

# Antioxidant Supplementation during Exercise Training Beneficial or Detrimental?

*Tina-Tinkara Peternelj and Jeff S. Coombes*

School of Human Movement Studies, The University of Queensland, Brisbane, QLD, Australia

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## Abstract

High levels of reactive oxygen species (ROS) produced in skeletal muscle during exercise have been associated with muscle damage and impaired muscle function. Supporting endogenous defence systems with additional oral doses of antioxidants has received much attention as a noninvasive strategy to prevent or reduce oxidative stress, decrease muscle damage and improve exercise performance. Over 150 articles have been published on this topic, with almost all of these being small-scale, low-quality studies. The consistent finding is that antioxidant supplementation attenuates exercise-induced

oxidative stress. However, any physiological implications of this have yet to be consistently demonstrated, with most studies reporting no effects on exercise-induced muscle damage and performance. Moreover, a growing body of evidence indicates detrimental effects of antioxidant supplementation on the health and performance benefits of exercise training. Indeed, although ROS are associated with harmful biological events, they are also essential to the development and optimal function of every cell. The aim of this review is to present and discuss 23 studies that have shown that antioxidant supplementation interferes with exercise training-induced adaptations. The main findings of these studies are that, in certain situations, loading the cell with high doses of antioxidants leads to a blunting of the positive effects of exercise training and interferes with important ROS-mediated physiological processes, such as vasodilation and insulin signalling. More research is needed to produce evidence-based guidelines regarding the use of antioxidant supplementation during exercise training. We recommend that an adequate intake of vitamins and minerals through a varied and balanced diet remains the best approach to maintain the optimal antioxidant status in exercising individuals.

## 1. Introduction

Antioxidant supplementation is a common practice amongst both professional athletes and amateur sportspersons, and the market offering various nutrient supplements is immense.<sup>[1]</sup> Although these products have been touted as a means of preventing exercise-induced oxidative damage and enhancing performance, consistent evidence of their efficacy is lacking. Moreover, it is clear that reactive oxygen species (ROS) produced during exercise play important roles in various cellular processes and, therefore, suppressing their formation with high doses of antioxidants might have a deleterious impact on cell function.

The studies included in the review were identified by a systematic search using the PubMed database. Search terms were 'reactive oxygen species', 'oxidative stress', 'antioxidant', 'exercise', 'skeletal muscle', 'muscle damage' and 'performance'. Further searching was performed by using the 'related citations' function of PubMed and scanning of the reference lists. We located over 150 studies investigating the effects of antioxidant supplementation on exercise-induced oxidative stress, muscle damage, recovery and performance. A number of excellent reviews are already available that contain a greater discussion of these studies.<sup>[2-11]</sup> In addition,

more detail on the effects of antioxidant therapy in human disease was beyond the scope of this review and can be found elsewhere.<sup>[12-17]</sup> The aim of this review is to discuss the studies that have shown negative effects of antioxidant supplements in exercising individuals, thus demonstrating the importance of ROS in skeletal muscle function.

## 2. Basic Mechanisms of Oxidative Damage

### 2.1 Redox Reactions

Reactions of oxidation and reduction, known as redox reactions, refer to all chemical reactions in which an atom in a compound has its oxidation number changed. The oxidation number is the effective charge that the central atom in a compound would have if all the ligands, including shared electron pairs, were removed. Oxidation can be explained as the loss of electrons, or more accurately, an increase of the oxidation number. Reduction is the gain of electrons or a decrease of the oxidation number. An oxidant is a compound that can accept electrons and is therefore reduced causing another substance to be oxidized. A reductant, on the other hand, donates electrons and is oxidized causing another substance to be reduced. Oxidation and reduction, which represent

the basis for numerous biochemical pathways, always accompany one another in order to transfer electrons between species. In a biological environment, oxidants and reductants are often called pro-oxidants and antioxidants, respectively. A cell's redox state describes the pro-oxidant/antioxidant balance and plays an important role in signalling and metabolic processes.<sup>[18,19]</sup>

While oxygen is obviously vital for the life of aerobic organisms, the by-products of its metabolism can be harmful to cells. During normal metabolism, oxygen is utilized in the mitochondria for energy production. In the process of oxidative phosphorylation the majority of oxygen consumed is bound to hydrogen to form water. A small percentage of oxygen is not completely reduced, which leads to the production of oxygen intermediates known as ROS.<sup>[8]</sup> When reactants are derived from nitrogen, they are called reactive nitrogen species. Reactive species can be classified into two categories: free radicals and nonradical derivatives. A radical is any chemical compound capable of independent existence possessing one or more unpaired electrons in the outer-atomic or molecular orbital. These species have an enhanced affinity to donate or obtain another electron to become more stable, which leads to the formation of new free radicals, setting up a chain reaction. The free radical group includes compounds such as the superoxide anion radical ( $O_2\bullet^-$ ), nitric oxide radical ( $NO\bullet$ ), nitric dioxide radical ( $NO_2\bullet$ ), hydroxyl radical ( $OH\bullet$ ), alkoxy ( $RO\bullet$ ) and peroxy ( $RO_2\bullet$ ) radicals. Most typical nonradical reactive species relevant to biological systems are singlet oxygen ( $^1O_2$ ), ozone ( $O_3$ ), hydrogen peroxide ( $H_2O_2$ ), peroxyxynitrite ( $ONNO_2^-$ ), hypochlorous acid ( $HOCl$ ), organic peroxides and aldehydes. Reactive species readily react with various organic substrates and play important roles in biological environments.<sup>[20]</sup>

Cells and extracellular spaces are exposed to a large variety of reactive species from both exogenous and endogenous sources. The exogenous sources include exposure to oxygen, radiation, air pollutants, xenobiotics, drugs, alcohol, heavy metals, bacteria, viruses, sunlight, food and exercise. Nonetheless, exposure to endogenous sources is much more important and extensive,

because it is a continuous process during the life span. Reactive species are generated by all aerobic cells as part of normal metabolism. Mitochondria have been known as the dominant source of ROS production.<sup>[18]</sup> However, it has been suggested that the actual fraction of oxygen transformed into ROS accounts for only around 0.15% of total oxygen consumption ( $\dot{V}O_2$ ),<sup>[21]</sup> which is considerably less than original estimate of 2–5%.<sup>[22,23]</sup> Enzymes, such as nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), nitric oxide synthase (NOS) and xanthine oxidase (XO), are now recognized as the main endogenous source of reactive species.<sup>[24]</sup> Furthermore, transition metals have been shown to catalyze ROS formation<sup>[25]</sup> and in order to combat bacteria and other invaders white blood cells also produce a significant amount of reactive species.<sup>[26]</sup>

The most vulnerable targets of reactive species are proteins, lipids and DNA.<sup>[27]</sup> ROS can oxidize proteins and alter their structure, impair their function and affect genetic transcription.<sup>[28,29]</sup> Fragmentation or loss of certain amino acids and aggregation make proteins more susceptible to proteolytic degradation.<sup>[30]</sup> Reactive species have the ability to oxidize polyunsaturated free fatty acids and initiate lipoprotein oxidation.<sup>[31]</sup> Disruption of the lipid bilayer changes fluidity and permeability of the cell membrane and may lead to inactivity of membrane bound proteins. Free radicals cause DNA strand breaks, loss of purines and damage to deoxyribose sugar.<sup>[32]</sup> They can impair the DNA repair system and provoke mutagenesis. Oxidative damage promotes inflammation<sup>[33]</sup> and apoptosis<sup>[34]</sup> and may eventually lead to decreased cellular and physiological functioning.

## 2.2 The Antioxidant Defence

To counter reactive species, we are equipped with highly effective antioxidant defence systems. These include nonenzymatic, enzymatic and dietary antioxidants. Glutathione, uric acid, lipoic acid, bilirubin and coenzyme  $Q_{10}$  are examples of nonenzymatic antioxidants that originate from endogenous sources and are often by-products of

cellular metabolism. Principal enzymatic antioxidants are superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX) and glutathione reductase, while most known examples of dietary antioxidants are tocopherols (vitamin E), ascorbic acid (vitamin C) and carotenoids ( $\beta$ -carotene). In addition, various polyphenolic compounds have recently been promoted as nutrient antioxidants.  $\alpha$ -Lipoic acid and pharmaceuticals *N*-acetylcysteine and allopurinol have also been used in supplementation studies.

Vitamin E refers to a group of fat-soluble compounds that include tocopherols and tocotrienols.  $\alpha$ -Tocopherol is the most biologically active form, and has been shown to protect the cells from lipid peroxidation<sup>[35,36]</sup> and play a role in prevention of chronic diseases associated with oxidative stress.<sup>[37,38]</sup> The oxidized form can be recycled back to the active form by other antioxidants, such as vitamin C, retinol, ubiquinol, glutathione, cysteine and  $\alpha$ -lipoic acid.<sup>[39]</sup> It has been suggested that vitamin E has other functions apart from its antioxidative one. For instance,  $\gamma$ -tocopherol acts as a nucleophile and is able to trap electrophilic mutagens in lipophilic compartments.<sup>[40]</sup>

Vitamin C or L-ascorbic acid is an antioxidant and a co-factor in a range of essential metabolic reactions in humans (e.g. collagen synthesis).<sup>[41]</sup> This water-soluble vitamin is produced endogenously by almost all organisms, excluding humans, several other mammalian groups and some species of birds and fish. L-ascorbate, an ion form of ascorbic acid, is a strong reducing agent and its oxidized form is reduced back by enzymes and glutathione.

$\beta$ -Carotene belongs to a group of red, orange and yellow pigments called carotenoids.<sup>[42]</sup> Others include  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, lycopene, lutein and zeaxanthin. These fat-soluble substances are found in plants and play a part in photosynthesis.  $\beta$ -Carotene is the most active carotenoid; after consumption it converts to retinol, a readily usable form of vitamin A. In addition to its provitamin A function,  $\beta$ -carotene is believed to have antioxidant properties,<sup>[43]</sup> and may positively impact the immune system<sup>[44]</sup> and exhibit anticarcinogenic effects.<sup>[37]</sup>

Coenzyme Q<sub>10</sub>, also known as ubiquinone, is a fat-soluble, vitamin-like substance, present in most eukaryotic cells, primarily in mitochondria.<sup>[45]</sup> It is a component of the electron transport chain and plays a part in the energy production of a cell. Its reduced form, ubiquinol, acts as an important antioxidant in the body. Coenzyme Q<sub>10</sub> is synthesized endogenously, and its dietary uptake is limited.

Polyphenols are a group of water-soluble, plant-derived substances, characterized by the presence of more than one phenolic group.<sup>[46]</sup> Several thousand polyphenols have been identified and they are divided into different groups according to their structure and complexity (flavonoids, lignans, stilbenes, coumarins and tannins). Flavonoids are the largest group of phenolic compounds and include anthocyanins, flavones, isoflavones, flavonols, flavanones and flavanols. Fruits and vegetables are a particularly rich source of polyphenols. For instance, red wine contains various polyphenolic compounds, such as stilbene resveratrol and flavonol quercetin, which have been well studied and have been shown to possess pharmacological properties in the treatment of chronic diseases.<sup>[47,48]</sup> The antioxidant potential of polyphenols has been well established and is exhibited through their chain-breaking and single-electron transfer abilities. However, there is compelling evidence that the protective actions of polyphenols are not simply because of their redox properties, but rather as a result of their ability to modulate cellular signalling cascades by binding to specific target proteins.<sup>[46]</sup>

$\alpha$ -Lipoic acid is an organosulfur compound derived from octanoic acid. It is an essential co-factor of the four mitochondrial enzyme complexes, therefore, is crucially involved in aerobic metabolism.  $\alpha$ -Lipoic acid may have potent antioxidant potential and can recycle vitamin E;<sup>[49]</sup> however, its accumulation in tissues is limited. Micronutrient functions of  $\alpha$ -lipoic acid may act more as an effector of cellular stress response pathways.<sup>[50]</sup>

*N*-acetylcysteine is a by-product of an endogenously synthesized antioxidant glutathione. It is a cysteine derivative and plays a role in glutathione maintenance and metabolism. *N*-acetylcysteine has been proposed to have

antioxidant effects and is used as a pharmaceutical drug (mucolytic agent) and a nutritional supplement.<sup>[51]</sup>

Allopurinol, a structural isomer of hypoxanthine, is an inhibitor of XO. It is a drug primarily used to treat hyperuricaemia, as it decreases uric acid formation and purine synthesis.<sup>[52]</sup>

Antioxidants are often divided into two groups: those that act either through stabilizing ROS or by removing reactive intermediates. The former, also known as preventative antioxidants, stabilize free radicals by donating electrons and become oxidized themselves, forming less active radicals. The latter, 'scavengers', help slow or stop the damaging chain reaction by removing free radical intermediates. In addition, transition metal sequestration and oxidative damage-repairing mechanisms support the body's defence system. Endogenous antioxidant systems respond rapidly to an increased production of reactive species. Cells can modulate gene expression and the activity of antioxidant enzymes to cope with oxidative stress.<sup>[18,53]</sup>

### 2.3 Oxidative Stress

Despite the extensive defence system, an increase in ROS production or diminished antioxidants can lead to progressive cell damage and a decline in physiological function. When oxidant capacity exceeds the antioxidant capacity, homeostatic balance is disturbed and the redox state becomes more pro-oxidizing. This imbalance is called oxidative stress.<sup>[54]</sup> As we now know that individual signalling and control events occur through discrete redox pathways, rather than through global balances, the classic definition of oxidative stress has been refined and also considers oxidative stress as a disruption of redox signalling and control.<sup>[55]</sup> Therefore, oxidative stress may occur without an overall imbalance of pro-oxidants and antioxidants and can cause organ-specific and pathway-specific toxicity.

Under usual lifestyle conditions we are exposed to high levels of reactive species from exogenous sources (e.g. environmental pollution)<sup>[56]</sup> and oxidative stress has been implicated in a growing list of human diseases, such as cardio-

vascular, inflammatory, metabolic and neurodegenerative diseases, as well as cancer and the ageing process.<sup>[57]</sup> A diet rich in antioxidants has been identified as a potentially noninvasive means of controlling oxidative stress.<sup>[58,59]</sup> Antioxidant supplementation has received much attention because of its capacity to support the endogenous defence by scavenging additional ROS and, therefore, by reducing oxidative damage.<sup>[60-62]</sup> However, there is little evidence for the efficacy of antioxidant supplements to treat ROS-associated diseases. This has led to considerable debate regarding the beneficial health effects of this kind of supplementation in different types of patients and with different types of antioxidants.<sup>[13,63,64]</sup> Although observational epidemiological cohort studies with large numbers of subjects and diverse populations have been largely supportive of the health-promoting effects of antioxidants,<sup>[65-68]</sup> interventional trials have been controversial, with some positive findings,<sup>[37,38,69]</sup> many null findings<sup>[70-73]</sup> and some suggesting a detrimental effect of antioxidant supplementation, particularly vitamin E, on morbidity and mortality.<sup>[74-76]</sup>

### 2.4 Beneficial Roles of Reactive Species

Although reactive species are associated with harmful biological events, they are essential in cellular development and optimal function.<sup>[77,78]</sup> Cells have evolved strategies to utilize reactive species as biological stimuli. They act as subcellular messengers in important molecular signalling processes and modulate enzyme and gene activation.<sup>[77]</sup> Most antioxidant enzyme genes contain regulatory sequences in their promoter and intron regions that can interact with redox sensitive transcription factors.<sup>[79]</sup> Reactive species play significant roles in cellular growth and proliferation.<sup>[77]</sup> It has been shown recently that physiological levels of ROS are required to activate DNA repair pathways for maintaining genomic stability in stem cells.<sup>[80]</sup> Furthermore, ROS are involved in the biosynthesis of other molecules,<sup>[81]</sup> the immune response of cells<sup>[26]</sup> and drug detoxification.<sup>[77]</sup> They are a requisite for vasodilation,<sup>[82]</sup> optimal muscular contraction<sup>[83]</sup> and initiation of apoptosis.<sup>[34]</sup>

### 3. Exercise-Induced Oxidative Stress

#### 3.1 Reactive Species in Skeletal Muscle

During contraction, skeletal muscle is a major source of ROS, as well as one of the main targets.<sup>[24]</sup> Exercise increases  $\dot{V}O_2$  by up to 20 times above resting values.<sup>[84]</sup> In the mitochondria of exercising muscle cells, this translates to a 200-fold greater oxygen usage.<sup>[84]</sup> Exercise-induced oxidative stress was first described in the late 1970s when increased levels of lipid peroxidation products were found in the expired air of exercising humans<sup>[35]</sup> and the tissues of exercised rats.<sup>[85]</sup> In 1982, Davies et al.<sup>[86]</sup> provided the first direct evidence that high-intensity exercise significantly increased ROS production in the muscles and liver of rats, and caused damage to mitochondrial membranes. It was suggested that this could, at the same time, deliver a stimulus to mitochondrial biogenesis. However, the majority of following studies focused on the damaging effects of oxidants in muscle and looked for the potential benefits of antioxidants. Over the last 30 years, an understanding of the sources and consequences of exercise-produced ROS has advanced markedly. It is now clear that reactive species play important roles in skeletal muscle function and metabolism. Redox signalling in contracting muscle is considered one of the basic elements in exercise biology.<sup>[24]</sup>

#### 3.2 Adaptation to Exercise-Induced Oxidative Stress

Cells adapt to increased ROS production to become more resistant to the adverse effects of oxidative stress.<sup>[87]</sup> It has to be emphasized, however, that the effects of a single bout of exercise and regular exercise are quite different. Regular physical activity brings about numerous beneficial effects and the body adapts to elevated oxidant levels, whilst with acute exercise, the adaptation is only marginal. Acute adjustment involves increased vasodilation to enhance blood flow and fuel transport and a kinetic shift via the allosteric activity of enzymes, which may not be sufficient to restore oxidant-antioxidant homeostasis.<sup>[88]</sup> Long-term stimulation of endogenous

defence mechanisms requires the continuous presence of physiological stimuli that maintain a certain degree of pro-oxidative milieu, and effectively overload the antioxidant systems.<sup>[89]</sup> With exercise training the body adapts to exercise-induced oxidative stress and becomes more resistant to subsequent oxidative challenges. This is achieved through a number of different mechanisms, such as upregulation of redox-sensitive gene expression and antioxidant enzymes levels,<sup>[90,91]</sup> an increase in enzyme activity,<sup>[92,93]</sup> stimulation of protein turnover,<sup>[94]</sup> improvement in DNA-repair systems,<sup>[95,96]</sup> and increased mitochondrial biogenesis<sup>[97]</sup> and muscle content of heat shock proteins (HSPs).<sup>[98,99]</sup> In addition, adaptation positively affects remodelling of skeletal muscle after injury and attenuates inflammation and apoptosis.<sup>[88,100,101]</sup>

Moderate levels of reactive species appear necessary for various physiological processes, whereas, an excessive ROS production causes oxidative damage. This may be described by the concept of hormesis, a dose-response relationship in which a low dose of a substance is stimulatory or beneficial and a high dose is inhibitory or toxic.<sup>[102]</sup> The adaptive response of mitochondria to increased formation of ROS is termed mitochondrial hormesis or mitohormesis.<sup>[103]</sup> The hormetic action of reactive species could represent a mechanism underlying the health and performance benefits of regular physical activity.<sup>[102]</sup> This can be seen in the role of reactive species as endogenous regulators of skeletal muscle function. Indeed, they appear obligatory for optimal contractile activity. Muscle myofibrils, such as myosin and troponin, and proteins in the sarcoplasmic reticulum are redox-sensitive, which gives ROS the ability to alter muscle contraction.<sup>[104]</sup> Based on Reid's model for the role of redox state on muscle force production, reaction to ROS can be described by a bell-shaped curve.<sup>[104,105]</sup> At baseline, low oxidant levels appear to be suboptimal for the contraction of unfatigued muscle. The data from Reid's studies suggest modest augmentation in ROS levels causes muscle force to increase, while antioxidants deplete oxidant levels and depress force. At higher ROS concentrations this is reversed and

force production decreases in a time- and dose-dependent manner.<sup>[105-107]</sup>

### 3.3 Oxidative Stress and Muscle Damage

Despite skeletal muscle being relatively resistant to exercise-induced oxidative damage, it is clear that intense and/or prolonged muscular activity can result in harmful outcomes.<sup>[9]</sup> Repetitive eccentric contractions, if unaccustomed in particular, place skeletal muscle under considerable stress that may cause muscle damage.<sup>[108,109]</sup> Damaging exercise also induces an inflammatory response, which further increases ROS formation.<sup>[110]</sup> However, the studies often lack the information about the subjects' redox status and therefore fail to provide evidence for the causal role of ROS in muscle damage.

The majority of studies have measured indirect and nonspecific indices of muscle damage, such as muscle soreness and reduction in the muscle force production. Eccentric exercise was shown to cause structural changes of muscle fibres,<sup>[108,109,111,112]</sup> and has been associated with muscular soreness,<sup>[110,113,114]</sup> reduced range of motion<sup>[110]</sup> and loss of torque and force production.<sup>[109,111,112,115,116]</sup> This may result in muscle fatigue and development of muscular atrophy.<sup>[117-119]</sup> Extreme fatigue can lead to muscle injury and, possibly, irreversible cell alterations.<sup>[119,120]</sup>

## 4. Antioxidant Supplementation and Exercise

### 4.1 Overview

It is common practice for athletes to use antioxidant supplements with the notion that they prevent the deleterious effects of exercise-induced oxidative stress, hasten recovery of muscle function and improve performance.<sup>[1,121-125]</sup> Indeed, there is now an enormous range of vitamins, minerals and extracts marketed as antioxidant supplements. None have undergone adequate testing, and therefore lack scientific evidence regarding efficacy and long-term safety.

The popularity of antioxidant supplements with athletes has led to a plethora of small research studies in this area. As expected, the

studies varied considerably in terms of research design, exercise protocol, population groups, supplementation regimen and analysis methods. Importantly, the studies are also of generally low quality. As commonly found in sports nutrition research, the vast majority do not adhere to all the accepted features of a high-quality trial (e.g. placebo-controlled, double-blind, randomized design with an intent-to-treat analysis). Indeed, most studies fail to provide sufficient detail regarding inclusion and exclusion criteria, justification of sample size, adverse events, data gathering and reporting, randomization, allocation and concealment methods, and an assessment of blinding success. The poor quality of the majority of studies in this field increases the possibility for bias and needs to be always considered when evaluating the findings.

Supplements used in the studies include vitamin E, vitamin C,  $\beta$ -carotene, coenzyme Q<sub>10</sub>,  $\alpha$ -lipoic acid, *N*-acetylcysteine, allopurinol, quercetin, resveratrol and several other polyphenolic compounds. A number of studies have used combinations of these. The range of dosages across the supplements was wide and duration of supplementation varied from acute (1–2 days) to chronic administration (from 1 week to up to 6 months). Blood, urine, breath and muscle tissue samples were collected pre-, during and post-supplementation and exercise. The most common outcome measure was a marker of oxidative stress with lipid peroxidation products predominating, followed by oxidized proteins, DNA damage markers and alterations in endogenous antioxidant systems. Direct measurement of reactive species concentration (e.g. electron spin resonance spectroscopy) was only performed in a small number of studies because of the instability of ROS, high costs and extensive work-up requirements.

### 4.2 Antioxidant Supplementation and Exercise-Induced Oxidative Stress

The majority of studies have used measures of oxidative stress as their main outcome, and most have demonstrated that antioxidants attenuate exercise-induced increases in oxidative stress.

Most common antioxidants in these positive studies were vitamin E<sup>[35,36,62,126-132]</sup> and vitamin C,<sup>[60,116,133-137]</sup> followed by different combinations of antioxidants<sup>[61,138-147]</sup> and, most recently, polyphenolic compounds.<sup>[148-156]</sup> Furthermore, lower levels of oxidative stress markers have been reported after  $\beta$ -carotene,<sup>[157]</sup>  $\alpha$ -lipoic acid,<sup>[158]</sup> *N*-acetylcysteine<sup>[159]</sup> and selenium<sup>[160]</sup> administration. However, there have been many studies showing no significant effect of antioxidant supplements on exercise-induced oxidative stress<sup>[110,161-172]</sup> and several indicating increased oxidative stress levels following antioxidant administration.<sup>[144,173-176]</sup>

Although the majority of studies report that antioxidants can reduce oxidative stress levels, the physiological implications of these effects are unknown. In an attempt to determine the importance of reducing oxidative stress, investigators have studied the role of antioxidant supplementation in exercise performance and muscle damage.

#### 4.3 Antioxidant Supplementation and Muscle Damage

Strong evidence to support the role of antioxidant supplementation in protecting against muscle damage is lacking. The majority of investigations have focused on the effects of vitamin C and E and looked at oxidative stress markers and plasma concentrations of intramuscular enzymes, e.g. creatine kinase (CK) and lactate dehydrogenase, rather than indices of muscle damage such as force loss, muscle soreness, structural changes of myoproteins and their plasma concentration.<sup>[6]</sup> As a result of the lack of direct measurement of specific indices of muscle damage, it is unclear to what extent muscle damage was induced in those studies. There are reports that antioxidant supplementation could offer some protection from exercise-induced cell damage,<sup>[127,177-181]</sup> attenuate the inflammatory response to exercise,<sup>[147,151,182-186]</sup> and reduce muscle force loss<sup>[154,156,177,187]</sup> and fatigue.<sup>[188-191]</sup> Other investigations, however, found no significant effect of antioxidants on indices of cell damage,<sup>[111,113,161,192-194]</sup> muscle soreness<sup>[114,195-199]</sup>

and inflammation.<sup>[111,114,127,169,194,200,201]</sup> A number of studies suggested that antioxidant supplementation may promote muscle damage and possibly hinder recovery.<sup>[165,175,197,202]</sup> These studies are the focus of this review and discussed in section 5.

#### 4.4 Antioxidant Supplements as Ergogenic Aids

There has been a general inconsistency of outcomes when investigating the role of antioxidant supplementation in exercise performance with the majority of the studies reporting no benefits. In the early 1970s, Sharman and colleagues<sup>[203]</sup> showed that supplementation with vitamin E had no beneficial effect on endurance performance of adolescent male swimmers. Moreover, the placebo group demonstrated greater improvements of cardiorespiratory function with exercise training compared with the antioxidant group, which may be the first report of the unfavourable effect of supplementation. In the studies that followed, vitamin E proved ineffective in improving performance in swimmers,<sup>[204]</sup> professional cyclists,<sup>[132,205,206]</sup> non-resistance-trained men,<sup>[202]</sup> athletic students<sup>[167]</sup> and marathon runners.<sup>[207]</sup> Furthermore, vitamin E supplements had no additive effect beyond that of aerobic training on indices of physical performance in a group of older sedentary adults.<sup>[208]</sup> Supplementation with coenzyme Q<sub>10</sub> did not exhibit any significant effects on exercise performance of men,<sup>[162,209,210]</sup> regardless of their age and training status. Quercetin supplements also failed to show any ergogenic effects in sedentary individuals<sup>[199,211]</sup> or cyclists.<sup>[212]</sup> Polyphenol resveratrol did not improve muscle force output and muscle fatigability in mice subjected to electrically stimulated isometric contractions.<sup>[213]</sup> In a study by Marshall et al.,<sup>[214]</sup> vitamin C was shown to slow racing greyhounds.

Despite the presumption that antioxidants work synergistically and may therefore be more efficient in combating oxidative stress, combinations of vitamins E, C, coenzyme Q<sub>10</sub> and other vitamins and minerals failed to improve the exercise performance of competitive male



runners,<sup>[215]</sup> cyclists,<sup>[144,216]</sup> triathletes,<sup>[217,218]</sup> soccer players,<sup>[146,219]</sup> resistance-trained men,<sup>[220]</sup> ultraendurance runners,<sup>[221]</sup> moderately trained men,<sup>[222]</sup> and trained and untrained males and females.<sup>[166]</sup>

Nonetheless, there have been a number of studies showing positive, albeit, modest effects of antioxidant supplementation on physical performance. Coenzyme Q<sub>10</sub> was associated with improved maximal  $\dot{V}O_2$  ( $\dot{V}O_{2max}$ ) and aerobic and anaerobic threshold of professional cross-country skiers that resulted in an increased exercise capacity and a faster recovery rate.<sup>[223]</sup> Similarly, supplementation with coenzyme Q<sub>10</sub> indicated beneficial effects on performance, fatigue sensation and recovery during fatigue-inducing workload trials in a group of healthy volunteers.<sup>[189]</sup> Furthermore, results from supplementation studies that involved male cyclists,<sup>[224]</sup> trained and untrained individuals<sup>[225]</sup> and sedentary men<sup>[226]</sup> supported the performance-enhancing effect of coenzyme Q<sub>10</sub>. Vitamin E supplementation was proposed to have a beneficial effect on the performance of climbers at high altitude<sup>[128]</sup> and endurance performance of mice,<sup>[227]</sup> rats<sup>[228]</sup> and sled dogs.<sup>[229]</sup> In two early studies, supplementation with vitamin C was associated with an improved exercise capacity of untrained male students<sup>[230]</sup> and athletes.<sup>[231]</sup> In a study by Aguilo et al.,<sup>[232]</sup> male athletes supplemented with a combination of vitamin E, C and  $\beta$ -carotene exhibited lower blood lactate levels after a maximal exercise test and exhibited a more significant increase more in  $\dot{V}O_{2max}$  after 3 months of exercise training than the placebo group. Supplementation with different combinations of antioxidants also positively affected the exercise performance of students,<sup>[233]</sup> elderly endurance-trained athletes<sup>[234]</sup> and aged rats.<sup>[139]</sup>

Medved and colleagues<sup>[235]</sup> have studied the effect of *N*-acetylcysteine on muscle fatigue and performance in untrained and trained men. Although *N*-acetylcysteine was shown to modulate blood redox status during high-intensity intermittent exercise, it did not affect time to fatigue in a group of untrained men. Similarly, *N*-acetylcysteine infusion during prolonged submaximal exercise had no effect on time to fatigue

in a group of team-sport athletes and endurance-trained cyclists. Nonetheless, the antioxidant improved regulation of plasma K<sup>+</sup> concentration and it was suggested the ergogenic effect of *N*-acetylcysteine depends on an individual's training status.<sup>[236]</sup> Finally, *N*-acetylcysteine infusion during prolonged submaximal exercise was reported to augment time to fatigue in a group of well trained individuals, possibly by increasing muscle cysteine and glutathione availability.<sup>[237]</sup>

Recently, there have been a number of investigations showing the performance enhancing effects of polyphenols, including quercetin,<sup>[201,238-240]</sup> resveratrol,<sup>[241]</sup> and polyphenolic compounds from grape extract,<sup>[152]</sup> beetroot juice,<sup>[242-245]</sup> *Rhodiola rosea* plant<sup>[246]</sup> and *Ecklonia cava* algae.<sup>[247]</sup> Emerging evidence suggests that the antioxidant potential of phenolic compounds is unlikely to be the sole mechanism responsible for their protective action, which could also be mediated by their interaction with various key proteins in the cell-signalling cascades.<sup>[248]</sup>

As mentioned above in section 4.1, many of the studies evaluating the effects of antioxidants on exercise performance have been of low quality with small subject numbers. In addition, most have had important methodological details left out of the articles (e.g. recruitment, randomization, allocation and concealment methods) leading to the assumption that they were not considered. This creates a potentially dangerous bias in regards to subject selection and the assessment of performance effects.

## 5. Antioxidant Supplementation Interferes with the Beneficial Effects of Exercise Training

Recent studies have indicated that antioxidant supplements have a detrimental effect on the health and performance benefits of exercise training. Considering the multifunctional beneficial roles of ROS in living organisms discussed above in section 2.4, reports of unfavourable effects of antioxidant supplementation should not come as a surprise. The studies reporting negative outcomes are discussed in sections 5.1–5.3 with more details presented in table I.

**Table I.** Studies with negative outcomes using antioxidant supplementation during exercise training

Study (y)	Subjects	Supplements (daily dose)	Duration	Study design	Findings
Malm et al. <sup>[249]</sup> (1996)	15 M	Coenzyme Q <sub>10</sub> (120 mg)	20 d	Placebo-controlled trial: Exercise tests: anaerobic test (Wingate test, 5 min recovery, 10 × 10 sec all-out cycling), $\dot{V}O_2$ submax and max test. Exercise training: 9 sessions (15 × 10 sec all-out cycling sprints). Samples: plasma CK activity	After exercise, CK levels ↑ only in the supplemented group. Subjects taking supplements showed smaller training-induced improvements in physical performance than the placebo group
Malm et al. <sup>[250]</sup> (1997)	18 M	Coenzyme Q <sub>10</sub> (120 mg)	22 d	Placebo-controlled double-blind trial: Exercise tests: anaerobic test (30 sec all-out cycling, 5 min recovery, 10 × 10 sec all-out cycling), submax and peak cycling $\dot{V}O_2$ test, $\dot{V}O_{2max}$ running test. Exercise training: 7 sessions (15 × 10 sec all-out cycling sprints). Samples: plasma lactate	There was a greater increase in anaerobic performance in the placebo group compared with the supplemented group. Moreover, supplementation was associated with reduced exercise training-induced increase in power output and recovery rate between cycling sprints. Coenzyme Q <sub>10</sub> had no effect on submax and peak cycling $\dot{V}O_2$ , running $\dot{V}O_{2max}$ and lactate levels
Childs et al. <sup>[175]</sup> (2001)	14 M	Vitamin C (12.5 mg/kg BW) + NAC (10 mg/kg BW)	1 wk (post-exercise)	Double-blind placebo-controlled trial: Exercise protocol: eccentric arm exercise (3 × 10 repetitions, 80% of 1RM). Samples: serum free iron levels, plasma lipid hydroperoxides, F2-isoprostanes, myeloperoxidase and IL-6, plasma CK and LDH activities, serum SOD and GPX	Exercise ↑ inflammatory indicators, free iron concentration and the levels of oxidative stress and muscle damage markers. The amount of iron, levels of lipid hydroperoxides and isoprostanes and LDH and CK activities were higher in the supplemented group than in the placebo group
Coombes et al. <sup>[251]</sup> (2001)	28 F rats	Vitamin E (10 000 IU/kg diet) + $\alpha$ -lipoic acid (1.65 g/kg diet)	8 d	<i>In situ</i> experiment: Contractile measurements (tibialis anterior): P <sub>o</sub> , P <sub>t</sub> and force-frequency curve, 60 min fatigue protocol. Samples: muscle MDA and lipid hydroperoxide	Contracted muscles of supplemented animals had lower levels of oxidative stress than the muscles from the control group. Vitamin E and $\alpha$ -lipoic acid supplementation had no effect on muscle fatigue but was associated with decreased muscle force production at low stimulation frequencies ( <i>in situ</i> ). <i>In vitro</i> experiments indicated that vitamin E depressed force production at low stimulation frequencies
	32 F rats	Vitamin E: 100, 200, 400 $\mu$ M/DHLA; 100 $\mu$ M/vitamin E; 400 $\mu$ M + DHLA; 100 $\mu$ M		<i>In vitro</i> experiment: contractile measurements (costal diaphragm): P <sub>o</sub> , P <sub>t</sub> and force-frequency curve, 30 min fatigue protocol	
Marshall et al. <sup>[214]</sup> (2002)	5 F racing greyhounds	Vitamin C (1 g)	4 wk (each treatment)	Crossover controlled trial: Treatments: no supplementation; supplementation after racing; supplementation 1 h before racing. Exercise training: 2 × 500 m races/wk. Samples: plasma TBARS and antioxidant capacity	Vitamin C showed no effect on oxidative stress and antioxidant capacity. The dogs ran slower when supplemented

Continued next page

Table I. Contd

Study (y)	Subjects	Supplements (daily dose)	Duration	Study design	Findings
Avery et al. <sup>[202]</sup> (2003)	18 untrained M	Vitamin E (1200 IU)	3 wk	Randomized placebo-controlled double-blind trial: Exercise Protocol: 3 resistance exercise sessions separated by 3 days of recovery. Measurements: muscle soreness, muscle strength and power assessment. Samples: plasma MDA and CK activity	There was no effect of supplementation on muscle soreness, performance indices and MDA levels. CK levels were greater in the supplemented group than in the placebo group
Bryant et al. <sup>[144]</sup> (2003)	7 M cyclists	Vitamin C (1 g)/vitamin C (1 g) + vitamin E (200 IU/kg)/vitamin E (400 IU/kg)	3 wk (each treatment)	Controlled crossover single-blind trial: Treatments: placebo; vitamin C; vitamin C + vitamin E; vitamin E. Exercise tests: 60 min steady state ride (70% $\dot{V}O_{2max}$ ) and 30 min performance ride (70% $\dot{V}O_{2max}$ ). Samples: plasma MDA and lactic acid	Supplementation had no effect on exercise performance. Vitamin E ↓ MDA levels, the combination of vitamins E and C had no effect, vitamin C alone ↑ MDA levels
Khassaf et al. <sup>[98]</sup> (2003)	16 untrained M	Vitamin C (500 mg)	8 wk	Randomized controlled trial: Muscle samples (exercise protocol: 45 min single leg cycling, 70% $\dot{V}O_{2max}$ , vastus lateralis): HSP60 and HSP70 content. Lymphocytes (treated with H <sub>2</sub> O <sub>2</sub> for 30 min): SOD and CAT activity, HSP60 and HSP70 content	Supplementation with vitamin C was associated with attenuated exercise-induced increase in HSP content and SOD and CAT activity
Nieman et al. <sup>[176]</sup> (2004)	36 triathletes (26 M, 10 F)	Vitamin E (800 IU)	2 mo	Randomized placebo-controlled double-blind trial: Ironman Triathlon race – samples: plasma and urinary F <sub>2</sub> -isoprostanes, urinary 8-OHdG and 8-oxoG, plasma lipid hydroperoxides and cytokines	Post-race concentrations of isoprostanes, lipid hydroperoxides, IL-6, IL-1ra and IL-8 increased more in the vitamin E group than in the placebo group. Supplementation had no effect on race time
Gomez-Cabrera et al. <sup>[252]</sup> (2005)	20 M rats	Allopurinol (32 mg/kg)	Admin prior to exercise	Randomized controlled trial: Exercise protocol: progressive intensity treadmill test, exercise to exhaustion. Samples: plasma lactate and XO activity, muscle GSH, GSSG, carbonylated proteins, p38, ERK1 and ERK2, NF-κβ DNA-binding activity and Mn-SOD, iNOS and eNOS	Allopurinol treated rats exhibited ↓ oxidative stress levels and ↓ exercise-mediated increase in XO activity and induction of MAPKs. This was associated with ↓ DNA binding of NF-κB and blunted upregulation of <i>Mn-SOD</i> , <i>eNOS</i> and <i>iNOS</i> gene expression
Gomez-Cabrera et al. <sup>[253]</sup> (2006)	25 marathon runners	Allopurinol (300 mg)	2 h prior to marathon race	Randomized placebo-controlled trial: Marathon race - samples: lymphocyte NF-κβ p50 activation, plasma MDA and XO activity	Allopurinol prevented XO activation and lipid peroxidation. Inhibitor of XO-derived ROS formation prevented NF-κB activation. Allopurinol had no effect on race time

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Table I. Contd

Study (y)	Subjects	Supplements (daily dose)	Duration	Study design	Findings
Close et al. <sup>[197]</sup> (2006)	20 M	Vitamin C (1 g)	2 h prior to and for 2 wk post-exercise	Randomized placebo-controlled double-blind trial: Exercise protocol: downhill running test (30 min, 60% $\dot{V}O_{2max}$ ). Measurements: pain assessment (visual analogue scale, pressure algometry) and muscle function (quadriceps torque assessment). Samples: serum MDA	Supplementation with vitamin C ↓ exercise-induced increase in MDA levels but had no effect on DOMS. Delayed recovery of muscle function was noted in the supplemented group
Fischer et al. <sup>[99]</sup> (2006)	21 M	$\alpha$ -Tocopherol (400 IU) + vitamin C (500 mg) $\alpha$ -Tocopherol (290 IU) + $\gamma$ -tocopherol (130 IU) + vitamin C (500 mg)	4 wk	Randomized placebo-controlled single-blind trial: Exercise protocol: 3 h, 2-legged dynamic knee extensor exercise. Samples: muscle HSP72 mRNA and protein, plasma HSP72 and $F_2$ -isoprostanes	$\alpha$ -Tocopherol + vitamin C treatment attenuated ↑ in lipid peroxidation post-exercise. Exercise-induced increase in HSP72 levels in skeletal muscle and circulation was abolished in $\alpha$ -tocopherol + $\gamma$ -tocopherol + vitamin C group
Knez et al. <sup>[93]</sup> (2007)	16 half-Ironman triathletes (13 M, 3 F)	Vitamin C (1095 ± 447 mg) + vitamin E (314 ± 128 mg)	Vitamin C: 4.9 ± 4.7 y; vitamin E: 5.6 ± 5.2 y	Observational study: subjects recruited 4 wk before the race, controls active <3h/wk: Triathletes: training and competing for 4.7 ± 2.4 y, 14.5 ± 3.4 h/wk, 10 taking supplements; race: 1.9 km swim, 90.1 km cycle, 21.1 km run. Samples: plasma MDA and erythrocyte SOD, GPX and CAT activities	Dose-response relationship between adaptations of antioxidant enzymes and responses to ultraendurance exercise. Ultraendurance training upregulated endogenous antioxidant system (GPX and CAT activity). Triathletes taking supplements had elevated post-race MDA levels compared with nonsupplementers
	29 Ironman triathletes (23 M, 6 F)	Vitamin C (558 ± 350 mg) + vitamin E (702 ± 756 mg)	Vitamin C: 0.8 ± 0.6 y; vitamin E: 1.6 ± 0.8 y	Triathletes: training and competing for 6.9 ± 6.4 y, 17.19 ± 3.4 h/wk, 8 taking supplements; race: 3.8 km swim, 180 km cycle, 42.2 km run. Samples: plasma MDA and erythrocyte SOD, GPX and CAT activities	
Richardson et al. <sup>[254]</sup> (2007)	25 M	Dose: $\alpha$ -lipoic acid (300 mg) + vitamin C (500 mg) + vitamin E (200 IU) Dose: $\alpha$ -lipoic acid (300 mg) + vitamin C (500 mg) + vitamin E (400 IU)	2 h and 1.5 h prior to exercise	Randomized placebo-controlled crossover double-blind trial: Exercise protocol: forearm handgrip exercise at low-intensity workload (3, 6 and 9 kg at 0.5 Hz) for 3 min. Measurements: plasma FR, vasodilation.	Antioxidant administration ↑ total antioxidant capacity and ↓ exercise-induced oxidative stress but ↓ brachial artery vasodilation during submaximal exercise.
Gomez-Cabrera et al. <sup>[97]</sup> (2008)	14 sedentary M	Vitamin C (1 g)	8 wk	Randomized double-blind controlled trial: Exercise test: $\dot{V}O_{2max}$ test (bicycle ergometer). Exercise training: 40 min cycling 3 d/wk (65% → 80% $\dot{V}O_{2max}$ )	

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Table I. Contd

Study (y)	Subjects	Supplements (daily dose)	Duration	Study design	Findings
	36 M rats	Vitamin C: 0.24 mg/cm <sup>2</sup> body surface area	3 wk; 6 wk	Untrained group, trained group, trained + supplemented group: RT-PCR experiment: 3 wk training. Western blotting and performance experiments: 6 wk training. Exercise training: 5 d/wk, treadmill (75% $\dot{V}O_{2max}$ , 25 → 85 min/d). Endurance test (run to exhaustion), $\dot{V}O_{2max}$ test (treadmill run). Samples: muscle mTFA and NRF-1 mRNA and protein, cyt c and PGC-1 protein, Mn-SOD and GPX mRNA	Moderate intensity exercise enhanced endogenous antioxidant defence (↑ expression of Mn-SOD and GPX) and mitochondrial biogenesis (upregulation of PGC-1 → NRF-1 → mTFA → cyt c pathway) and increased endurance capacity. Vitamin C prevented these training induced adaptations
Copp et al. <sup>[255]</sup> (2009)	19 M rats	Vitamin C (76 mg/kg) + tempol (52 mg/kg)	Acute infusion (after first exercise protocol)	Exercise protocol (right spinotrapezius muscle): 1 Hz twitch contractions for 180 sec (2 sessions: pre- and post-antioxidant administration); 13 rats: blood flow and $P_{O_{2,mv}}$ measurements; 6 rats: muscle force measurements	Antioxidant administration ↑ serum antioxidant capacity but ↓ blood flow, baseline $P_{O_{2,mv}}$ , muscle oxygen utilization and muscle force production
Lamprecht et al. <sup>[174]</sup> (2009)	8 trained M cyclists	Vitamin E (107 IU) + vitamin C (450 mg) + β-carotene (36 mg) + Se (100 μg)	2 wk	Randomized double-blind placebo-controlled crossover trial: Exercise test: cycle ergometer, 90 min cycling (45% $\dot{V}O_{2max}$ ) + 30 min cycling (75% $\dot{V}O_{2max}$ ). Samples: plasma MDA and GPX	MDA concentrations were ↑ and GPX levels ↓ after antioxidant treatment (pre- and post-exercise)
Ristow et al. <sup>[91]</sup> (2009)	20 untrained M (<2 h of exercise/wk), 20 pretrained M (>6 h of exercise/wk)	Vitamin C (1 g) + vitamin E (400 IU)	4 wk	Controlled trial, 2 part-study – open-label study; double blind placebo-controlled study: 4 groups: untrained nonsupplemented, trained nonsupplemented, untrained supplemented, trained supplemented. Exercise training – 5 d/wk, session: 20 min biking/running, 45 min circuit training. Measurements: GIR. Samples: plasma adiponectin, muscle <i>PGC-1α</i> , <i>PGC-1β</i> , <i>PPARγ</i> , <i>SOD1</i> and <i>SOD2</i> , and <i>GPX</i> gene levels	Exercise training ↑ insulin sensitivity, ↓ fasting plasma insulin levels, ↑ gene expression of <i>PGC-1α</i> , <i>PGC-1β</i> , <i>PPARγ</i> , <i>SOD1</i> and <i>SOD2</i> , <i>GPX</i> (irrespective of training status). Supplementation with vitamins E and C was shown to prevent these health promoting effects
Teixeira et al. <sup>[165]</sup> (2009)	20 competitive kayakers (14 M, 6 F)	α-Tocopherol (272 mg) + vitamin C (400 mg) + β-carotene (30 mg) + lutein (2 mg) + Se (400 μg) + Zn (30 mg) + mg (600 mg)	4 wk	Randomized double-blind placebo-controlled trial: Exercise test: maximal flat-water kayaking trial (1000 m). Samples: plasma antioxidants, TBARS, IL-6 and CK, SOD, GR, GPX activities	Antioxidant supplementation ↑ antioxidant capacity but had no effect on oxidative stress and inflammation markers. Supplemented athletes showed a blunted decrease in CK activity post-exercise

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Table I. Contd

Study (y)	Subjects	Supplements (daily dose)	Duration	Study design	Findings
Wray et al. <sup>[256]</sup> (2009)	6 older, mildly hypertensive M	Dose: $\alpha$ -lipoic acid (300 mg), vitamin C (500 mg), vitamin E (200 IU)  Dose: $\alpha$ -lipoic acid (300 mg), vitamin C (500 mg), vitamin E (400 IU)	Prior to and after 6 wk of training; 2 h before exercise protocol  Prior to and after 6 wk of training; 30 min after 1	Double-blind placebo-controlled crossover trial: Exercise protocol – d 1, 2: antioxidant efficacy test; d 3–6: FMD procedure followed by knee extensor exercise, subjects crossed over, returned after 24 h. Exercise training: 3 $\times$ wk-single leg knee-extensor exercise. Measurements: plasma FR, BP and FMD	Antioxidant administration reduced FR levels pre- and post-exercise. Exercise training reduced BP and improved vasodilation, supplementation after training negated these effects
Bailey et al. <sup>[110]</sup> (2010)	38 M	Vitamin C (800 mg) + vitamin E (536 mg) + vitamin B6 (4 mg) + vitamin B9 (400 $\mu$ g) + zinc sulphate monohydrate (10 $\mu$ g) + vitamin B12 (2 $\mu$ g)	6 wk (including 2 d post-exercise)	Randomized placebo-controlled double-blind trial: Exercise test (d 40): 90 min intermittent high-intensity shuttle-running. Measurements: pre- and post-exercise ratings of perceived muscle soreness and assessment of muscle function (peak isometric torque of the knee flexors and extensors, range of motion at the knee joint). Samples: urine F2-isoprostanes, serum IL-6 and cortisol	Antioxidant supplementation was associated with attenuated exercise-induced $\uparrow$ in cortisol concentration but $\uparrow$ post-exercise IL-6 and F2-isoprostane levels (compared with the placebo). Treatment had no effect on indices of muscle damage, muscle function measures and perception of muscle soreness
Matsumoto et al. <sup>[257]</sup> (2011)	48 M rats	$\alpha$ -Tocopherol (1000 IU/kg diet) + $\alpha$ -lipoic acid (1.6 g/kg diet)	14 wk	Controlled trial: 4 groups: untrained nonsupplemented, trained nonsupplemented, untrained supplemented, trained supplemented. Exercise training: 90 min treadmill run 4 d/wk. Samples: left ventricular and coronary artery endothelial cells (gene analysis)	IL-6 gene levels were $\downarrow$ by all treatments. <i>RhoA</i> gene expression was $\downarrow$ by exercise training, $\uparrow$ by antioxidant supplementation. The combination of exercise and supplementation resulted in a blunted $\downarrow$ of <i>RhoA</i> gene levels (compared with the exercise training effect)

**1RM** = repetition maximum; **8-OHdG** = 8-hydroxy-2-deoxyguanosine; **8-oxoG** = 7,8-dihydro-8-oxoguanosine; **BP** = blood pressure; **BW** = bodyweight; **CAT** = catalase; **CK** = creatine kinase; **cyt c** = cytochrome c; **DOMS** = delayed onset muscle soreness; **DHLA** = dihydrolipoic acid; **ERK** = extracellular signal-regulated protein kinases; **F** = female; **FMD** = flow-mediated vasodilation; **FR** = free radical; **GIR** = glucose infusion rate; **GPX** = glutathione peroxidase; **GR** = glutathione reductase; **GSH** = reduced glutathione; **GSSG** = oxidized glutathione; **H<sub>2</sub>O<sub>2</sub>** = hydrogen peroxide; **HSP** = heat shock protein; **IL-1ra** = interleukin 1 receptor antagonist; **IL-6(8)** = interleukin-6(8); **LDH** = lactate dehydrogenase; **M** = male; **MAPK** = mitogen activated protein kinase; **max** = maximal; **MDA** = malondialdehyde; **mRNA** = messenger RNA; **mTFA** = mitochondrial transcription factor A; **NAC** = N-acetyl cysteine; **NF- $\kappa$ B** = nuclear factor kappa-light chain-enhancer of activated B cells; **NOS** = nitric oxide synthase; **NRF-1** = nuclear respiratory factor 1; **p38** = a member of MAPKs; **p50** = a subunit of NF- $\kappa$ B complex; **PGC-1** = peroxisome proliferator-activated receptor gamma coactivator 1; **PPAR $\gamma$**  = peroxisome proliferator-activated receptor gamma; **P<sub>O<sub>2</sub>mv</sub>** = microvascular O<sub>2</sub> partial pressure; **P<sub>o</sub>** = max specific tension; **P<sub>t</sub>** = twitch tension; **RhoA** = Ras homolog gene family member A; **RT-PCR** = real-time reverse transcriptase-polymerase chain reaction; **Se** = selenium; **SOD** = superoxide dismutase; **submax** = submaximal; **TBARS** = thiobarbituric acid reactive substances; **VO<sub>2</sub>** = oxygen uptake; **VO<sub>2</sub>max** = maximal VO<sub>2</sub>; **XO** = xanthine oxidase; **Zn** = zinc;  $\uparrow$  indicates increase;  $\downarrow$  indicates decrease;  $\rightarrow$  indicates 'leads to'/outcome.

### 5.1 Antioxidant Supplements Promote Exercise-Induced Oxidative Stress

Antioxidants, especially when present in high amounts, have been shown to increase markers of exercise-induced oxidative stress. After high-intensity exercise, coenzyme Q<sub>10</sub> supplementation was associated with an increase in a marker of cell damage (CK)<sup>[249]</sup> and a decrease in exercise-training induced improvements in physical performance.<sup>[249,250]</sup> A number of important methodological details were omitted from the articles, indicating low quality. A study by Childs et al.<sup>[175]</sup> found that vitamin C and *N*-acetylcysteine following eccentric arm exercise increased oxidative stress and cell damage above levels induced by muscle injury alone. The effects of vitamins E and C alone and in combination were investigated in seven male cyclists.<sup>[144]</sup> Vitamin E decreased malondialdehyde, an oxidative stress marker, whereas the combination of both had no effect and vitamin C increased malondialdehyde. This indicates that the type of antioxidant (e.g. water vs lipid soluble) is likely to be an important factor. In another study, an increase in the serum CK levels following a 3-day resistance exercise was greater after the use of vitamin E supplements compared with a placebo group.<sup>[202]</sup> However, the increase was both modest and transient with no effect of supplementation on muscle soreness and exercise performance. Furthermore, variability in the baseline CK levels between groups and the large interindividual variability of the measure need to be considered.

Two months of supplementation with high doses of vitamin E had no effect on the race time of Ironman Triathlon participants but was associated with increased lipid peroxidation and inflammation.<sup>[176]</sup> Knez et al.<sup>[93]</sup> demonstrated that ultraendurance training upregulated the resting activity of several antioxidant enzymes and reduced resting levels of oxidative stress, whilst supplementation with vitamins C and E had no effect on these values. Moreover, athletes taking supplements had elevated post-race malondialdehyde levels compared with nonsupplementers. It is important to recognize that this was only an observa-

tional study; although, when a randomized controlled crossover design was used, similar findings were reported with 2 weeks of supplementation with an antioxidant concentrate (vitamins E, C,  $\beta$ -carotene and selenium) associated with increased lipid peroxidation and decreased plasma glutathione peroxidase concentration pre- and post-exercise.<sup>[174]</sup> Finally, in a recent study by Bailey et al.,<sup>[110]</sup> young men were supplemented with a combination of vitamins C and E for 6 weeks before and 2 days after a 90-minute intermittent shuttle run. The supplemented subjects had increased markers of oxidative stress and inflammation compared with the placebo group. However, although the overall change in isoprostane levels (baseline vs post-exercise) approached significance, the tendency for slightly higher isoprostane levels in the placebo group at baseline precluded establishment of any significant differences at the final recovery timepoint. The authors noted that a large inter-individual variability in the responses of isoprostanes and interleukin (IL)-6 after supplementation could have impacted on the findings. Indeed, in all of the above mentioned studies there were no attempts to provide sample size or power calculations to assess the likelihood that the findings were real.

### 5.2 Antioxidant Supplementation Hinders Cell Adaptation to Exercise-Induced Oxidative Stress

Cells adapt to increased exposure to oxidation, thereby reducing the risk of tissue damage.<sup>[90,98,258]</sup> Five small studies now show that antioxidant supplements hinder the beneficial cell adaptations to exercise.<sup>[97-99,252,253]</sup> In a group of untrained males, supplementation with vitamin C resulted in the inactivation of redox-sensitive transcription factors responsible for the expression of cytoprotective proteins, including HSPs.<sup>[98]</sup> Such suppression of cell adaptation may negatively impact cell viability over the longer term. Similarly, supplementation with  $\gamma$ -tocopherol inhibited an exercise-induced increase of HSP levels in skeletal muscle and the circulation.<sup>[99]</sup>

A research group at the University of Valencia, Valencia, Spain has published a number of important studies on this topic. In one of their

first studies they used allopurinol in rats and found it attenuated the exercise-induced increase of XO activity and ROS formation.<sup>[252]</sup> This was associated with a decreased activation of mitogen-activated protein kinases (MAPKs) and blunted DNA-binding of nuclear factor kappa B (NF- $\kappa$ B). MAPKs respond to extracellular stimuli, including oxidative stress, and regulate cell development and survival. Transcription factor NF- $\kappa$ B mediates gene expression of enzymes such as *Mn-SOD*, *eNOS* and *iNOS*. Therefore, impairing the exercise training effects on MAPKs and NF- $\kappa$ B would likely impact on these positive benefits. Indeed, in humans, administration of allopurinol prior to a marathon race did suppress the exercise-induced increase of antioxidant enzyme expression.<sup>[253]</sup> In another study, Gomez-Cabrera et al.<sup>[97]</sup> showed that chronic supplementation with vitamin C impacted on exercise performance by decreasing exercise training efficiency. This was shown in both humans and rats. Analysis of animal muscles showed that the antioxidant supplementation inhibited upregulation of *Mn-SOD* and *GPX* gene expression. Moreover, attenuated mitochondrial biogenesis in the supplemented rats was indicated by reduced protein levels of cytochrome c (cyt c) and transcription factors peroxisome proliferator-activated receptor co-activator 1 (PGC-1), nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription factor A (mTFA). Cyt c, a protein in the inner membrane of mitochondria, is an essential component of the electron transport chain and serves as a marker of mitochondrial content. PGC-1 is a transcriptional coactivator of the genes involved in cellular energy metabolism. It induces messenger RNA expression of NRF-1 and mTFA and provides a link between external physiological signals and mitochondrial biogenesis.

In a recent study from our laboratory,<sup>[257]</sup> the effects of 14 weeks of antioxidant supplementation ( $\alpha$ -tocopherol and  $\alpha$ -lipoic acid) and treadmill exercise on myocardial and vascular endothelium gene expression were investigated in rats. Both antioxidant therapy and exercise training downregulated IL-6 gene expression, while the expression of the RAS homolog gene family

member A (*RhoA*), a gene involved in cardiovascular disease progression, was upregulated by antioxidant supplementation and downregulated by exercise. The combination of supplementation and exercise resulted in a blunted downregulation of *RhoA* expression. These findings confirmed an unfavourable effect of antioxidants on exercise-induced cardiovascular protection.

### 5.3 Reactive Oxygen Species Elimination and Physiological Processes

Given that reactive species play an important role in the regulation of muscle contractile activity, their elimination with high doses of antioxidants may result in negative effects on muscle function. We have shown that supplementation of rats with vitamin E and  $\alpha$ -lipoic acid decreased lipid peroxidation after a fatigue protocol but had no effect on fatigue resistance.<sup>[251]</sup> Moreover, high levels of vitamin E depressed muscle force production at low stimulation frequencies. Acute supplementation of rats with vitamin C and tempol, a radical scavenger, reduced skeletal muscle blood flow, oxygen utilization and force production at rest and during electrically stimulated contractions.<sup>[255]</sup>

Close and colleagues<sup>[197]</sup> found consumption of high doses of vitamin C in the days post-exercise delayed the recovery of muscle function in humans. Chronic supplementation of competitive kayakers with a mixture of vitamins and minerals failed to protect from exercise-induced oxidative stress and inflammation, and hindered the recovery of muscle damage after a 1000 m race.<sup>[165]</sup> Together, these findings suggest that ROS produced post-exercise play a role in muscle regeneration.

Physical activity is known to improve insulin sensitivity as the transient rise in ROS production efficiently counteracts insulin resistance.<sup>[91]</sup> In one of the most interesting studies on this topic, Ristow et al.<sup>[91]</sup> reported that supplementation with vitamins E and C inhibited the insulin sensitizing effects of exercise training, regardless of previous training status. They found that exercise-induced oxidative stress increased expression of ROS-sensitive transcriptional regulators



of insulin sensitivity PGC-1 $\alpha$ , PGC-1 $\beta$  and peroxisome proliferator-activated receptor- $\gamma$ , a nuclear receptor protein involved in fatty acid storage and glucose metabolism. Exercise training also decreased fasting plasma insulin levels and caused an adaptive response promoting endogenous antioxidant defence capacity by up-regulation of *SOD1*, *SOD2* and *GPX* gene expression. Supplementation with antioxidants precluded these health promoting effects of exercise in both pre-trained and untrained men.

Reactive species act as potent vasodilators and may be an important part of the vasodilatory response during exercise. Administration of an antioxidant cocktail (vitamins C, E and  $\alpha$ -lipoic acid) augmented plasma antioxidant capacity and reduced circulating levels of free radicals in a group of healthy young males.<sup>[254]</sup> Importantly, brachial artery vasodilation was decreased during a submaximal handgrip exercise in the supplemented group. The direct measurement of oxidative stress is a strength of this study. Wray et al.<sup>[256]</sup> from the same research group, showed that 6 weeks of single leg knee-extensor exercise lowered blood pressure at rest and during exercise in a group of mildly hypertensive older men. Acute administration of  $\alpha$ -lipoic acid, vitamin C and vitamin E after the training period returned blood pressure to pre-training values. Furthermore, with exercise training, vasodilation improved significantly, but the effect was blunted after consuming antioxidants. It was concluded that antioxidant administration negated the health benefits of exercise training in older individuals. Although the study only included six subjects, the authors state they had sufficient statistical power.

Negative outcomes following the combination of two potentially beneficial interventions emphasize the complex nature of oxidative stress. Reactive species in skeletal muscle are generated in response to physiological and pathophysiological stimuli and are not solely by-products of aerobic metabolism. Attempts to decrease their levels, such as, for example, through antioxidant supplementation, may lead to a blunting of positive effects of exercise and even deleterious health effects.

## 6. Limitations of the Studies and Future Directions

An obvious limitation of the current body of research on this topic is the lack of studies investigating antioxidants other than vitamin E, vitamin C and coenzyme Q<sub>10</sub>. Despite the vast range of antioxidant supplements commercially available, many of these compounds have not been studied based on our systematic search. Therefore, generalizing the results to all antioxidant supplements may be problematic. Furthermore, numerous methodological issues interfere with the ability to interpret the effects of antioxidant supplementation on exercise. These include differences in exercise protocols, subject population, dosage and form of supplements, duration and timing of supplementation, and the methodology used to assess oxidative stress. It should be made clear that detection of differences between treatment and control groups in measured indices does not imply cause and effect of antioxidant supplements. Most studies investigated the effect of supplementation in small groups of subjects and did not employ a crossover design that could easily lead to type I and type II errors.<sup>[99,202,249,250,259]</sup>

Null findings in supplementation studies could be partially explained by insufficient dosages or treatment durations and the lack of sensitive detection techniques. Most studies lacked information on the redox state of the subjects to confirm whether their endogenous defence system was actually overwhelmed by increased ROS formation. For instance, highly trained individuals may experience an attenuated oxidative stress response, especially with long-duration, low-intensity exercise protocols. This is likely due to an enhanced endogenous antioxidant defence that is sufficient to combat an increased free radical production, thus masking any potential effect of supplementation. However, prolonged vigorous exercise can lead to a very large increase in ROS production, overwhelming antioxidant systems. In such conditions, additional doses of antioxidants may not exert any significant effect on oxidative stress levels.

Furthermore, detection depends, to a large degree, on the tissue/biofluid sampled, the timing

of sampling and the sensitivity and specificity of the chosen biomarker. For example, in some studies, oxidative stress may have occurred preceding or following the sample collection and was therefore not detected. Importantly, nearly all of the studies included in the review did not determine the actual levels of ROS but, rather, measured indirect markers of oxidative stress, such as by-products of lipid, protein and DNA damage.<sup>[93,144,174,175,197,202,259]</sup> In addition, in the majority of the studies, a single assay analysis of oxidative stress was used. Indeed, investigating only a particular oxidative stress marker does not represent universal oxidative stress status. Given the complexity of oxidative stress, a number of markers should be chosen (e.g. lipid peroxidation and protein oxidation measures). Moreover, changes in redox status within cells may be compartmentalized and regulated via specific signalling pathways. It seems highly unlikely that various potential targets in cells would show an equivalent sensitivity to specific ROS. In addition, ROS are present in low concentrations in biological systems, have short half-lives and are highly reactive. Thus, direct measurement is difficult and as reactive species cannot be targeted easily exogenous antioxidants may not scavenge the relevant ROS.

Difficulty in quantifying oxidative stress and understanding the health implications of oxidative stress measures are important issues when establishing appropriate intervention strategies. Despite the increasing awareness of the importance of reactive species, screening and monitoring of oxidative stress has not yet become routinely available. Individuals are often recommended antioxidant therapy, although there is no test that advises whether to assess if they are exposed to increased levels of free radicals or have depleted antioxidant capacities.

Careful reassessment of the existing evidence is warranted to better understand the conflicting data and design future studies appropriately. There is a need for more rigorous clinical trial designs with populations under high levels of oxidative stress and carefully chosen outcomes. Large randomized controlled trials with exercising individuals consuming a variety of anti-

oxidant supplements and using hard endpoints, such as onset of disease, would need to be conducted to adequately address the question of the impact of antioxidant supplementation on exercise-induced oxidative stress. Bioavailability and pharmacokinetics of antioxidants should be examined closely to establish the dosage, timing and duration of supplementation that would significantly reduce oxidative stress levels in the study participants. In addition, nutrigenomic issues might be considered as people respond differently to particular antioxidants based on their genetic profile. Further research, supported by improved techniques to measure oxidative stress and target specific ROS, will help to clarify the potential roles of antioxidant supplements in exercise-training.

## 7. Optimizing Nutrition

### 7.1 Summary

Studies included in this review have demonstrated disparate results with regards to the effects of antioxidant supplementation on exercise-induced oxidative stress. In summary, there is insufficient evidence to recommend antioxidant supplements for exercising individuals who consume the recommended amounts of dietary antioxidants through food. Antioxidant supplements generally do not improve physical performance. There is little proof to support their role in prevention of exercise-induced muscle damage and enhancement of recovery. Although ingesting supplemental antioxidants can decrease exercise-induced oxidative stress, there is no evidence that this confers health benefits. Further work is warranted to illuminate the interactive effects of exercise training and antioxidant supplementation.

### 7.2 Current Recommendations

The outcomes of supplementation studies have important implications for nutritionists, physicians, practitioners, exercise trainers and athletes, as well as for the general population. Reports that high doses of antioxidants preclude health-promoting effects of exercise training and interfere with ROS-mediated physiological pro-

cesses suggest caution in the use of antioxidant supplements. Physically active individuals need to optimize their nutrition rather than use supplements. Diets rich in antioxidants should be attained by consuming a variety of fruits, vegetables, whole grains and nuts. Whole foods, rather than capsules, contain antioxidants presented in beneficial ratios and numerous phytochemicals that may act in synergy with the former to optimize the antioxidant effect. Antioxidant supplementation may be warranted when individuals are exposed to high levels of oxidative stress and struggle to meet the dietary antioxidant requirements. Athletes, who restrict their energy intake, use severe weight loss practices and eliminate one or more food groups from their diet or consume unbalanced diets with low micronutrient density, are at risk of suboptimal antioxidant status. A qualified sports dietitian would need to provide individualized nutrition direction and advice subsequent to blood analysis and comprehensive nutritional assessment. Careful product evaluation is required prior to adopting an antioxidant regimen, which should be clinically supervised and should only represent a short-term solution while dietary changes are being implemented.

## 8. Conclusions

The multifunctional role of reactive species in living organisms, and the beneficial and deleterious effects of antioxidant supplementation demonstrate the complexity of exercise-induced oxidative stress. Interactions of antioxidants and reactive species should be carefully considered as the redox state will dictate cell functioning. More detailed research and critical appraisal of the situations that may warrant antioxidant supplementation in exercise training are required. A balanced diet including a variety of fruits and vegetables remains the best nutritional approach to maintain optimal antioxidant status.

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Correspondence: *Tina-Tinkara Peternelj*, School of Human Movement Studies, University of Queensland, St Lucia, Brisbane, QLD, 4072, Australia.  
E-mail: [tpeternelj@hms.uq.edu.au](mailto:tpeternelj@hms.uq.edu.au)