exercise. SOD training adaptation is not limited to locomotive muscle. Rigorous swim training was shown to induce myocardial and diaphragm SOD in rats (111, 112). Several detailed reviews are available on this topic (8–11, 87).

CAT activity has been shown to increase after training in skeletal muscle by some authors (95, 96, 105, 110). However, most studies have reported no change in muscle CAT with training (7, 8, 10), and a few studies even reported a decrease (99, 107). In contrast, more consistent training adaptation has been reported on GPX (20, 24, 65, 70, 85, 96, 99, 106, 107). GPX adaptation also demonstrates a muscle fiber–specific pattern with type 2a muscle being the most responsive to training. Powers *et al.* (96) showed a 45% increase in GPX activity in red gastrocnemius muscle (type 2a) after endurance training in rats, whereas soleus and white gastrocnemius muscles revealed no training effect. Leeuwenburgh *et al.* (65) reported a 62% increase in GPX activity in DVL muscle in response to treadmill training, whereas soleus and myocardium showed no GPX training effect. Although GPX is expressed as a uniform enzyme in

the various cellular compartments, evidence exists that mitochondrial GPX undergoes a greater training adaptation than the cytosolic GPX in rat skeletal muscle (24).

Why do different antioxidant enzymes display different characteristics of training adaptation? The answer may be multifaceted and depend on the specific pattern of gene expression for each enzyme, the threshold required for induction, and their interactions. *De novo* synthesis of an enzyme is energy-demanding and relatively slow, and probably is reserved as the last means to cope with oxidative stress. SOD activities appear sufficiently high and relatively uniform across tissues and various muscle types, suggesting that the removal of superoxide anion may not be a rate-limiting step. In comparison, GPX destroys the end products of ROS generation pathway (i.e., hydrogen peroxide and organic peroxide, including lipid and nucleotide peroxides), and its activity is relatively low. This may explain why GPX usually displays a greater training adaptation than SOD and CAT (113).

Conclusion

Heavy physical exercise enhances free radical production in skeletal muscle and other tissues. Although increased oxygen flux through mitochondrial electron transport chain is probably the main source for free radical generation, other pathways may also be involved under specific physiological conditions and in specific tissues. Furthermore, these free radical generating mechanisms are not mutually exclusive; therefore, oxidative injury may be escalated during and after an acute bout of strenuous exercise.

The antioxidant defense system is of vital importance in the protection against oxidative stress. Perhaps an important concept that needs to be developed is the distinction between inducible and noninducible antioxidants. The former, including antioxidant enzymes and the GSH system, have demonstrated prominent adaptive responses to chronic exercise in at least skeletal muscle, provided that adequate nutritional status is maintained. However, the latter heavily depends upon dietary intake and is thus susceptible to deficiency. Understanding the unique characteristics and regulatory mechanisms of various antioxidants will guide us in developing proper strategies to enhance cellular antioxidant capacity through physiological and nutritional means. However, there appears to be no single strategy that can improve every antioxidant system.

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