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# Antioxidants in Exercise Nutrition

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## Abstract

Physical exercise may be associated with a 10- to 20-fold increase in whole body oxygen uptake. Oxygen flux in the active peripheral skeletal muscle fibres may increase by as much as 100- to 200-fold during exercise. Studies during the past 2 decades suggest that during strenuous exercise, generation of reactive oxygen species (ROS) is elevated to a level that overwhelms tissue antioxidant defence systems. The result is oxidative stress. The magnitude of the stress depends on the ability of the tissues to detoxify ROS, that is, antioxidant defences. Antioxidants produced by the body act in concert with their exogenous, mainly dietary, counterparts to provide protection against the ravages of reactive oxygen as well as nitrogen species. Antioxidant supplementation is likely to provide beneficial effects against exercise-induced oxidative tissue damage. While universal recommendations specifying types and dosages of antioxidants are difficult to make, it would be prudent for competitive athletes routinely engaged in strenuous exercise to seek an estimate of individual requirement.

A new dimension in oxidant biology has recently unfolded. Although excessive oxidants may cause damage to tissues, lower levels of oxidants in biological cells may act as messenger molecules enabling the function of numerous physiological processes. It is plausible that some exercise-induced beneficial effects are actually oxidant-mediated. Such developments call for an even more careful analysis of the overall significance of types and amounts of antioxidants in diet. While these complexities pose significant challenges, experts agree that if used prudently, oxidants and antioxidants may serve as potent therapeutic tools. Efforts to determine individual needs of athletes and a balanced diet rich in antioxidant supplements are highly recommended.

The study of exercise physiology and biochemistry has had a revolutionary overall impact on biomedical research. Physical exercise has been used as a model for studying the response of physiological regulatory mechanisms to stress. In exercise physiology, a common approach to measure physical fitness is based on the ability of an individual to utilise atmospheric oxygen in a given interval of time per kilogram of bodyweight, that is, the aerobic capacity. Therefore, athletes aim to boost their aerobic capacity to the highest possible limit. Supply of more and more oxygen to active tissues fuels oxidative metabolism that produces higher amounts of energy rich phosphates compared with anaerobic metabolism, and avoids the formation of lactate during the energy supply process. Physical exer-

cise may be associated with a 10- to 20-fold increase in whole body oxygen uptake.[1] Oxygen flux in the active peripheral skeletal muscle fibres may increase by as much as 100- to 200-fold during exercise.[2] Does this markedly enhanced consumption of oxygen by the tissue at exercise contribute to oxidative stress? This question, first addressed in 1978, resulted in the observation that strenuous physical exercise causes oxidative damage to lipids in various tissues.[3] One of the early studies that kindled strong motivation for further research in the area of exercise and oxygen toxicity was reported by Davies et al.[4] Using electron paramagnetic or spin resonance (EPR or ESR) spectroscopy for the direct detection of free radical species in tissues, it was shown that exhaustive exercise results in a 2- to 3-fold increase in free radical levels in the muscle and liver of rats exercised on a treadmill. Since then a considerable body of research has accumulated showing that strenuous physical exercise may be associated with oxidative stress.[5,6]

During strenuous exercise, the generation of reactive oxygen species (ROS, highly reactive, partially reduced metabolites of oxygen) is elevated to a level that overwhelms tissue antioxidant defence systems.[7,8] The result is oxidative stress.[5,6] The magnitude of the stress depends on the ability of the tissues[9] to detoxify ROS, that is, antioxidant defences. Endurance training enhances such defence in various tissues,[10] especially in skeletal muscle and heart.[5,6,11] In the circulation, lactate[12] and urate[13] generated during exercise seem to have antioxidant properties. Antioxidants produced by the body act in concert with their exogenous (mainly dietary) counterparts to provide protection against the ravages of reactive oxygen as well as nitrogen species.[6,14] Susceptibility to oxidative stress is likely to vary considerably from person to person. Given that oxidative stress represents an imbalance between oxidant insult and antioxidant defences in favour of the former, it is important to note that both determinants, that is, oxidant exposure and antioxidant defence, are highly variable and are not likely to be uniform for any given general population. Variables such as dietary habit, lifestyle (smoking or alcohol consumption, physical activity, exposure to environmental stressors such as UV), age and genetic disposition (determinant of numerous antioxidant protein expression) are key contributors to the oxidant-antioxidant balance.[6,15] Thus, efforts to determine individual antioxidant needs are highly recommended.

The Food and Nutrition Board of the National Institute of Medicine (USA) has formulated the following definition for dietary antioxidants: 'a dietary antioxidant is a substance in foods that significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in humans.' This proposed definition is based on several criteria: (i) the substance is found in human diets; (ii) the content of the substance has been measured in foods commonly consumed; and (iii) in humans, the substance decreases the adverse effects of reactive oxygen and nitrogen species *in vivo*. To meet the definition of a dietary antioxidant proposed here, nutrients and food components must be found in typical human diets. Many substances have been shown to have antioxidant activity *in vitro*. However, *in vitro* findings are of uncertain relevance to the *in vivo* situation in healthy humans. The definition of a dietary antioxidant focuses on antioxidant effects of substances when consumed by humans. Therefore, *dietary* antioxidants are substances that have been shown to decrease the effects of reactive oxygen and nitrogen species in humans.

The chemical nature of any antioxidant determines its solubility, and thus its localisation in biological tissues. For example, lipid soluble antioxidants are localised in membranes and function to protect against oxidative damage of membranes. Water-soluble antioxidants, located for example, in the cytosol, mitochondrial matrix or extracellular fluids, may not have access to ROS generated in membranes. Tocopherol (vitamin E) and retinol (vitamin A), coenzyme Q, carotenoids, flavonoids and polyphenols represent the most extensively studied natural fat-soluble antioxidants. Ascorbic acid (vi-

tamin C), glutathione, uric acid and lipoic acid are the most commonly known water-soluble antioxidants. For a detailed discussion of the properties of these and other antioxidants, the reader is referred to other recent review articles.[6] The objective of this work is to derive the significance of antioxidant strategies to attenuate exercise-induced oxidant challenge in light of recent findings.

## 1. Exercise-Induced Oxidative Stress: What Is at Stake?

### 1.1 Lipids

Multiple unsaturation points in polyunsaturated fatty acids (PUFA) make them highly susceptible to ROS attack and oxidative damage. Uncontrolled and autocatalytic oxidative destruction of PUFA, commonly referred to as lipid peroxidation, is initiated when a ROS having sufficient energy to abstract a H-atom of a methylene ($-CH_2$) group (of the PUFA backbone) reacts with a PUFA.[5] 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, lipid peroxidation is commonly studied to estimate oxidative stress. In 1978, Dillard et al.[3] first reported that in humans, physical exercise at 75% maximal oxygen uptake ($\dot{V}O_{2max}$) 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 individuals. Since then considerable evidence has accumulated showing that strenuous physical exercise may trigger lipid peroxidation in several tissues including skeletal muscles, heart, liver, erythrocytes and plasma.[16]

### 1.2 Protein

Oxidative protein damage is widespread within the body at rest. It has been estimated that, at rest, 0.9% of the total oxygen consumed by a cell contributes to protein oxidation.[17] Most of this damage is irreparable, and by-products of such damage are either stored or degraded. Proteins that have been damaged by ROS are highly susceptible to proteolytic degradation. The amount of oxidised protein in various tissues increases with age.[18] Certain components of protein such as tyrosine, methionine, tryptophan, histidine and sulfhydryl residues are highly susceptible to oxidative damage. Following reactive oxygen attack, amino acid residues are converted to carbonyl derivatives. Alternatively, reducing sugars linked with the ε amino group of Lys residues can be oxidised. As a result, protein carbonyl formation is widely used as an index of oxidative protein damage. Other specific markers of oxidative amino acid modification are dityrosine cross-linking and formation of disulphide bridges (-S-S-) and mixed disulphides in cysteine residues. For example, in dystrophic muscle the protein disulphide **:** sulfhydryl (SS **:** SH) ratio has been observed to be increased, suggesting the possible involvement of oxidative stress.[5] Oxidative modification of proteins may cause receptor modification, disturbance in intracellular ionic homeostasis and altered signal transduction, and may also influence other fundamental cell-regulatory processes. Reznick et al.[19] has reported that exhaustive exercise triggers skeletal muscle protein oxidation in rats. In another study where rats were subjected to exhaustive exercise, Sen and co-workers[20] observed consistent effects of physical exercise on tissue protein oxidation. Protein carbonyl levels in the red gastrocnemius muscle were roughly 3-fold greater in exercised rats. In the vastus lateralis muscle, exercise increased the carbonyl content by 69%. Exhaustive exercise also increased protein oxidation in the liver, but the effect was much less pronounced compared with that in the muscles.[20] In another study, 10 to 15 minutes of swim exercise resulted in oxidation of rat erythrocyte membrane protein. Following exercise, skeletal muscle microsomes contained decreased sulfhydryls and protein cross-linking was extensive.[21] Sen and colleagues[22] observed that in skeletal muscle cells certain mem-

brane $K^+$ transport proteins are highly sensitive to oxidant exposure.

### 1.3 Nucleic Acids

In humans, the number of oxidative hits to the DNA per cell per day has been estimated to be as high as 10 000.[23] Oxidative lesions of DNA accumulate with age. A 2-year-old rat is estimated to have 2 million oxidative DNA lesions per cell, which is about twice that in a young rat. In mammals, oxidative DNA damage appears to be roughly related to the metabolic rate.[23] Such a trend, suggesting a relationship between metabolic rate and oxidative DNA damage, makes it important to study the effect of exercise on oxidative DNA modifications. Information regarding exercise-induced oxidative DNA damage is limited, however. Ten hours after marathon running, the ratio of urinary oxidised nucleosides per creatinine increased 1.3-fold above rest.[24] Neutrophils represent 50 to 60% of the total circulating leucocytes, and Smith et al.[25] 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 leucocyte DNA in humans. Results obtained in our study[26] indicated the possibility that exercise-associated oxidative stress may initiate DNA damage in leucocytes. In another study,[27] no significant increase in the urinary level of the oxidised RNA adduct 8-hydroxyguanosine of young healthy men was observed after 90 minutes of bicycle exercise. In a later study,[28] the single cell gel test or COMET assay was employed to detect exercise-induced DNA damage in human white blood cells with increased sensitivity. Incremental exercise on a treadmill performed by healthy nonsmoking participants caused DNA damage that was detected after 24 hours of exercise.[28] Strenuous exercise for ≈10 hours a day for 30 days also increased the rate of oxidative DNA modification by 33% (95% confidence limits, 3 to 67%; $p < 0.02$) in 20 men. It was suggested that oxidative DNA damage may increase the risk for the development of cancer and premature aging in humans performing strenuous exercise on a regular basis.[29]

### 1.4 Antioxidant Depletion

Another line of evidence that supports the hypothesis that physical exercise may induce oxidative stress is the lowering of tissue levels of antioxidants during exercise. In view of the above-mentioned increases in tissue oxidative stress indices following exercise, such lowering of tissue antioxidant levels in response to physical exercise is thought to be a result of increased antioxidant consumption in oxidative-stress challenged tissues. Several studies have shown that physical exercise decreases tissue levels of tocopherol.[5] It is thought that exercise-induced mobilisation of free fatty acids from the adipose tissues is accompanied by the loss of tocopherols from the tissue. As a result, tocopherol levels increased in human blood following intense cycling. This elevation of tocopherol levels in the circulation is transient and the level returns to baseline values in the early phase of recovery.[30] Treadmill exercise induced decreases in total antioxidant capacity of the blood has also been evident in male patients with claudication.[31]

It has been consistently reported from several laboratories[26,32-35] that physical exercise induces blood glutathione oxidation even at submaximal intensities. This response is relatively rapid and can be observed after only a few minutes of exercise. Given the critical role of glutathione in the antioxidant defence network and other physiological functions,[6,36,37] this effect of exercise on blood glutathione may be expected to have important implications in defending against oxidative damage as well as in influencing redox-sensitive signal transduction processes.[37,38]

### 1.5 Critical Considerations

In contrast to the conventional idea that reactive oxygen is mostly a trigger for oxidative damage of biological structures, we now know that low physiologically relevant levels of ROS can regulate a variety of key molecular mechanisms that may be

linked with important processes such as immune function and angiogenesis. Oxidation-reduction (redox) based regulation of gene expression appears to be a fundamental regulatory mechanism in cell biology.[39] Therefore, it is plausible that oxidants generated during the course of physical exercise, may serve as cellular messenger molecules. It would, therefore, not be far-fetched to hypothesise, for example, that oxidants generated during exercise promote redox-sensitive responses such as vasculogenesis. Research along these lines focusing on redox-regulated signal transduction processes[15,40] during exercise has the potential to define new frontiers in the field. Having outlined the numerous potential damaging consequences of exercising it is important to note that most of the evidence showing exercise induced lipid, protein or DNA damages come as a result of strenuous exercise. The consequence of moderate intensity aerobic exercise seems to be limited to changes in tissue glutathione status. Such changes in thiol redox state in the cell is likely to serve as a modulator of several key signal transduction pathways such as inducible nuclear factor kappa B activation and adhesion molecule expression.[15,38,41]

## 2. Nutritional Manipulations

The 1988 US Surgeon General's report on Nutrition and Health stated that 'for the two out of three adult Americans who do not smoke and do not drink excessively, one personal choice seems to influence long term health prospect more than any other: what we eat'. Epidemiological studies have emphasised the relevance of antioxidants in the prevention of health disorders that may have an oxidative stress related aetiology.[42] It is not only what we eat but also how much we eat that may have marked implications in the management of oxidative stress. For example, dietary restriction is known to effectively strengthen cellular antioxidant defences and protect against oxidative stress.[43]

### 2.1 Dietary Restriction

Dietary restriction delays the loss of several cellular immune functions, retards age-related functional disorders and has been proven to significantly extend life span in laboratory animals.[44] Activities of certain components of the physiological antioxidant defence system are up-regulated during the course of aging, perhaps to cope with age related increased oxidative stress. In the skeletal muscle, activities of catalase and glutathione peroxidase increased progressively and markedly with aging in rats fed *ad libitum*. Dietary restriction clearly suppressed such responses suggesting that the aging tissue may have been exposed to lesser oxidative stress challenge compared with that of rats fed *ad libitum*.[45] In mice, aging has been observed to be associated with marked oxidative protein damage in organs such as the brain, heart and kidney. This adverse effect could be considerably limited when mice were fed with diet 40% lower in energy. Aging increases the resting respiratory rate of mitochondria resulting in increased generation of mitochondrial superoxides and hydrogen peroxide. A protective effect of dietary restriction under such conditions has been also evident.[44] In rats, dietary restriction has been shown to suppress ROS generation in hepatic microsomes. A recent study,[46] utilising the yeast model of dietary restriction, has identified that nicotinamide adenine dinucleotide (NAD)-dependent activation of a Sir2P protein is required to extend life span in diet-restricted yeast.[46]

A synergistic effect of dietary restriction and exercise has been observed to protect mitochondrial membrane fluidity against oxidative damage.[47] Another study[48] investigated the effect of dietary restriction and physically active lifestyle on lipid peroxidation and antioxidant defences of the rat heart. Diet restricted rats were fed 60% of the *ad libitum* level for 18.5 months. Both dietary restriction and a physically active lifestyle decreased lipid peroxidation damage in cardiac mitochondria. Dietary restriction significantly increased the activities of cytosolic antioxidant enzymes such as superoxide dismutase, selenium dependent glutathione perox-

idase and glutathione S-transferase. Thus, it is evident that long term dietary restriction and a physically active lifestyle may alleviate the extent of free radical damage in the heart by strengthening endogenous antioxidant defences.[48]

Food deprivation, on the other hand, may adversely affect liver glutathione reserves and whole body glutathione metabolism. Starvation is followed by reduced glutathione levels in the plasma, lung and skeletal muscles.[49,50] The influences of food deprivation and refeeding on glutathione (GSH) status, antioxidant enzyme activity and lipid peroxidation in response to an acute bout of exercise have been investigated in the liver and skeletal muscles of male rats. Food deprivation depleted tissue glutathione stores and caused increased lipid peroxidation in the liver and skeletal muscles. Leeuwenburgh and Ji[51] showed that both food deprivation-refeeding and exhaustive exercise influence liver and skeletal muscle glutathione status and that these changes may be controlled by hepatic glutathione synthesis and release caused by hormonal stimulation.

### 2.2 Antioxidant Deficiency

The antioxidant deficiency model has been used to test the significance of various antioxidants in exercise induced oxidative stress. Several studies have consistently indicated that tocopherol deficiency can lead to enhanced free radical formation resulting in compromised exercise performance and increased tissue lipid peroxidation.[3,52-57] These studies suggest that inadequate amounts of dietary tocopherol may decrease endurance performance by as much as 40% and lead to enhanced oxidative lipid damage of several tissues.[4,53,58,59] Furthermore, tocopherol deficiency was associated with increased fragility of lysosomal membranes and greater haemolysis of red blood cells.[4,53] Tocopherol deficiency also decreased oxidative phosphorylation[55,58] in skeletal muscle, liver and adipose tissues. However, in female rats, tocopherol deficiency does not appear to influence the ability to run nor does it enhance tissue lipid peroxidation.[60] It has been suggested that female rats may be less susceptible to free radical damage compared with male rats because of higher levels of estrogen, a potential antioxidant, in the circulation.[4,57,61] The effects of an ascorbate-depleting diet on run time were examined in guinea-pigs that do not synthesise ascorbic acid. Run time of ascorbate-deficient guinea-pigs was significantly less than ascorbate-adequate animals.[62]

Dietary selenium deficiency impairs tissue antioxidant defences by markedly down-regulating glutathione peroxidase activity in tissues such as the liver and muscle. This effect on the antioxidant enzyme did not influence endurance to treadmill run, however. This suggests that muscle glutathione peroxidase activity is not a limiting factor in physical performance.[63] Selenium deficiency has also been found to enhance lipid peroxidation in skeletal muscle mitochondria of rats that were exercised for 1 hour.[64] Activity of antioxidant enzymes in both liver and skeletal muscle has been observed to adapt in response to selenium deficiency, suggesting that the organs may have encountered and responded to oxidant challenge.

The role of endogenous GSH in the circumvention of exhaustive exercise-induced oxidative stress has been investigated using GSH-deficient rats. GSH synthesis was inhibited by intraperitoneally administered L-buthionine-sulfoximine (BSO) to produce glutathione deficiency. The BSO treatment resulted in: (i) ≈50% decrease in the total glutathione pools of the liver, lung, blood and plasma; and (ii) 80 to 90% decrease in the total glutathione pools of the skeletal muscle and heart. Compared with controls, glutathione-deficient rats had higher levels of tissue lipid peroxides. Glutathione-deficient rats could run for only about half the interval when compared with the saline injected controls. This observation underscores the critical role of tissue glutathione in the circumvention of exercise-induced oxidative stress and as a determinant of exercise performance.[65] Increased susceptibility to oxidative stress was also observed in muscle-derived cells pretreated with BSO.[66]

Leeuwenburgh and Ji[67] studied the effect of chronic *in vivo* glutathione depletion by BSO on intracellular and inter-organ GSH homeostasis in mice both at rest and after an acute bout of exhaustive swim exercise. BSO treatment for 12 days decreased levels of GSH in the liver, kidney, quadriceps muscle and plasma to 28, 15, 7 and 35%, respectively, compared with GSH-adequate mice. GSH depletion was associated with adaptive changes in the activities of several enzymes related to GSH metabolism. Exhaustive exercise in the GSH-adequate state severely depleted the GSH content of the liver (–55%) and kidney (–35%), whereas plasma and muscle GSH levels remained unchanged. However, exercise in the GSH-depleted state exacerbated the GSH deficit in the liver (–57%), kidney (–33%), plasma (–65%), and muscle (–25%) in the absence of adequate reserves of liver GSH. Hepatic lipid peroxidation increased by 220 and 290%, respectively, after exhaustive exercise in the GSH-adequate as well as GSH-depleted mice. It was concluded that GSH homeostasis is an essential component of the pro-oxidant–antioxidant balance during prolonged physical exercise.

## 2.3 Antioxidant Supplementation

Venditti and Di Meo[68] observed that free radical–induced damage in muscle could be one of the factors terminating muscle effort. They suggested that greater antioxidant levels in the tissue should allow the trained muscle to withstand oxidative processes more effectively, thus lengthening the time required so that the cell function is sufficiently damaged as to make further exercise impossible. Whether oxidative stress is the single most important factor determining muscle performance is certainly a debatable issue. The contention that strengthened antioxidant defence of the muscle may protect against exercise-induced oxidative-stress–dependent muscle damage is much more readily acceptable.[69] Animal experiments studying the effect of tocopherol have shown mixed results on the prevention of lipid peroxidation[16] with the general trend that such supplementation may diminish oxidative tissue damage. Brady and co-workers[70] examined the effects of tocopherol supplementation (50 IU/kg diet) on lipid peroxidation in liver and skeletal muscle at rest and following exhaustive swim exercise. Tocopherol effectively decreased lipid peroxidation in the liver independent of selenium supplementation, whereas skeletal muscle lipid peroxidation response was unaffected by the supplementation. Goldfarb et al.[71] observed that tocopherol supplementation can protect against run-induced lipid peroxidation in the skeletal muscle and blood. The effect in skeletal muscle was muscle fibre type dependent. The protective effect of tocopherol was more clearly evident when the animals were exposed to an additional stressor, prasterone (dehydroepiandrosterone). Prasterone stimulates peroxisomal fatty acid oxidation leading to lipid peroxidation.

### *2.3.1 Tocopherol Supplementation*

Jackson et al.[72] examined the effect of both tocopherol deficiency and supplementation on the contractile activity of muscle. Male rats and female mice were given either a standard diet, a tocopherol-deficient diet with 500 μg/kg selenium or a diet supplemented with 240mg α-tocopherol acetate/kg diet. The animals were given this diet for 42 to 45 days. Tocopherol deficiency, in both mice and rats, was associated with increased susceptibility to contractile damage. Tocopherol supplementation clearly protected against such damage. Despite the fact that tocopherol supplementation protected the muscles from damage as indicated by creatine kinase and lactate dehydrogenase leakage there was no apparent effect on muscle lipid peroxidation. Kumar et al.[73] noted that tocopherol supplementation for 60 days in female adult albino rats completely abolished the increase in free radical-mediated lipid peroxidation in the myocardium as a result of exhaustive endurance exercise. They reported that exercise-induced lipid peroxidation increased in heart tissue in control rats but did not increase in the tocopherol-supplemented rats. It has also been consistently observed that tocopherol supplementation for 5 weeks attenuated exercise induced increases in myocardial lipid peroxidation.[71,74,75]

A tocopherol supplemented diet prevented prasterone-induced increases in peroxisomal fatty acid oxidation and leakage of alanine amino transferase and aspartate amino transferase into the plasma.[76,77] Acute exercised animals fed normal diets demonstrated similar peroxisomal fatty acid oxidation profiles and plasma enzyme levels as the tocopherol-supplemented group. Novelli et al.[78] examined the effects of intramuscular injections of 3 spin-trappers and tocopherol on endurance swimming to exhaustion in mice. Mice were injected on 3 successive days. It was observed that, compared with either the control or placebo saline-injected animals, the spin-trap– and tocopherol–injected groups had significantly increased swim endurance. In a study reported by Quintanilha and Packer,[56] rats were given one of the following 3 diets and compared for liver mitochondrial respiration and lipid peroxidation: a diet deficient in tocopherol, a diet with 40IU tocopherol/kg, or a diet with 400IU tocopherol/kg. Hepatic mitochondrial function was best in the group supplemented with 400 IU/kg. Additionally, liver lipid peroxidation in nuclei and microsomes was lowest in the tocopherol-supplemented group, especially when reduced nicotinamide adenine dinucleotide phosphate (NADPH) was present. Warren et al.[79] studied the effects of tocopherol supplementation (10 000 IU/kg diet for 5 weeks), on muscle damage and free radical damage to membranes as indicated by alterations in plasma enzymes. Susceptibility of the skeletal muscles to oxidative stress was markedly decreased in response to tocopherol supplementation but this did not attenuate muscle injury triggered by eccentric contractions. It was concluded that tocopherol supplementation may be beneficial in protecting against free radical damage, but that the hepatic injury caused by eccentric exercise may not be ROS-mediated.

The effect of dietary tocopherol on exercise-induced oxidative protein damage has been investigated in the skeletal muscle of rats. For a period of 4 weeks, rats were fed with a high tocopherol diet (10 000 IU/kg diet), a α-tocopherol and tocotrienol-rich (7000mg tocotrienol/kg diet) palm oil diet or a control diet with basal levels of α-tocopherol (30 IU/kg bodyweight). Uphill exhaustive treadmill exercise caused oxidative protein damage in skeletal muscles. A protective effect of tocopherol supplementation against exercise-induced protein oxidation in skeletal muscles was clearly evident.[19] While tocopherol supplementation protects against oxidative damage, it does not influence exercise-induced expression of nitric oxide synthase, heme oxygenase or cytokines.[80]

#### 2.3.2 Fish Oil and Tocopherol Supplementation

Fish oils have been shown to have a beneficial effect on cardiovascular mortality based on numerous epidemiological studies,[81] presumably *via* effects on triglyceride levels, membrane fluidity and on platelet and leucocyte function.[82] Not all studies show beneficial effects, however.[83] Because the (n-3) fatty acids making up fish oil are highly polyunsaturated, concerns have been raised regarding increased oxidative stress from fish oil intake.[84-88] Furthermore, fish oils induce peroxisomal β-oxidation, in which fatty-acyl oxidation yields hydrogen peroxide ($H_2O_2$) as a normal by-product, and up-regulates the activity of the $H_2O_2$ decomposing enzyme catalase.[84,85,89] Under normal conditions up to 20% of cellular oxygen consumption has in fact been estimated to occur in the peroxisome.[90] The beneficial effects of regular exercise on cardiovascular and overall mortality[91] may be decreased by exercise-induced oxidative stress. This may be particularly concerning in groups predisposed to oxidative stress, including that induced by fish oil.[86-88] Our laboratory assessed the effect of fish oil and tocopherol supplementation, compared with placebo soy oil and tocopherol supplementation, on physiological antioxidant defences and resting and exercise-induced oxidative stress in rat liver, heart and skeletal muscle. The effects of 8 week tocopherol and fish oil supplementation on resting and exercise-induced oxidative stress were examined. Lipid peroxidation was indeed 33% higher in fish oil fed rats compared with the placebo group in the liver, but oxidative protein damage remained similar in both liver and red gastrocnemius muscle.

Tocopherol supplementation markedly decreased liver and muscle lipid peroxidation induced by the fish oil diet. Tocopherol supplementation also markedly decreased oxidative protein damage in the liver and red gastrocnemius muscle. Exhaustive treadmill exercise increased liver and muscle lipid peroxidation, and muscle oxidative protein damage. Tocopherol effectively decreased exercise-induced lipid peroxidation and protein oxidation.[20]

A separate report documented another aspect of this same study testing the effect of fish oil and tocopherol supplementation on tissue antioxidant defences.[92] Fish oil supplementation increased the activity of catalase, glutathione peroxidase, and glutathione-S-transferase in the liver and red gastrocnemius muscle. This could be viewed as adaptive responses to fish oil-induced oxidant insult. Consistent with results showing fish oil-induced lipid peroxidation, hepatic total glutathione levels were lower in fish oil fed rats. Part of these effects of fish oil on tissue antioxidants were negated by tocopherol supplementation. Consistent with the concept of antioxidant network,[36] tocopherol supplementation increased total glutathione levels in tissues such as the liver, heart and skeletal muscle. In this study,[92] regression analysis showed tissue total glutathione levels to be a negative determinant of protein oxidative damage as measured by carbonyl levels. Sparing of glutathione appeared to be an important mechanism by which tocopherol decreased tissue protein oxidative damage caused by a fish oil diet and exercise.[92]

#### *2.3.3 Studies in Humans*

A limited number of studies have examined the effect of tocopherol supplementation in humans. In this context it should be noted, however, that exercise performance and physical fitness have multifactorial determinants and may not serve as reasonable end-points to test the efficacy of antioxidant supplementation. Tocopherol supplementation (900 IU/day for 6 months) in trained swimmers did not alter their swim performance nor their lactate response in plasma.[93] Furthermore, tocopherol supplementation (800 IU/day for 4 weeks) did not alter the work load needed to run at 80% maximal oxygen uptake ($\dot{V}O_{2max}$) in trained and untrained males.[94] Volunteers administered 400 IU/day of tocopherol for 6 weeks demonstrated no influence on cycle time, swim time, or step time.[95] Additionally, no changes in $\dot{V}O_{2max}$, a marker of physical fitness, were noted in humans following tocopherol supplementation.[94,96,97] However, Cannon et al. [98] reported that 400 IU/day of tocopherol supplementation for 48 days attenuated the amount of creatine kinase leakage from muscles during recovery from a down-hill run. Sumida et al.[96] examined the effects of 4 weeks of tocopherol supplementation in 21 healthy college-aged males. The participants ingested 300mg of tocopherol daily and blood levels of several enzymes and lipid peroxides were determined before and for up to 3 hours after cycling exercise to exhaustion. Exercise increased the level of lipid peroxidation by-products in plasma immediately after the cycling and recovered to baseline values at 1 and 3 hours of recovery. Tocopherol supplementation significantly decreased the baseline resting level of lipid peroxides in the blood plasma.

Meydani et al.[99] reported that urinary excretion of lipid peroxidation by-products tended to be lower in tocopherol-supplemented individuals (400IU doses, twice daily, for 48 days) compared with the corresponding placebo group, but this effect was only significant 12 days after downhill running. The participants ran at a 16% down-hill inclination at 75% of their maximum heart rate for three 15-minute periods. Skeletal muscle biopsies were obtained from the vastus lateralis of young participants. It was observed that exercise increased the level of lipid peroxidation by-products in the muscle of the placebo group whereas in the muscle of the tocopherol-supplemented group no such oxidative lipid damage was evident. Another study examined the effect of tocopherol supplementation (800 IU/day for 4 weeks) and compared this with a placebo treatment in the same individuals at a specific exercise intensity.[94] Participants were randomly assigned to either a placebo or tocopherol-

treatment group in a counter-balanced design. Participants were exercised for 30 minutes at 80% $\dot{V}O_{2max}$ and blood was collected before and after the run. Tocopherol treatment attenuated the level of resting plasma lipid peroxidation by-products and also protected against the exercise response. The effects of 5 months of α-tocopherol supplementation have been studied in 30 top-class cyclists. Although the supplementation protocol did not improve physical performance, it was evident that exercise-induced muscle damage was less in response to antioxidant supplementation.[100]

In 1980, the US recommended that the daily allowance of tocopherol should be reduced from 30IU (recommended in 1968) to 15IU. In the same year, it was estimated that in the US, the amount of tocopherol supplied by a 'normal' diet is about 11IU (7.4mg). Packer and Reznick[101] have observed that such dosages are insufficient for active athletes and that dosages of up to 400 IU/day may be a reasonable recommendation for active athletes engaged in moderate to heavy exercise. Tocopherol is proven to be well tolerated at levels of intake of up to 3000mg for prolonged periods of time.[102] However, individuals taking anticoagulants should refrain from taking very high doses (>4000IU) of tocopherol because it can act synergistically with this class of drug.[103]

#### 2.3.4 Ascorbic Acid Supplementation

Although ascorbic acid is expected to protect against exercise-induced oxidative stress,[104] studies testing the effects of ascorbic acid alone are not as convincing as those reporting the effects of tocopherol. In designing experiments testing the effects of ascorbic acid supplementation it is important to note that plasma ascorbic acid attained on a given dose depends on bodyweight (or dose per kilogram of bodyweight) and on whether or not any prior depletions have been repleted adequately.[105] Ascorbic acid supplements (3 g/kg diet) given to rats who were placed on a tocopherol-deficient diet did not alter the run time to exhaustion in the tocopherol-deficient animals.[53] In rats, dietary ascorbic acid was unable to counter the deleterious effects of tocopherol deficiency. In a preliminary report, the effect of ascorbic acid supplementation in humans was documented. A mild protective effect of ascorbic acid supplementation, based on elevated total antioxidant capacity of the plasma, has been suggested.[106] Exercise-induced rapid depletion of plasma ascorbic acid levels was recently noted in professional basketball players. Exercise-induced depletion of plasma ascorbic acid levels and oxidative stress was effectively checked by supplementation with an antioxidant cocktail containing 600mg α-tocopherol, 1000mg ascorbic acid and 32mg β-carotene.[107] Ashton et al.[108] examined the effect of short term ascorbic acid supplementation on exercise-induced free radical production in healthy individuals. Using ESR spectroscopy as well as markers of lipid peroxidation, it was observed that exercise-induced oxidative stress was circumvented by ascorbic acid supplementation in humans.[108]

#### 2.3.5 Other Supplementation

Other nutrients ascribed to be beneficial as antioxidants such as selenium and β-carotene have not been examined individually but have been assessed in conjunction with either tocopherol deficiency or in combination with other antioxidants. The effects of selenium supplementation (0.5 ppm diet) or deprivation have been tested in the liver, muscle and blood of swim-exercised rats.[70] Some rats were additionally supplemented with tocopherol (50 IU/kg). Selenium supplementation increased the activity of the hydroperoxide metabolising enzyme glutathione peroxidase in the liver. A tight regulation of tissue glutathione peroxidase activity by dietary selenium was observed because a selenium-deficient diet markedly down-regulated the activity of the enzyme. Muscle glutathione peroxidase activity demonstrated similar responses to selenium intervention compared with the liver. Increased tissue lipid peroxidation was evident when both selenium and tocopherol were deficient. However, selenium deficiency had little effect when tocopherol was present. Selenium appeared to have minimal effects on swim-induced lipid peroxidation in the liver or muscle. Dietary selenium supplementation

in horses (0.15 ppm daily for 4 weeks) had minimal effects on exercise-induced lipid peroxidation as indicated by blood levels of lipid peroxidation by-products.[109] In a double-blind human study, no effect of selenium supplementation on human physical performance was observed.[35] Selenium poisoning is rare in the US, but the case of a man who was poisoned by selenium-containing vitamin tablets has been described.[110]

A few studies have examined the effects of coenzyme $Q_{10}$ to determine if additional amounts of this factor in the electron transport chain would be beneficial in preventing free radical damage.[111-113] Dietary coenzyme $Q_{10}$ supplementation protected against leakage of creatine kinase and lactate dehydrogenase from the muscles to serum following a downhill run.[111] In 2 human studies, however, this beneficial effect of coenzyme $Q_{10}$ could not be observed.[112,113] The effects of ubidecarenone (ubiquinone) supplementation (120 mg/day for 6 weeks) on aerobic capacity and lipid peroxidation during exercise has been investigated in 11 younger (aged 22 to 38 years) and 8 older (aged 60 to 74 years) trained men. This cross-over study was double-blind and placebo-controlled. Ubidecarenone supplementation effectively increased serum concentrations of the supplement in both age groups but did not influence maximal aerobic capacity. Consistent with previous reports, oral ubidecarenone supplementation was ineffective as an ergogenic aid in both the younger and older trained men.[114] A more recent study weakly suggested that antioxidant activity of coenzyme $Q_{10}$ may be responsible for a limited improvement of tolerance to higher workloads in humans.[115]

Endogenous thiol antioxidants are highly sensitive to exercise-induced consumption[26,32,116] and nutritional thiol antioxidants have remarkable potential in sports nutrition.[36,37] Two brief rodent studies have shown that exogenous GSH may markedly increase endurance to physical exercise.[117,118] Compared with controls treated with placebo, intraperitoneal doses of GSH 0.5, 0.75 and 1 g/kg increased endurance to swimming by a marked 102.4, 120 and 140.7%, respectively.[118] At a dose 0.25 g/kg, GSH did not affect endurance when injected once but such a dose could significantly increase endurance when injected once a day for 7 consecutive days. In another study, oral GSH at doses of 0.25 to 1 g/kg caused a dose-dependent significant improvement in swim endurance.[117] Both above-mentioned studies employed brief bursts of swimming as the exercise challenge and did not report any biochemical data related to either glutathione metabolism or other indices of oxidative stress.

Our laboratory sought to determine the possible mechanisms underlying the beneficial effects of GSH supplementation on work performance.[65] Almost all evidence supporting the finding that a single bout of exercise induces oxidative stress has been obtained from studies using exercise types that were long in duration, and mostly running or cycling in nature. Because we aimed to test the efficacy of exogenous GSH in controlling exercise-induced oxidative stress, an enduring (≈2 hours) treadmill run protocol was used. Intraperitoneal injection of GSH solution (1 g/kg bodyweight) to rats resulted in a rapid appearance of GSH in the plasma and was followed by a rapid clearance of the thiol. Following the injection, excess plasma GSH was rapidly oxidised. GSH injection did not influence the GSH status of other tissues studied. Following repeated administration of GSH, blood and kidney total glutathione levels were increased. The plasma total glutathione levels of GSH-supplemented animals were rapidly cleared during exhaustive exercise. The GSH administration protocol, as used in this study,[65] did not influence endurance to exhaustive physical exercise. In a previous study, Sen et al.[119] observed that treadmill run to exhaustion was associated with a remarkable increase in immunoreactive manganese superoxide dismutase (Mn-SOD, a mitochondrial protein) in the plasma. Glutathione supplementation (500 mg/kg bodyweight) marginally suppressed release of the mitochondrial protein to the plasma.[119]

The inability of exogenous GSH to provide added antioxidant protection to tissues may be largely at-

tributed to the poor availability of exogenous GSH to the tissues. Atalay et al.[120] tested the effect of GSH supplementation on exercise-induced leucocyte margination and neutrophil oxidative burst activity. Exercise-associated leucocyte margination was prevented by GSH supplementation. Peripheral blood neutrophil counts were significantly higher in GSH-supplemented groups compared with the placebo control groups. In addition, exercise induced increases in peripheral blood neutrophil oxidative burst activity (as measured by luminol-enhanced chemiluminescence per volume of blood), tended to be higher in the GSH-supplemented group, and lower in the GSH-deficient rats suggesting that high plasma GSH may have augmented exercise dependent neutrophil priming. In these experiments, for the first time it was shown that GSH supplementation can induce neutrophil mobilisation and decrease exercise-induced leucocyte margination, and that exogenous and endogenous GSH can regulate exercise-induced priming of neutrophils for oxidative burst response.[120] In another human study, the effect of oral N-acetyl-L-cysteine (NAC) on exercise-associated rapid blood GSH oxidation in healthy adult males who performed 2 identical maximal bicycle ergometer exercises 3 weeks apart was investigated. Before the second maximal exercise test, men took effervescent NAC tablets (200mg 4 times per day) for 2 days, and an additional 800mg on the test morning. The NAC supplementation protocol used in the study: (i) increased the net peroxyl radical scavenging capacity of the plasma; and (ii) spared exercise-induced blood glutathione oxidation.[26]

While ROS are thought to regulate numerous physiological aspects of skeletal muscle contractility, under certain conditions oxidants may limit muscle function.[121-123] Khawli and Reid[124] have shown that antioxidant enzymes are able to depress contractility of unfatigued diaphragm fibre bundles and inhibit development of acute fatigue. NAC has been tested for similar effects. Fibre bundles were removed from diaphragms and stimulated directly using supramaximal current intensity. Studies of unfatigued muscle showed that 10 mmol/L NAC reduced peak twitch stress, shortened time to peak twitch stress, and shifted the stress-frequency curve down and to the right. Fibre bundles incubated in 0.1 to 10 mmol/L NAC exhibited a dose-dependent decrease in relative stresses developed during 30Hz contraction with no change in maximal tetanic (200Hz) stress. NAC (10 mmol/L) also inhibited acute fatigue. In a later experiment by Reid and colleagues[125] this effect of NAC was tested in humans. Healthy volunteers were studied on 2 occasions each. Participants were pretreated with NAC 150 mg/kg or 5% dextrose in water by intravenous infusion. During fatiguing contractions stimulated at 10Hz, NAC increased force output by ≈15%, an effect that was evident after 3 minutes of repetitive contraction and persisted throughout the 30-minute protocol. Thus, it was evident that NAC pretreatment can improve performance of human limb muscles during fatiguing exercise, suggesting that oxidative stress plays a causal role in the fatigue process and identifying antioxidant therapy as a novel intervention that may be useful clinically.[124,125]

α-Lipoic acid boosts cellular GSH levels[34] as well as stimulates glucose uptake by skeletal muscle cells.[126] Khanna et al.[127] studied the effect of intragastric lipoate supplementation (150 mg/kg bodyweight for 8 weeks) on lipid peroxidation and glutathione dependent antioxidant defences in the liver, heart, kidney and skeletal muscle of male Wistar rats. Lipoate supplementation significantly increased total glutathione levels in the liver and blood. This finding is consistent with *in vitro* results,[128] and shows that lipoate supplementation may increase glutathione levels of certain tissues *in vivo*. Lipoate supplementation, however, did not affect the total glutathione content of organs such as the kidney, heart and skeletal muscles. Lipoate supplementation-dependent increases in the hepatic glutathione pool were associated with increased resistance to lipid peroxidation. This beneficial effect against oxidative lipid damage was also observed in the heart and red gastrocnemius skeletal muscle. Lower lipid peroxide levels in certain tissues of lipoate fed rats suggest a strengthening of the antioxidant network

defence in these tissues.[127] Recently, a modified form of α-lipoic acid, positively charged α-lipoic acid, that is more effectively reduced to dihydrolipoic acid and more efficiently retained by human cells has been reported.[129] *In vivo* studies are yet to be performed with this novel product.

#### *2.3.6 Antioxidant Combinations*

From the biochemistry of antioxidant action it is evident that antioxidants function in a network and interactions between several major antioxidants have been experimentally demonstrated.[36,37] As a result, some studies have attempted to investigate the efficacy of a combination of several antioxidants as supplements.[130-132] Supplementation of individuals with a vitamin mixture containing 37.5mg β-carotene, 1250mg ascorbic acid and 1000IU of tocopherol for 5 weeks decreased the level of lipid peroxidation by-products in the serum and breath, both at rest and following exercise at both 60 and 90% $\dot{V}O_{2max}$.[131] In contrast, a previous study which used a similar mixture of antioxidants and exercised the participants at 65% of maximal heart rate in a downhill run was unable to demonstrate any positive effects.[130] This inconsistency in observation was explained by differences in the nature and intensity of the exercise in the 2 studies. The effects of an antioxidant mixture (10mg β-carotene, 1000mg ascorbic acid and 800IU of tocopherol) on the human blood glutathione system and muscle damage has been investigated.[132] A protective effect on the blood glutathione system and muscle damage was evident. A randomised and placebo-controlled study was carried out on 24 trained long-distance runners who were supplemented with α-tocopherol (400 IU/day ) and ascorbic acid (200 mg/day) for 4.5 weeks before a marathon race. Serum levels of ascorbic acid as well as α-tocopherol were elevated in supplemented individuals. In this study,[133] the antioxidant supplementation protocol was observed to significantly protect against exercise-induced muscle damage as manifested by the loss of creatine kinase from the muscle to the serum. Beneficial effects of a mixture of tocopherol, ascorbic acid and β-carotene were also observed in a recent study of professional basketball players involved in habitual training.[107]

## 3. Conclusion and Implications

Several lines of evidence consistently support the concept that strenuous physical exercise may induce oxidative stress. The relationship between physical activity, physical fitness and total radical trapping antioxidant potential was examined in the Northern Ireland Health and Activity Survey.[134] This was a large cross-sectional population study (n = 1600) using a 2-stage probability sample of the population. A necessity for antioxidant supplementation, especially in physically active and fit individuals, was indicated. Depending on nutritional habits, lifestyle and genetic disposition susceptibility to oxidative stress may vary considerably from individual to individual. Determination of the tissue antioxidant status of individuals is thus recommended. Such information will be necessary to identify specific requirements and formulate effective antioxidant therapy strategies. Nutritional antioxidant supplements are known to be bioavailable to tissues and may strengthen defence systems. Results from antioxidant supplementation studies vary considerably depending on the study design and measures of outcome. Physical performance is regulated by multi-factorial processes and may not serve as a good indicator to test the effect of antioxidant supplementation. The general trend of results shows no effect of antioxidant supplementation on physical performance. However, in a large number of studies it has been consistently demonstrated that antioxidant supplementation protects against exercise-induced tissue damage.

The diets of laboratory animals are often heavily enriched with antioxidant vitamins, particularly tocopherol. This may be one reason why antioxidant supplementation to animals fed regular diets does not influence several measures of outcome. At present there is a growing trend among people to avoid fat-containing diets. While this markedly decreases caloric intake, in many cases, it may also contribute to marked decreases in the intake of fat

Insert table 1 here

soluble essential nutrients including vitamins. On the other hand, it is of importance to note that consumption of mega-doses of antioxidant supplements may pose significant risks under certain conditions (table I). Generally, antioxidants such as α-tocopherol, ascorbic acid and β-carotene are well tolerated and free from toxicity even when consumed at doses several-fold higher than the recommended dietary allowances.[136] In view of this and the tremendous potential of antioxidant therapy, the consumption of a diet rich in a mixture of different antioxidants may be expected to be a prudent course of action.[137]

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**Table I.** Recommended dietary allowances and adequate intakes of tocopherol, ascorbic acid and selenium. Values are presented in mg/day[a,b,c]

| Antioxidants | Infants | | Children | | Male | | Female | | Pregnancy | Lactation |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0-6mo | 7-12mo | 1-3y | 4-8y | 9-13y | 14+y | 9-13y | 14+y | | |
| Tocopherol[b] | 4[d] | 6[d] | 6 | 7 | 11 | 15 | 11 | 15 | 15 | 19 |
| Ascorbic acid[e] | 40[d] | 50[d] | 15 | 25 | 45 | 75 (14-18y)<br>90 (18+y) | 45 | 65 (14-18y)<br>75 (18+y) | 80 (≤18y)<br>85 (18+y) | 115 (≤18y)<br>120 (18+y) |
| Selenium[f] | 0.015[d] | 0.02d | 0.02 | 0.03 | 0.04 | 0.055 | 0.04 | 0.055 | 0.06 | 0.07 |

a Reprinted with permission from *Dietary Reference Intakes for Dietary Antioxidants and Related Compounds*.[135] Copyright 2000 by the National Academy of Sciences. Courtesy of the National Academy Press. Washington, D.C.

b 15mg of tocopherol corresponds to 22IU. The upper level, based only on intake from vitamin supplements, is 1000mg of α-tocopherol per day for adults. This amount is equivalent to roughly 1500IU of 'd-α-tocopherol,' sometimes labelled as 'natural source' tocopherol, or 1100IU of 'dl-α-tocopherol,' a synthetic version of tocopherol. People who consume more than this amount place themselves at greater risk of haemorrhagic damage because the nutrient can act as an anticoagulant.

c β-carotene and other carotenoids: In laboratory tests, these nutrients have been shown to act as antioxidants, but the results have not been consistently duplicated in humans. In addition, data on the adverse effects of carotenoid overconsumption are contradictory. For these reasons, the report does not recommend a daily intake level or an upper intake level for consumption of carotenoids. It is noted that people should use caution before taking them in high doses; the report recommends β-carotene supplementation only for the prevention and control of retinol (vitamin A) deficiency. The report does not present any specific guidelines regarding the use of thiol antioxidants.

d Adequate intake values. All others are recommended dietary allowance values.

e Because smokers are more likely to experience oxidative damage, the report[135] states that they need an additional 35mg of ascorbic acid per day. No specific recommendations have been made for individuals engaged in strenuous competitive sports. The report sets the upper intake level for ascorbic acid, from both food and supplements, at 2000 mg/day for adults. Intakes above this amount may cause diarrhoea.

f The report[135] also set the upper intake level for selenium at 400 μg/day. The level is based on nutrients from all sources. More than this amount could cause selenosis, a toxic reaction marked by hair loss and nail sloughing.

**mo** = months; **y** = years.

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