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Dietary antioxidants and exercise

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Muscular exercise promotes the production of radicals and other reactive oxygen species in the working muscle. Growing evidence indicates that reactive oxygen species are responsible for exercise-induced protein oxidation and contribute to muscle fatigue. To protect against exercise-induced oxidative injury, muscle cells contain complex endogenous cellular defence mechanisms (enzymatic and non-enzymatic antioxidants) to eliminate reactive oxygen species. Furthermore, exogenous dietary antioxidants interact with endogenous antioxidants to form a cooperative network of cellular antioxidants. Knowledge that exercise-induced oxidant formation can contribute to muscle fatigue has resulted in numerous investigations examining the effects of antioxidant supplementation on human exercise performance. To date, there is limited evidence that dietary supplementation with antioxidants will improve human performance. Furthermore, it is currently unclear whether regular vigorous exercise increases the need for dietary intake of antioxidants. Clearly, additional research that analyses the antioxidant requirements of individual athletes is needed.

Keywords: antioxidants, exercise, oxidative stress, performance, reactive oxygen species.

Introduction

Radicals are molecules or fragments of molecules that possess an unpaired electron in their outer orbital. Because of this molecular instability, radicals are highly reactive and can promote damaging oxidation reactions with cellular proteins, lipids or DNA, leading to oxidative stress and impaired cellular function. Although regular physical exercise has many beneficial effects, it is now clear that muscular exercise results in increased production of radicals and other reactive oxygen species (Davies *et al.*, 1982; Reid *et al.*, 1992b; Borzone *et al.*, 1994; O'Neill *et al.*, 1996; Halliwell and Gutteridge, 1999). Furthermore, strong evidence indicates that reactive oxygen species are the primary cause of exercise-induced disturbances in muscle oxidation–reduction status (i.e. redox balance). Severe disturbances in cellular redox balance have been shown to contribute to oxidative injury and muscle fatigue (Ji *et al.*, 1988; Shindoh *et al.*, 1990; Reid *et al.*, 1992a; Nashawati *et al.*, 1993; O'Neill *et al.*, 1996). Given the

potential role of reactive oxygen species in contributing to oxidative stress and muscle fatigue, it is not surprising that skeletal muscle fibres contain defence mechanisms to reduce the risk of oxidative damage. Two major classes of endogenous protective mechanisms, the enzymatic and non-enzymatic antioxidants, work to reduce the harmful effects of reactive oxygen species in cells. Furthermore, dietary antioxidants interact with endogenous antioxidants to form a cooperative antioxidant network.

The aim of this review is to discuss our current understanding of the relationship between dietary antioxidants and muscular exercise. We begin with a short discussion of exercise-induced oxidative stress. We then provide an overview of common dietary antioxidants and address the important questions, 'Do antioxidants improve exercise performance?' and 'Does exercise increase the need for dietary antioxidants?'

Exercise-induced oxidant production

Davies *et al.* (1982) were the first to report that skeletal muscles produce radicals during contractile activity. Since then, many investigations have confirmed these observations and have explored the potential sources of

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exercise-induced radicals and reactive oxygen species (O'Neill *et al.*, 1996; Jackson, 1998; Reid, 2001; Ji, 2002; Reid and Durham, 2002). Determining the primary sites of reactive oxygen species production in skeletal muscle is a complex undertaking, as many pathways are capable of generating radicals in skeletal muscle. Nonetheless, current evidence indicates that the primary sources of radical production in skeletal muscle are the mitochondria, xanthine oxidase, NAD(P)H oxidase and the production of nitric oxide by nitric oxide synthase (Davies *et al.*, 1982; Jackson *et al.*, 1985; Reid *et al.*, 1992a,b; Borzone *et al.*, 1994; O'Neill *et al.*, 1996; Jackson, 1998). Secondary sources for radical production during exercise include autoxidation of catecholamines, radical generation by phagocytic white cells and radical formation due to the disruption of iron-containing proteins (Jackson, 1998; Halliwell and Gutteridge, 1999). Most investigators have concluded that radical production in the mitochondria is the primary source of radical production in contracting skeletal muscles. Indeed, while 95–98% of the oxygen consumption of skeletal muscle results in the formation of adenosine trisphosphate (ATP) and water, the remaining 2–5% of this oxygen undergoes one electron reduction to produce superoxide radicals (Jackson, 1998; Halliwell and Gutteridge, 1999). It follows that increased muscular activity results in an elevation in oxidative metabolism and a proportional increase in superoxide production. Hence, this increased production of radicals must be balanced by the antioxidant capacity of the muscle to prevent oxidant-mediated damage to proteins, lipids and DNA.

Overview of antioxidants

Oxidative stress occurs due to an imbalance between oxidant production and the antioxidant capacity of the cell (Fig. 1). Cells are protected against oxidant injury by a complex network of antioxidants. Specifically, enzymatic and non-enzymatic antioxidants exist in both the intracellular and extracellular environments and work as complex units to remove different reactive oxygen species. To provide maximum intracellular protection, these scavengers are strategically compartmentalized throughout the cell. Figure 2 illustrates the cellular locations of important antioxidants and Tables 1 and 2 provide a brief overview of the antioxidant function of these molecules.

Several strategies are applied by both endogenous and exogenous antioxidants to protect against reactive oxygen species-mediated injury. These include conversion of reactive oxygen species into less active molecules (i.e. scavenging) and prevention of the transformation of the least reactive oxygen species into more damaging
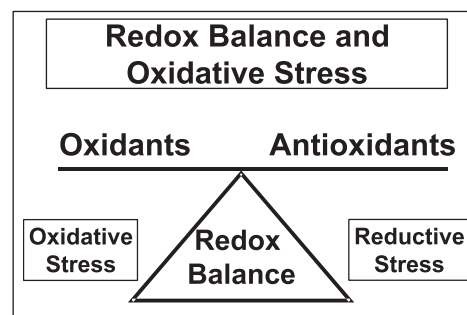

Fig. 1. Illustration of the relationship between oxidants and antioxidants in the determination of cellular redox balance. Note that an increase in oxidants or antioxidants results in a disturbance in cellular redox balance. Oxidative stress occurs when oxidants outnumber the available antioxidants. In contrast, reductive stress occurs when antioxidants outnumber the oxidants present in the cell.

forms (i.e. conversion of hydrogen peroxide to the hydroxyl radical). In the following sections, we discuss important dietary antioxidants and define their role in maintaining muscle redox balance.

Non-enzymatic and dietary antioxidants

Many non-enzymatic antioxidants exist in cells. Important non-enzymatic defences include, but are not limited to, glutathione, vitamin E, vitamin C, lipoic acid, carotenoids, uric acid, bilirubin and ubiquinone.

Glutathione

Glutathione is the most abundant non-protein thiol source in muscle cells (Meister and Anderson, 1983). Glutathione is primarily synthesized in the liver and transported to tissues via the circulation. Because of the peptide structure of glutathione, it is degraded in the small intestine when ingested; hence, cellular concentrations of glutathione are not directly influenced by diet.

Glutathione concentration in the cell is typically in the millimolar range, but there is wide variability in glutathione content across organs depending on their basal levels of radical production. For example, the two highest concentrations of glutathione in the body are found in the lens of the eye ($10 \ mmol \cdot l^{-1}$) and the liver ($5–7 \ mmol \cdot l^{-1}$) (Halliwell and Gutteridge, 1999). Other key organs such as the lung, kidney and heart contain about $2–3 \ mmol \cdot l^{-1}$ of glutathione (Ji, 1995a). Skeletal muscle glutathione concentration varies depending on muscle fibre type and animal species. In rats, (slow) type I fibres contain 600% more glutathione ($\sim 3 \ mmol \cdot l^{-1}$)
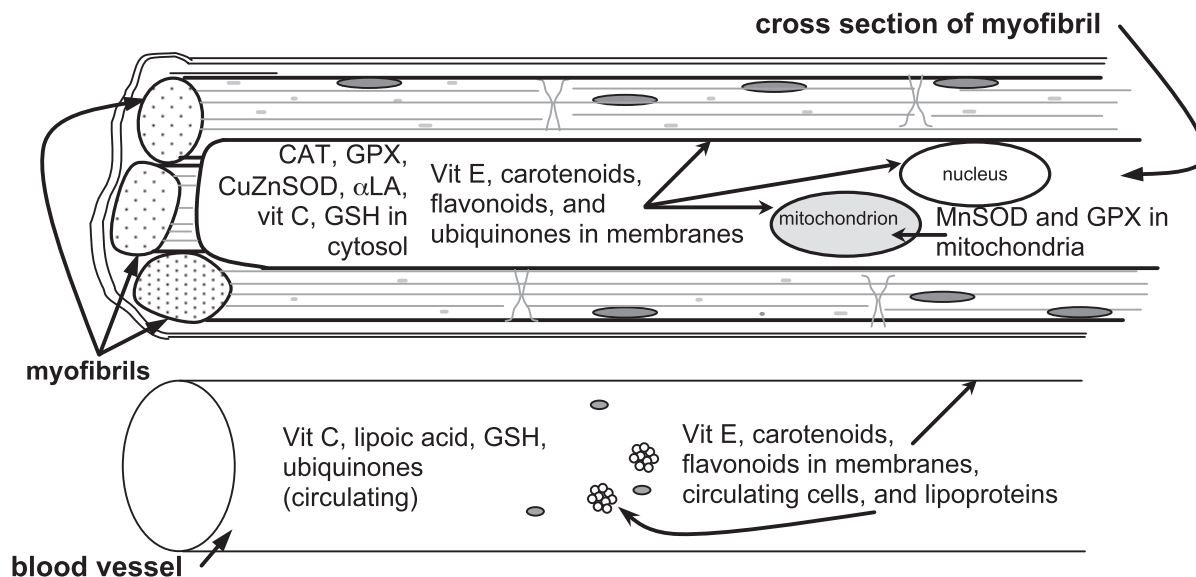
Fig. 2. Locations of important intracellular and extracellular antioxidants within the skeletal muscle myocyte. See text and Table 1 for details on antioxidant function. Vit E = vitamin E, Vit C = vitamin C, αLA = α = lipoic acid, GSH = glutathione, CAT = catalase, GPX = glutathione peroxidase.

Table 1. Important enzymatic antioxidants

Enzymatic antioxidants	Properties
Superoxide dismutase	Located in both mitochondria and cytosol; dismutates superoxide radicals
Glutathione peroxidase	Located in both mitochondria and cytosol; removes hydrogen peroxide and organic hydroperoxides
Catalase	Located in cytosol (and in mitochondria of heart); removes hydrogen peroxide

than (fast) type IIb fibres $(0.5 \text{ mmol} \cdot 1^{-1})$ (Ji, 1995a,b; Ji *et al.*, 1992).

Glutathione serves several roles in the cellular antioxidant defence system. First, glutathione directly scavenges a variety of radicals, including hydroxyl and carbon centred radicals, by donating a hydrogen atom (Yu, 1994). A second key antioxidant function of glutathione is to remove both hydrogen and organic peroxides (e.g. lipid peroxide) during a reaction catalysed by the enzyme glutathione peroxidase. During this reaction, glutathione donates a pair of hydrogen atoms and two glutathione are oxidized to form glutathione disulphide. Glutathione has also been shown to be involved in reducing or 'recycling' a variety

of antioxidants in the cell. For example, glutathione has been postulated to reduce vitamin E radicals that are formed in the chain-breaking reactions with alkoxyl or lipid peroxyl radicals (Packer, 1991). Furthermore, glutathione can also reduce the semi-dehydroascorbate radical (vitamin C radical) derived in the recycling of vitamin E and to reduce alpha-lipoic acid to dihydrolipoate. This reaction has recently been hypothesized to play an important role in the recycling of ascorbic acid (Packer, 1991). More will be said about these functions of glutathione in later sections.

Vitamin E

The generic term vitamin E refers to at least eight structural isomers of tocopherols or tocotrienols. Among these, α-tocopherol is the best known and possesses the most potent antioxidant activity (Burton and Ingold, 1989; Janero, 1991). From an antioxidant perspective, vitamin E is the primary chain-breaking antioxidant in cell membranes (Burton and Ingold, 1989; Janero, 1991).

Because of its high lipid solubility, vitamin E is associated with lipid-rich structures such as mitochondria, sarcoplasmic reticulum and the plasma membrane. Under most dietary conditions, the concentration of vitamin E in tissues is relatively low. For example, the ratio of vitamin E to lipids in the membrane may range from 1:1000 in red blood cells to 1:3000 in other tissues and organelles (Janero, 1991; Packer, 1991). Note, however, that vitamin E concen-

Table 2. A list of selected dietary antioxidants

Antioxidant	Properties	DRI[a]	UL[b]
Vitamin E	Lipid-soluble phenolic compound; major chain-breaking antioxidant found in cell membranes	15 mg	1000 mg
Vitamin C	Located in aqueous phase of cell; acts as radical scavenger and recycles vitamin E	90 mg	2000 mg
Glutathione	Non-protein thiol in cells; serves multiple roles in cellular antioxidant defence; can be consumed in diet but is degraded in the gut	NE	NE
α-Lipoic acid	Endogenous thiol; effective as an antioxidant and in recycling vitamin C; may also serve as a glutathione substitute	NE	NE
Carotenoids	Lipid-soluble antioxidants located primarily in membranes of tissues	NE	NE
Flavonoids	Amphipathic antioxidants located throughout cell; able to scavenge radicals in lipid and aqueous environments	NE	NE
Ubiquinones	Lipid-soluble quinone derivatives; reduced forms are efficient antioxidants	NE	NE

[a]Dietary reference intakes (DRI) are the most recent set of dietary recommendations established for Canadians and Americans by the Food and Nutrition Board of the Institute of Medicine, 1997–2001. The values shown are the highest DRI for each nutrient.
[b]The tolerable upper intake level (UL) is the upper level of intake considered to be safe for adults in Canada and the USA. In some cases, lower ULs have been established for children. NE = DRI or UL not established.
Source: Council for Responsible Nutrition, 1828 L. Street NW, Suite 900, Washington, DC 20036-5114, USA.

trations in tissues and organelles can be elevated with dietary supplementation (Janero, 1991).

As an antioxidant, vitamin E is particularly important because of its ability to convert superoxide, hydroxyl and lipid peroxyl radicals to less reactive forms. Vitamin E can also break lipid peroxidation chain reactions that occur during free radical reactions in biological membranes (Burton and Traber, 1990).

Although vitamin E is an efficient radical scavenger, the interaction of vitamin E with a radical results in a decrease in functional vitamin E and the formation of a vitamin E radical. Indeed, oxidative stress has been shown to significantly decrease tissue vitamin E concentrations (Burton and Traber, 1990; Janero, 1991; Packer, 1991). However, the vitamin E radical can be 'recycled' back to its native state by a variety of other antioxidants (Packer *et al.*, 1979; Burton and Traber, 1990). Therefore, it is postulated that the ability of vitamin E to serve as an antioxidant is synergistically connected to other antioxidants, such as glutathione, vitamin C and α-lipoic acid, which are capable of recycling vitamin E during periods of oxidative stress. This point is discussed in more detail in the next sections on vitamin C and α-lipoic acid.

Vitamin C

In contrast to vitamin E, vitamin C (ascorbic acid) is hydrophilic and functions better in aqueous environments than vitamin E. Because the pKa of ascorbic acid is 4.25, the ascorbate anion is the predominant form that exists at physiological pH (Yu, 1994). Ascorbate is widely distributed in mammalian tissues, but is present in relatively high amounts in the adrenal and pituitary glands (Yu, 1994).

The role of vitamin C as an antioxidant is two-fold. Vitamin C can directly scavenge superoxide, hydroxyl and lipid hydroperoxide radicals. Additionally, vitamin

C plays an important role in recycling the vitamin E radical back to its reduced state (Packer *et al.*, 1979). In the process of recycling vitamin E, reduced vitamin C is converted to a vitamin C (semiascorbyl) radical (Packer *et al.*, 1979). Recycling of the vitamin C radical can be achieved by NADH semiascorbyl reductase, or cellular thiols such as glutathione and dihydrolipoic acid (Sevanian *et al.*, 1985).

In light of the role of vitamin C in the recycling of vitamin E, increased cellular concentrations of vitamin C should provide protection against radical-mediated injury (Yu, 1994). However, in high concentrations (i.e. ~ 1 mmol·l^{-1}) vitamin C can exert pro-oxidant effects in the presence of transition metals such as Fe^{3+} or Cu^{2+}. The pro-oxidant action of vitamin C stems from its ability to reduce ferric iron (Fe^{3+}) to the ferrous (Fe^{2+}) state. Ferrous iron is known to be a potent catalyst in the production of free radicals. Therefore, the wisdom of mega-dose vitamin C supplementation has been questioned by some investigators due to its pro-oxidant potential (Yu, 1994).

α-Lipoic acid

α-Lipoic acid is an endogenous thiol that serves as a co-factor for α-dehydrogenase complexes and participates in S–O transfer reactions (Packer, 1994). Normally, α-lipoic acid is present in very small quantities (5–25 nmol·g^{-1}) in animal tissues and is generally bound to an enzyme complex that renders α-lipoic acid unavailable as an antioxidant (Packer, 1994). However, unbound α-lipoic acid may be effective as an antioxidant and in recycling vitamin C (Kagan *et al.*, 1992; Packer, 1994). α-Lipoic acid can be consumed in the diet and has no known toxic side-effects (Packer, 1994). Following dietary supplementation, α-lipoic acid is reduced to dihydrolipoic acid (DHLA), which is a potent antioxidant against all major oxyradical species (Packer, 1994). Furthermore, DHLA is an important agent in recycling vitamin C during periods of oxidative stress and can be an effective glutathione substitute (Kagan *et al.*, 1992; Packer, 1994).

Figure 3 illustrates the role of vitamin C and DHLA in the recycling of vitamin E during periods of oxidative stress. After the recycling of vitamin E, the vitamin C radical can be reduced back to vitamin C by DHLA. Dihydrolipoic acid is then converted back to α-lipoic acid in this process and can be reconverted to DHLA by cellular enzymatic mechanisms (Packer, 1994).

Carotenoids

Carotenoids (e.g. β-carotene) are lipid-soluble antioxidants located primarily in biological membranes. The antioxidant properties of carotenoids come from their
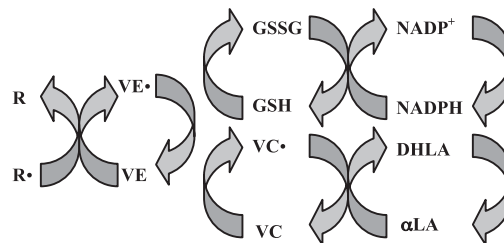

Fig. 3. Illustration of the interaction between α-lipoic acid (αLA), glutathione and vitamin C (VC) in the recycling of vitamin E (VE). VC• = ascorbate radical; VE• = vitamin E; DHLA = dihydrolipoic acid; GSSG = oxidized glutathione (adapted from Ji, 1995a).

structural arrangement consisting of long chains of conjugated double bonds; this arrangement permits the scavenging of several reactive oxygen species, including superoxide radicals and peroxyl radicals (Yu, 1994). Indeed, carotenoids display an efficient biological antioxidant activity, as evidenced by their ability to reduce the rate of lipid peroxidation induced by radical generating systems (Krinsky and Deneke, 1982).

Similar to vitamin C, β-carotene can function both as an antioxidant and a pro-oxidant. Under physiological oxygen partial pressures (i.e. < 100 mmHg), β-carotene exhibits radical scavenging activity. However, exposure to hyperoxic partial pressures (i.e. > 150 mmHg) results in β-carotene exerting pro-oxidant properties with a concomitant loss of its antioxidant capacity (Burton and Ingold, 1989; Palozza *et al.*, 1997).

Ubiquinone

Ubiquinones are lipid-soluble quinone derivatives that contain an isoprene or farnesyl tail. Ubiquinone homologues containing 1–12 isoprene units occur in nature. Reduced forms of ubiquinones are better antioxidants by several orders of magnitude (Mellors and Tappel, 1966).

The predominant form of ubiquinone in humans and many mammals is ubiquinone-10 (often called co-enzyme Q) (Karlsson, 1997). The major sources of ubiquinone-10 in the diet are soybean oil, meats, fish, nuts, wheat germ and vegetables (beans, garlic, spinach, cabbage) (Kamei *et al.*, 1986). The concentration of ubiquinone-10 in human plasma varies between 0.4 and 1.0 μmol·l^{-1}; approximately 80% is present in the reduced (ubiquinol) state (Stocker *et al.*, 1987; Aberg *et al.*, 1992). In human tissue, ubiquinone-10 is found in relatively high concentrations (60–110 μg·g^{-1}) in heart, liver and kidney, of which 70–100% is in the reduced state (Aberg *et al.*, 1992). With regard to the intracellular location, approximately 40–50% of the

Table 3. A list of trace minerals involved in antioxidant protection

Mineral	Function	DRI[a]	UL[b]
Copper	Co-factor for CuZnSOD-catalysed superoxide quenching	0.9 mg	10 mg
Iron	Co-factor for catalase-catalysed hydrogen peroxide decomposition	18 mg	45 mg
Manganese	Co-factor for MnSOD-catalysed superoxide quenching	2.3 mg	11 mg
Selenium	Co-factor for glutathione peroxidase-catalysed hydrogen peroxide decomposition	55 μg	400 μg
Zinc	Co-factor for CuZnSOD-catalysed superoxide quenching	11 mg	40 mg

[a]Dietary reference intakes (DRI) are the most recent set of dietary recommendations established for Canadians and Americans by the Food and Nutrition Board of the Institute of Medicine, 1997–2001. The values shown are the highest DRI for each nutrient.
[b]The tolerable upper intake level (UL) is the upper level of intake considered to be safe for adults in Canada and the USA. In some cases, lower ULs have been established for children.

total cellular ubiquinone is located within the mitochondria, 25–30% in the nucleus, 15–20% in the endoplasmic reticulum and the remaining 5–10% in the cytosol (Sustry *et al.*, 1961).

The antioxidant effect of ubiquinone is attributed to its phenol ring structure (Mellors and Tappel, 1966; Karlsson, 1997). Ubiquinones react with reactive oxygen species to prevent lipid peroxidation in membranes and other lipid structures in the cell (Karlsson, 1997). Also, some ubiquinones play an important role in the recycling of vitamin E during periods of oxidative stress via an NADPH-dependent system (Kagan *et al.*, 1990).

Flavonoids

Flavonoids are a large family of diphenylpropanes (over 4000 members have been identified) that are commonly found in plants consumed by humans. Family members include, but are not limited to, flavones, isoflavones, flavanones, anthocyanins and catechins (Das, 1994). Flavonoids have been reported to possess a wide variety of biological activities ranging from inhibition of inflammatory enzymes (e.g. lipoxygenase, cyclooxygenase, xanthine oxidase, NADH-oxidase, phospholipase A$_2$) to anti-tumoral, anti-viral, anti-mutagen, anti-inflammatory, anti-ischaemic and anti-allergic activities (Cao *et al.*, 1997). Many of these biological effects are thought to be a result of the antioxidant capacity of flavonoids (Bors *et al.*, 1994; Saija *et al.*, 1995; Scalbert *et al.*, 2002). Radical scavenging activities of flavonoids appear to vary greatly among family members, but include quenching of peroxyl, hydroxyl and superoxide radicals, as well as hydrogen peroxide and a variety of chemically generated radicals not naturally found in the body (Cao *et al.*, 1997).

Among the many flavonoids under investigation for their potential role in protecting against radical-mediated disease processes is the polyphenolic flavo-

noid family, the catechins, which are found in significant concentrations in green and black tea and red wine. Catechins are amphipathic and thus exert their antioxidant activities in both lipid and aqueous environments. They have been shown to be efficient scavengers of superoxide, hydroxyl and peroxyl radicals, to inhibit metal ion-mediated radical formation and to inhibit formation of lipid peroxyl radical species (Sichel *et al.*, 1991; Salah *et al.*, 1995; Lotito *et al.*, 2002). In addition to inhibiting lipid peroxidation, catechins were reported to prevent the radical-mediated depletion of vitamin E and β-carotene in human plasma in a dose-dependent manner (Lotito and Fraga, 1999).

Trace minerals associated with antioxidant defences

Several trace minerals play important but indirect roles in providing antioxidant protection in cells (Table 3). Trace minerals involved in antioxidant-related functions include copper (Cu), zinc (Zn), iron (Fe), selenium (Se) and manganese (Mn). These trace minerals contribute to the body's antioxidant defence system by acting as co-factors for antioxidant enzymes. In the following paragraphs, we provide a brief overview of the antioxidant function of each of these trace minerals. Supplementation of trace minerals is not generally necessary in well-nourished populations, but possible deficiencies of these minerals are addressed in the following discussion.

Copper contributes to cellular antioxidant protection as a co-factor for the antioxidant enzyme, CuZn-superoxide dismutase (CuZnSOD). This enzyme, located in the cytosol of cells, is responsible for eliminating superoxide radicals. Although copper deficiencies are uncommon in Western society, a copper deficiency would result in reduced levels of functioning CuZnSOD and an impaired cellular antioxidant defence system. Signs and symptoms of copper deficiency

include anaemia, a reduction in circulating neutrophils, bone loss and heart disease (Wardlaw and Insel, 1996; Halliwell and Gutteridge, 1999).

Zinc has been recognized as an essential nutrient since the early 1900s and is a co-factor for over 300 different enzymes (Wardlaw and Insel, 1996). In terms of the role of zinc as an antioxidant, it is an essential co-factor for CuZnSOD. As mentioned above, this enzyme is responsible for the removal of superoxide radicals from the cytosol of cells. It follows that a zinc deficiency would result in diminished CuZnSOD activity that would contribute to an impaired antioxidant capacity.

Iron is the most abundant transition metal in the body and plays a key role in the function of both haemoglobin and myoglobin (Halliwell and Gutteridge, 1999). Furthermore, iron is an essential co-factor in the antioxidant enzyme catalase. Catalase is located in both the cytosol and the mitochondria and is responsible for removing hydrogen peroxide from cells. An iron deficiency would not only impair oxygen transport in the body, but would also compromise the body's antioxidant capacity by lowering catalase activity in cells (Halliwell and Gutteridge, 1999).

Selenium plays a critical role in antioxidant defence as a co-factor for the antioxidant enzyme glutathione peroxidase. Glutathione peroxidase is located in both the cytosol and mitochondria of cells and is responsible for removing hydrogen peroxide and other organic hydroperoxides from the cell (Halliwell and Gutteridge, 1999). Selenium deficiency in humans has been reported in some areas of Europe and China and is associated with muscle pain, muscle wasting and cardiomyopathy (Wardlaw and Insel, 1996).

Manganese is a co-factor for several enzymes, including the important antioxidant enzyme, manganese-superoxide dismutase. This key antioxidant enzyme is located in the mitochondria and is responsible for eliminating superoxide radicals produced by oxidative phosphorylation (Halliwell and Gutteridge, 1999). Manganese deficiency in animals results in impaired brain development and reproduction. Nonetheless, manganese deficiency has not been reported in humans (Wardlaw and Insel, 1996).

Antioxidants and exercise performance

Antioxidant deficiencies and exercise performance

Antioxidant nutrient deficiencies are not widely reported among athletes (Clarkson, 1995). However, it is conceivable that an antioxidant nutrient deficiency could result in an increased susceptibility to exercise-induced damage by reactive oxygen species and thus lead to impaired exercise performance. Indeed, studies utilizing animal models have documented that vitamin E deficiency results in skeletal muscle degeneration and impaired exercise performance in rats (Davies *et al.*, 1982; Gohil *et al.*, 1986; Coombes *et al.*, 2002). Vitamin C deficiency in guinea pigs has also been shown to reduce times to exhaustion during treadmill running (Packer *et al.*, 1986). Failure to reverse the effects of vitamin E deficiency by vitamin C supplementation highlights the synergistic nature of antioxidant action (Gohil *et al.*, 1986).

It is important to emphasize caution when extrapolating results of animal studies to human populations. Although it is well established that a vitamin C deficiency impairs exercise performance in humans, marginally deficient individuals have not demonstrated similar adverse effects (van der Beek *et al.*, 1990). Additionally, in contrast to the findings of animal studies, vitamin E deficiency in humans does not appear to be associated with impaired exercise performance. Males who were made vitamin E deficient over a period of 13 months did not suffer from impaired performance or muscle weakness despite blood concentrations of vitamin E that were indicative of deficiency (Bunnell *et al.*, 1975). While the potential for antioxidant nutrient deficiencies do exist in athletes, the low incidence of vitamin deficiencies among athletes indicates that antioxidant deficiencies are not common (Clarkson, 1995).

Antioxidant effects on muscle contraction and exercise performance

It is well documented that exercise-related oxidant stress is associated with damage to lipids and protein in both muscle and blood cells (Alessio, 1993; Lawler *et al.*, 1993; Jackson, 1998; Mastaloudis *et al.*, 2001). In addition to imposing cellular damage, excessive reactive oxygen species has been shown to have an adverse effect on skeletal muscle contractile function and to exert a negative impact on performance (Reid and Durham, 2002). Pharmacologic antioxidant administration has been reported to decrease fatigue after electrically stimulated contractions of animal skeletal muscle (Barclay and Hansel, 1991; Reid *et al.*, 1992a; Supinski *et al.*, 1997). Additionally, infusion of *N*-acetylcysteine, a cysteine donor thought to increase the endogenous antioxidant glutathione, has been shown to attenuate muscle fatigue of the tibialis anterior and diaphragm muscles after low-frequency electrical stimulation in humans (Reid *et al.*, 1994; Travaline *et al.*, 1997). Collectively, these findings suggest that antioxidant supplementation may play a role in preserving skeletal muscle contractile function by scavenging exercise-induced reactive oxygen species and reactive nitrogen species.

The most convincing data suggesting ergogenic benefits from dietary antioxidant supplementation come from animal studies. Rodents with adequate nutritional status have demonstrated improved exercise performance after the administration of various forms of antioxidants (Novelli *et al.*, 1990, 1991; Asha Devi *et al.*, 2003). However, not all animal studies have demonstrated enhanced performance following antioxidant administration. For example, rats supplemented with vitamin E failed to improve treadmill endurance time to exhaustion (Mehlhorn *et al.*, 1989; de Oliveira *et al.*, 2003). One of these reports, however, was based on a preliminary experiment involving only one animal (Mehlhorn *et al.*, 1989).

In contrast to studies conducted on animals, studies in humans generally have not demonstrated enhanced exercise performance after antioxidant supplementation. The vast majority of studies investigating vitamin E supplementation have not demonstrated improvements in exercise performance (Shephard *et al.*, 1974; Lawrence *et al.*, 1975; Sumida *et al.*, 1989; Rokitzki *et al.*, 1994a,b). The main exception is a study conducted at high altitude in which the consumption of vitamin E was associated with a preservation of the anaerobic threshold (Simon-Schnass and Pabst, 1988). It was hypothesized that vitamin E supplementation at high altitude reduced red cell fragility and allowed for more efficient oxygen transport (Simon-Schnass and Pabst, 1988). Lack of a whole-body ergogenic effect for other antioxidants has also been reported in humans. In contrast to the findings of Reid *et al.* (1994), who found that administration of *N*-acetylcysteine resulted in decreased fatigue development in the tibialis anterior, infusion of *N*-acetylcysteine did not improve high-intensity cycling performance in untrained males (Medved *et al.*, 2003). Furthermore, other studies using antioxidant mixtures (Snider *et al.*, 1992) or selenium (Tessier *et al.*, 1995; Margaritis *et al.*, 1997) have not demonstrated improved exercise performance.

Investigations into the effects of vitamin C supplementation on exercise performance have demonstrated variable results. Vitamin C reportedly did not decrease markers of lipid peroxidation or improve recovery from unaccustomed exercise unless administered for 2 weeks prior to the exercise stress, which then resulted in modest improvements in muscle soreness (Thompson *et al.*, 2001, 2003). Other well-controlled studies with vitamin C have reported no beneficial effects on performance (Clarkson, 1995; Ashton *et al.*, 1999).

Ubiquinone-10 has been touted to possess ergogenic properties by increasing energy production via facilitating electron flux through the mitochondria and by functioning as an antioxidant. Nonetheless, among healthy individuals, only limited data illustrate the potential ergogenic properties of ubiquinone-10. In one study, a positive relationship between exercise capacity and the concentration of ubiquinone-10 in the vastus lateralis was reported in physically active males (Karlsson *et al.*, 1996). However, most studies investigating the effects of ubiquinone-10 supplementation on exercise performance have failed to authenticate these ergogenic claims. For example, supplementation of ubiquinone-10 alone (Braun *et al.*, 1991; Weston *et al.*, 1997; Bonetti *et al.*, 2000) or in combination with other antioxidants (Snider *et al.*, 1992) among groups of male athletes did not enhance performance. Additionally, male triathletes consuming ubiquinone-10 with ascorbic acid and vitamin E did not demonstrate altered energy metabolism or fatigue of the gastrocnemius muscle after plantar flexion exercise (Nielsen *et al.*, 1999). Furthermore, some studies have actually demonstrated impaired performance following high-intensity (Malm *et al.*, 1997) and endurance exercise tests (Laaksonen *et al.*, 1995) among males supplemented with ubiquinone-10. Collectively, these studies do not support the use of ubiquinone-10 as a dietary supplement for the purpose of enhancing exercise performance.

Summary

Many factors may account for the observed discrepancies between studies examining the effects of antioxidant supplementation on exercise performance, including: (1) the antioxidants delivered, (2) the antioxidant dose and (3) the type and intensity of the exercise performed. Furthermore, in many of these studies, it is unlikely that an optimal dosing strategy was identified or utilized. Evidence also suggests that high doses of antioxidants may shift the intracellular redox balance towards a reduced state and impair skeletal muscle contractile function and exercise performance (Coombes *et al.*, 2001; Marshall *et al.*, 2002). Therefore, from an exercise performance standpoint, indiscriminant antioxidant supplementation could be detrimental. Hence, to date, little evidence exists to recommend antioxidant supplementation for the purpose of performance enhancement.

Exercise and antioxidant requirements

As discussed earlier, current opinion holds that exercise-induced oxidative stress may be deleterious to exercise performance. This notion is based on cellular (Alessio, 1993; Lawler *et al.*, 1993) and extracellular (Mastaloudis *et al.*, 2001) indices of oxidant damage to lipids and proteins after exercise. Empirical data usually demonstrate that dietary antioxidant supplementation diminishes blood (Sumida *et*

al., 1989; Ashton *et al.*, 1999) and cellular markers (Goldfarb *et al.*, 1994) of radical-mediated damage during exercise. Excessive exposure to environmental pollutants during training may further support the need for antioxidant supplementation (Papas, 1996). However, the current consensus on antioxidant supplementation for athletes remains equivocal because of a paucity of well-designed studies that clearly outline the need for dietary antioxidants in highly trained populations. The remainder of this review will address athletes' risk for exercise-induced oxidative stress, existing evidence for supplementation efficacy and perspectives for future directions in antioxidant supplement research and athletics.

Exercise and oxidative stress: are athletes at increased risk?

Though not fully supported experimentally, a plausible rationale for supplementation of antioxidants does exist. The increased oxidant load experienced by competitive athletes during training is thought to necessitate antioxidant supplementation (van der Beek, 1985). Training-related oxidant stress is associated with adaptations that improve the ability of the muscle cells to quench reactive oxygen species. Well-characterized examples of these adaptations include increases in enzymatic antioxidants within active skeletal muscle (for reviews, see Powers *et al.*, 1999; Powers and Shanely, 2002). Despite these protective adaptations of exercise training against cellular oxidative stress, sustained exercise imposes an acute cellular oxidative stress even in highly adapted skeletal muscle (Higuchi *et al.*, 1985). The greater training loads of competitive athletes, compared with their recreationally fit counterparts, may further compound this oxidative stress (Tiidus, 1998). In support of this notion, the magnitude of blood oxidative stress markers appears to be dose-dependent relative to increased exercise duration (Hessel *et al.*, 2000) and intensity (Alessio *et al.*, 2000; Quindry *et al.*, 2003).

If the need for dietary antioxidant supplements exists, however, one would expect a causal relationship between acute physical activity and decreased concentrations of key plasma antioxidants. A recent investigation reported a 75% increase in a plasma marker of lipid peroxidation that corresponded with an increased rate of plasma vitamin E turnover after an ultramarathon run (Mastaloudis *et al.*, 2001). Similarly, Bergholm and colleagues reported that chronic endurance running resulted in decreases of 18, 20 and 15% of plasma concentrations of α-tocopherol, β-carotene and retinal, respectively (Bergholm *et al.*, 1999). Nonetheless, others have found either no change or even an increase in blood concentrations of both vitamin E and

ascorbate after an acute bout of endurance exercise (Liu *et al.*, 1999).

Importantly, caution is recommended when interpreting altered plasma antioxidant concentrations in response to acute exercise. Indeed, changes in plasma vitamin E and vitamin C during exercise may represent a complex redistribution between tissue and plasma antioxidant stores (Ji, 1995a; Liu *et al.*, 1999). One also cannot assume that plasma redox status is indicative of cellular (e.g. muscle) redox balance (Quindry *et al.*, 2003).

Extended exposure to environmental air pollutants, including ozone, sulphur dioxide and nitrogen dioxide, during endurance training may present an additional oxidant source (van Klaveren and Nemery, 1999). Moreover, high amounts of environmental air pollutants can limit exercise performance (Pierson *et al.*, 1986). Certainly, exposure to these and other environmental factors, including ultraviolet radiation, has led to the suggested need for antioxidant supplementation (Papas, 1996; Packer and Valacchi, 2002). However, logic would suggest that if exercise creates a clear need for dietary antioxidant supplementation, epidemiological and/or empirical data would directly link exercise and oxidative stress to some form of morbidity. On the contrary, epidemiological data generally indicate that fit individuals are healthier than their sedentary counterparts (Blair *et al.*, 2001).

Efficacy of antioxidant supplementation to reduce oxidative stress

Evidence addressing the efficacy of supplementation in athletic populations remains ambiguous. Existing studies generally report decreased oxidative damage after antioxidant supplementation. However, only limited data demonstrate the ability of antioxidant supplementation to prevent the exercise-associated rise in markers of oxidative stress. For example, Sumida *et al.* (1989) demonstrated that 4 weeks of vitamin E supplementation prevented the rise in plasma malondialdehyde and markers of muscle damage observed in non-supplemented controls after maximal-intensity cycle exercise. In contrast to these findings, daily supplementation with an antioxidant mixture (30 mg β-carotene, 592 mg α-tocopherol and 1000 mg ascorbate) did not prevent the exercise-induced rise in plasma malondialdehyde after moderate- to high-intensity treadmill running (Kanter *et al.*, 1993). Additionally, Helgheim *et al.* (1979) reported that vitamin E supplementation was ineffective in preventing a rise in blood markers of muscle damage. Collectively, these studies illustrate the mixed results that have been reported about the effectiveness of antioxidant supplementation in decreasing exercise-induced oxidative stress.

Perspectives and future directions

While a firm case for the necessity of dietary antioxidant supplementation in athletes cannot be made at this time, limited supplementation of antioxidants may not harm the athlete (Tiidus, 1998). Nonetheless, mega-dosing antioxidant supplements can be detrimental to health. For example, augmentation of cellular antioxidant status may attenuate the inflammatory response to muscle-damaging exercise, thereby limiting the rate of muscle regeneration (Tiidus, 1998). Furthermore, some investigators have also reported that antioxidant supplementation appears to allosterically decrease cellular and extracellular antioxidant enzyme activity during exercise (Goldfarb *et al.*, 1994; Reddy *et al.*, 1998). These findings may simply indicate that supplementation altered redox balance such that elevated endogenous antioxidant enzyme activity was unnecessary. Finally, transient pro-oxidant shifts in redox balance, as experienced during exercise, regulate signal transduction for gene transcripts, including heat shock proteins and endogenous antioxidants (Sen, 2001). Thus, high levels of antioxidant supplementation may blunt some cellular adaptations induced by exercise. Clearly, additional research is necessary before it can be stated with certainty that exercise creates a need for dietary antioxidant supplementation in athletes without nutritional deficiency.

Conclusions

Muscular exercise results in an increased production of radicals and other forms of reactive oxygen species. Furthermore, growing evidence implicates cytotoxic reactive oxygen species as an underlying aetiology in exercise-induced disturbances in muscle redox status that could result in muscle fatigue or injury.

Muscle cells contain complex cellular defence mechanisms to minimize the risk of oxidative injury. Two major classes of endogenous protective mechanisms work together to decrease the harmful effects of oxidants in cells: (1) endogenous (enzymatic and non-enzymatic antioxidants) and (2) exogenous (non-enzymatic) antioxidants. Important antioxidant enzymes include superoxide dismutase, glutathione peroxidase and catalase. The trace minerals copper, zinc, manganese, selenium and iron serve as co-factors for these essential antioxidants. Important non-enzymatic antioxidants include vitamins E and C, β-carotene, glutathione and ubiquinone. Collectively, these endogenous and exogenous antioxidants form a network to protect the cell against oxidative stress.

Although many animal experiments have demonstrated that the addition of antioxidants can improve muscular performance, to date there is limited evidence that dietary supplementation with antioxidants will improve human performance. Furthermore, it is not known if vigorous exercise training increases the need for dietary antioxidants. Therefore, dietary supplementation with antioxidants cannot be recommended at the present time.

Acknowledgements

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