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REVIEWS: CURRENT TOPICS

The influence of antioxidant supplementation on markers of inflammation and the relationship to oxidative stress after exercise

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Abstract

Interest in the relationship between inflammation and oxidative stress has increased dramatically in recent years, not only within the clinical setting but also in the fields of exercise biochemistry and immunology. Inflammation and oxidative stress share a common role in the etiology of a variety of chronic diseases. During exercise, inflammation and oxidative stress are linked via muscle metabolism and muscle damage. Because oxidative stress and inflammation have traditionally been associated with fatigue and impaired recovery from exercise, research has focused on nutritional strategies aimed at reducing these effects. In this review, we have evaluated the findings of studies involving antioxidant supplementation on alterations in markers of inflammation (e.g., cytokines, C-reactive protein and cortisol). This review focuses predominantly on the role of reactive oxygen and nitrogen species generated from muscle metabolism and muscle damage during exercise and on the modulatory effects of antioxidant supplements. Furthermore, we have analyzed the influence of factors such as the dose, timing, supplementation period and bioavailability of antioxidant nutrients.

Keywords: Cytokines; Reactive oxygen species; Muscle damage; Cortisol; C-reactive protein

1. Introduction

Exercise is known to have many benefits, including preventative and therapeutic effects on a variety of chronic disorders such as diabetes mellitus; dyslipidemia; hypertension; obesity; cardiovascular and pulmonary diseases; muscle, bone and joint diseases; cancer; and depression [1]. Many of these chronic disorders share a common link with chronic low-grade inflammation and overproduction of reactive oxygen and nitrogen species (RONS) [2]. Increasing evidence suggests that the health benefits of exercise are partly linked to reduced levels of inflammation and oxidative stress [3,4].

It is well accepted that the health benefits of exercise are enhanced by positive dietary modification. The relationship between inflammation and oxidative stress has generated interest in the benefits of antioxidant supplements in health and disease, as well as athletic performance and adaptation to training. Over the years, exercise scientists have examined the potential effect of antioxidant nutrients to counter the influence of RONS on muscle damage, muscle fatigue, lipid peroxidation and damage to cellular proteins and DNA during exercise. However, attention has more recently shifted toward the specific interaction between antioxidant nutrients, redox-sensitive signaling pathways and inflammatory responses to exercise. Evidence suggests that antioxidant supplementation may in fact attenuate some of the exercise-induced cellular signals that stimulate adaptations in vascular tissue and skeletal muscle also exists [5,6]. The number of studies that have investigated this issue has risen in recent years, and this has stimulated interesting debate [7–11].

The aim of this review article is to collate and review the findings of studies that have examined the effects of antioxidant supplements on inflammatory responses to exercise. In the first part of this review, we have briefly discussed the mechanisms that contribute to cytokine changes during exercise. This is followed by a short discussion of the mechanisms of production of RONS

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during exercise, the signaling pathways that may regulate cytokine production during exercise and, more specifically, how such pathways are influenced by RONS and antioxidant nutrients. In the second half of this review, we have provided a detailed description of the findings from studies that have investigated the interaction between antioxidants, RONS and inflammation after exercise. This is followed by a discussion of the nutritional and biochemical factors that may account for the disparate findings of studies in this area, including the dose, timing and period of supplementation, in addition to the bioavailability and activity of antioxidants used in these studies. The main focus of this review is on the relatively new issue of the relationship between antioxidants and inflammatory responses to exercise. Related issues such as the influence of antioxidant supplementation on markers of muscle damage and oxidative stress have been reviewed elsewhere. Consequently, we have not addressed these issues in detail in the present review.

2. Exercise and cytokine production

A basic representation of the interactions between exercise, RONS, antioxidants and cytokines is shown in Fig. 1. Cytokine production during exercise is influenced by a number of factors [12]. Muscle glycogen breakdown and Ca^{2+} are two important factors that regulate cytokine production within skeletal muscle during exercise [13]. Immune cells are mobilized and activated during exercise in response to muscle damage and also via the actions of stress hormones (catecholamines, growth hormone, cortisol) that are released in response to increasing metabolic demands and core temperature during exercise [14,15]. Interactions between immune cells and stress hormones contribute to alterations in cytokine production [16]. Elevated core temperature during exercise can promote the leakage of endotoxins (lipopolysaccharide) across the intestinal wall into the circulation, and this may alter cytokine production [17].

Exercise-induced oxidative stress is another factor that affects cytokine production. Oxidative stress may result from oxidative reactions within skeletal muscle as well as from muscle damage [18]. RONS generated through oxidative metabolism and muscle damage can activate redox-sensitive signal transduction pathways that control cytokine production, such as those involving nuclear transcription factor κB (NF-κB), calcinuerin-nuclear factor activated T cells (NFAT) and heat shock proteins (HSPs) [19–23]. During exercise, endogenous antioxidant enzymes and dietary antioxidant supplements can potentially attenuate cytokine production by directly neutralizing RONS [24] and/or inhibiting the activity of redox-sensitive signal transduction pathways [20,21]. Some cytokines themselves may cause production of RONS and/or the activation of NF-κB [25]. Finally, instead of acting as *anti*oxidants in some situations, α-tocopherol and ascorbic acid may actually act as '*pro*-oxidants,' thereby enhancing rather than reducing the formation of RONS [26].

3. Production of ROS and RNS during exercise

In response to electrical stimulation, cultured myotubes produce reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals and hydrogen peroxide (H_2O_2), as

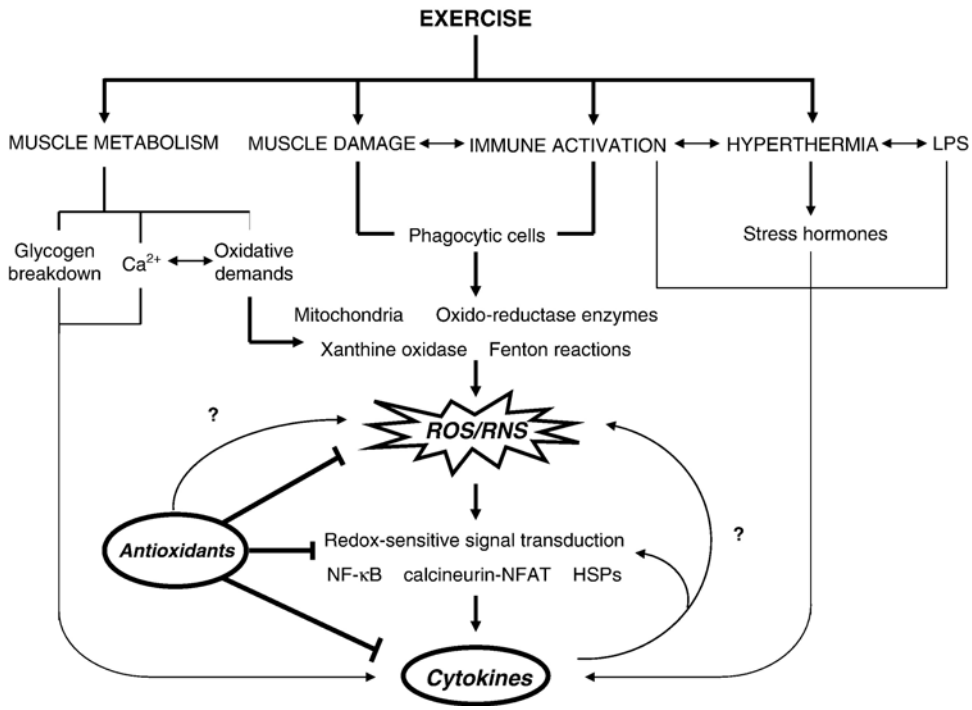

Fig. 1. A schematic that indicates the interactions between exercise, ROS and RNS, antioxidants and cytokines. LPS, lipopolysaccharide.

well as reactive nitrogen species (RNS) such as nitric oxide [27–29]. Voluntary muscle contractions also stimulate the release of free radicals from muscle into the bloodstream [24,30,31]. During muscle contractions, RONS may be produced as a result of aerobic metabolism and/or activation of phagocytic cells in response to muscle damage. Within skeletal muscle, potential sources of RONS include (a) mitochondria, (b) an unidentified oxidoreductase enzyme located in the plasma membrane of fibroblasts and immune cells present within muscle, (c) xanthine oxidase produced by endothelial cells and (d) iron-catalyzed reactions [28,32,33]. Although the majority of early studies investigated the potential for damaging contractile activity (eccentric exercise) to generate RONS, these reactive species are also produced in response to nondamaging contractions (concentric exercise) [27,28].

These findings have led to interest in researching the effects of antioxidant supplementation — and therefore the involvement of RONS — on changes in cytokines and other markers of inflammation (e.g., cortisol, C-reactive protein) following both eccentric and concentric exercises. Due to the greater amount of muscle damage that is typically associated with eccentric exercise, there may be differences in terms of the amount and source of RONS that are generated following eccentric versus concentric exercise. This might partially explain why antioxidant supplementation attenuates cytokine responses after some forms of exercise but not others.

4. Redox regulation of cytokine production

Redox regulation of cytokine production occurs at several levels including direct effects of oxidants, modulation by antioxidants, alterations in the redox equilibrium (e.g., ratio of reduced:oxidized glutathione and thioredoxin) and activation of oxidant- and redox-sensitive transcription cofactors such as NF-κB [19]. Among these regulatory pathways, two studies have directly implicated ROS and NF-κB with cytokine production by muscle cells in vitro [20,21], while the majority of exercise studies have focused on modulation of cytokine production by antioxidants. The following sections describe the involvement of redox-sensitive transcription cofactors in the regulation of cytokine production.

4.1. NF-κB as a redox-sensitive pathway for cytokine production

The NF-κB signaling pathway is one particular redox-sensitive signaling pathway that is proposed to regulate cytokine production during exercise [20,21]. Exercise activates the NF-κB signaling pathway in skeletal muscle [20,34,35] and lymphocytes [36]. This effect may be mediated in part by RONS [20,21]. Evidence in support of this concept comes from the data of two studies involving antioxidants. One study reported that in rats a 3-week diet high in α-tocopherol (500 mg/kg) attenuated the activation of NF-κB, the protein content of cytokine-induced neutrophil chemoattractant-1 (CINC-1), monocyte chemotactic protein-1 (MCP-1) and the activity of neutrophil-derived myeloperoxidase in skeletal muscle after exercise [20]. The same group also incubated myoblast cells with 100 μM D-α-tocopherol for 24 h and then exposed the cells to H_2O_2. D-α-tocopherol inhibited the translocation of NF-κB into the cell nucleus and the mRNA expression of CINC-1 and MCP-1 [20]. These data indicate, firstly, that RONS are involved in the activation of NF-κB and, secondly, that inhibition of NF-κB attenuates the production of cytokines in muscle cells.

In another study, myotubes exposed in vitro to H_2O_2 produced the cytokine interleukin (IL)-6 in a concentration-dependent manner [21]. This effect was blocked by the pretreatment of the myotubes with the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). Blocking of p38 mitogen-activated protein kinase (MAPK) and NF-κB also reduced the amount of IL-6 produced when the myotubes were stimulated with H_2O_2. When undifferentiated myoblast cells were stimulated with tumor necrosis factor (TNF)-α, IL-6 was produced. This effect was also blocked by the glutathione precursor *N*-acetylcysteine (NAC) [21]. These data indicate that IL-6 is produced in muscle cells via redox-sensitive pathways that involve p38 MAPK and NF-κB.

Despite this evidence for a role of RONS and NF-κB in the production of cytokines within skeletal muscle cells, there is also evidence suggesting that RONS may not be universal messengers in the activation of NF-κB. Instead, RONS may work as second messengers to activate NF-κB [37]. The ability of RONS to activate NF-κB depends on the cell type and may be linked to the levels of antioxidants within the cells. Even within one cell type, the effects of RONS can be variable [38]. Cytokines such as TNF-α can activate NF-κB independently of RONS, and in some cases, RONS actually inhibit these effects [38]. Lastly, antioxidants (e.g., ascorbic acid, NAC and pyrrolidine diothiocarbamate) can inhibit activation of NF-κB independently of any true antioxidant mechanisms [39,40].

4.2. Calcineurin-NFAT as a redox-sensitive pathway for cytokine production

Another redox-sensitive signaling pathway that has been proposed to regulate cytokine production during exercise is the calcineurin-NFAT pathway [13]. Calcineurin is highly concentrated in skeletal muscle [41]. When activated by calcium, calcineurin subsequently dephosphorylates and induces the nuclear localization of the cytosolic components of NFAT. Once in the nucleus, NFAT transcription complexes bind to DNA to regulate the expression of genes involved in immune responses [42]. Indeed, the calcineurin-NFAT signaling pathway plays a key role in the synthesis of TNF-α, IL-1β, IL-6 [43–45] and NF-κB [46]. Interaction between NFAT and the transcription factor activating protein-1 also promotes cytokine production [47].

Several studies have investigated the interaction between antioxidants, RONS and calcineurin-NFAT signaling.

Pretreatment of human MRC5 fibroblast cells with 10 μM DL-α-tocopherol reduced lipid peroxidation [48] and partially prevented an increase in free intracellular Ca^{2+} and NFAT binding activity following exposure to ultraviolet A radiation [22]. In another study by the same group, pretreatment of human Jurkat (T lymphocyte) cell lines with 10 μM DL-α-tocopherol also attenuated the production of RONS, the rise in free intracellular Ca^{2+} and NFAT binding activity following exposure to copper-oxidized and monocyte-oxidized low-density lipoproteins [49]. These data indicate that the calcineurin-NFAT signaling pathway is redox sensitive. In contrast to these findings, exposure of splenic T cells from rats to xanthine/xanthine oxidase and H_2O_2 actually reduced the binding activity of NFAT and NF-κB [50]. This result suggests that the effect of RONS on these transcription factors may be specific to certain cell types.

To date, no studies have investigated the involvement of the calcineurin-NFAT signaling pathway in cytokine synthesis during exercise. However, Holmes et al. [51] have demonstrated that incubation of muscle with the calcium ionophore, ionomycin, at a concentration of 10 μM increased IL-6 mRNA expression and protein release. Therefore, calcium release during exercise may stimulate IL-6 production via activation of the calcineurin-NFAT signaling pathway [13]. Further research is needed to determine whether exercise-induced alterations in this pathway are modified by antioxidants and whether this influences inflammatory responses to exercise.

4.3. HSPs as redox-sensitive mediators of cytokine production

Evidence showing that HSPs play an important role in exercise-induced immune changes is increasing [12,52]. Heat is a stimulus for the induction of HSPs, but RONS have been directly implicated as mediators of this response [23,53,54]. HSPs are induced by oxidative damage to the upstream signaling protein heat shock factor (HSF)-1 [55]. Antioxidants attenuate HSP induction in immune cells exposed to hot conditions [53] and also when immune cells are exposed directly to RONS [23]. It is unclear whether antioxidants work directly to modify HSF-1 activity or inhibit HSF-1 activation by preventing oxidative damage to HSF-1 [55]. Exercise studies are yet to confirm a link between oxidative modification of HSPs and subsequent inflammatory responses. However, antioxidants attenuate the release of HSPs into the circulation, in addition to HSP mRNA expression and protein content in skeletal muscle following exercise [23,56,57]. Therefore, future research could be aimed at investigating the relationships between exercise-induced oxidative stress, induction of HSPs and cytokine production.

5. Antioxidant supplements and cytokine responses to concentric exercise

Several studies have examined the efficacy of antioxidant supplements on cytokine responses to nondamaging exercise such as cycling and knee-extension exercise (Table 1). During these types of exercise, antioxidants might be expected to alter cytokine production via their effect on RONS produced from sources such as mitochondria and xanthine oxidase. Muscle damage is relatively minor following these types of exercise, and therefore, RONS produced by phagocytic cells probably have less influence on cytokine production after cycling and knee-extension exercise.

5.1. Supplementation with vitamin C

Davison and Gleeson [58,59] have carried out two studies to investigate the effect of vitamin C on changes in IL-6 following 2.5 h of cycling at 60% VO_{2max}. Acute supplementation with vitamin C during exercise did not influence changes in plasma IL-6 concentration after exercise in moderately trained males (Table 1) [58]. It should be noted in Table 1 that plasma IL-6 concentration was lower following exercise versus the placebo only when carbohydrate was combined with vitamin C. Vitamin C had no significant effect on plasma cortisol concentration after exercise. In their second study, supplementation with 1000 mg vitamin C per day for 2 weeks also had no significant effect on plasma IL-6 concentration following exercise in well-trained cyclists (Table 1) [59]. Vitamin C tended to reduce plasma cortisol concentration after exercise ($P=.08$). As a marker of oxidative stress, plasma malondialdehyde concentration remained unchanged after exercise. In vitro production of ROS by stimulated neutrophils decreased after exercise and was not affected by vitamin C.

5.2. Supplementation with antioxidant combinations

Vassilakopoulos et al. [60] investigated the effect of a complex antioxidant supplementation regimen on changes in the plasma concentrations and monocyte production of IL-1β, TNF-α and IL-6 after 45 min of cycling at 70% VO_{2max} (Table 1). The study involved a crossover design. Supplementation significantly attenuated plasma cytokine concentrations, while there was no effect of exercise or supplementation on monocyte cytokine production.

Fischer et al. [61] gave physically active males a combination of 500 mg vitamin C and 400 IU *RRR*-α-tocopherol per day for 4 weeks before and 1 day after 3 h of two-legged knee extension exercise at 50% maximum power output. The antioxidant supplement attenuated the release of IL-6 from contracting muscle. Furthermore, the plasma concentrations of IL-6, IL-1 receptor antagonist (IL-1ra), C-reactive protein and cortisol were lower following exercise in the supplement versus the placebo group (Table 1). As evidence of a reduction in oxidative stress, the plasma concentration of F_2-isoprostanes did not change significantly after exercise in the supplement group, whereas it increased significantly in the placebo group.

Hagobian et al. [62] examined the effect of antioxidant supplementation on cytokine responses to exercise at a high altitude (4800 m). Moderately trained males were given a

Table 1
Effects of antioxidant supplementation on changes in antioxidant status, cytokines, cortisol and oxidative stress markers after concentric exercise

Reference	Subjects	Exercise	Design	Supplement	Resting vitamin status[a]	Parameters	Maximum change from preexercise
Davison and Gleeson [58]	Moderately trained	2.5 h cycling 60% VO_{2max}	Crossover ($n=6$)	Placebo	No data available	IL-6	+8×
						Cortisol	+1.9×*
				Carbohydrate		IL-6	+3×*,†
						Cortisol	+1.1×†
				Vitamin C (0.15% w/v)		IL-6	+6×*
						Cortisol	+1.5×
				Carbohydrate + vitamin C (0.15% w/v)		IL-6	+3×*,†
						Cortisol	+1.1×†
Davison and Gleeson [59]	Well trained	2.5 h cycling 60% VO_{2max}	Crossover ($n=9$)	Placebo	Plasma (vitamin C) 47 μmol/L	Vitamin C	+1.1×
						IL-6	+12×*
						MDA	No change
						Cortisol	+2×*
				1000 mg vitamin C per day for 2 weeks before exercise	Plasma (vitamin C) 92 μmol/L†	Vitamin C	No change
						IL-6	+12×*
						MDA	No change
						Cortisol	+1.5×
Fischer et al. [61]	Physically active	3 h two-legged dynamic knee extensor exercise at 50% maximum power output	Placebo controlled ($n=14$)	Placebo	Plasma (vitamin E) 17 μmol/L	Vitamin E	No change
						Vitamin C	−6%
					Plasma (vitamin C) 51 μmol/L	IL-1ra	+6×*
						IL-6	+20×*
						F_2-IsoP	+2.3×*
						Cortisol	+2×*
				400 IU *RRR*-α-tocopherol and 500 mg vitamin C per day for 4 weeks before and 1 day after exercise	Plasma (vitamin E) 24 μmol/L	Vitamin E	+7%†
						Vitamin C	−1.2×*,†
					Plasma (vitamin C) 105 μmol/L†	IL-1ra	+4.4×
						IL-6	+12×*,†
						F_2-IsoP	+1.2×
						Cortisol	−1.1×
Vassilakopoulos et al. [60]	Untrained individuals	45 min cycling 70% VO_{2max}	Crossover ($n=6$)	200 mg vitamin E, 50,000 IU vitamin A and 1000 mg vitamin C for 60 days prior to exercise; 600 mg allopurinol per day for 15 days before exercise and 2 g NAC for 3 days and 800 mg in the morning before exercise	No data available	Before supplement:	
						TNF-α	+1.6×*
						IL-1β	+3.1×
						IL-6	+6×*
						After supplement:	
						TNF-α	+1.2×
						IL-1β	No change
						IL-6	+2.8×*

Abbreviations: F_2-IsoP, F_2-isoprostanes. MDA, malondialdehyde.
[a] Data are means.
* Significantly different from preexercise values, $P<.05$.
† Significantly different from placebo group, $P<.05$.

placebo or an antioxidant supplement containing 250 mg vitamin C, 10,000 IU β-carotene, 200 IU α-tocopherol, 50 μg selenium and 15 mg zinc for 3 weeks before cycling at 55% VO_{2max} for approximately 3 h. Supplementation did not influence changes in the plasma concentrations of IL-6 and C-reactive protein, while plasma TNF-α concentration remained unchanged following exercise.

5.3. Discussion

The results of studies by Vassilakopoulos et al. [60] and Fischer et al. [61] indicated that antioxidant supplements attenuate inflammatory responses to concentric exercise during which muscle damage and inflammation are minimal. Both of these studies used a combination of antioxidants, and it is possible that this is a more effective supplementation regimen than using single antioxidants on their own [60,63]. However, this concept cannot account for the findings in the study by Hagobian et al. [62].

The lack of any effect of vitamin C in the first study by Davison and Gleeson [58] could be due to the fact that participants were only given vitamin C *during* and not before exercise. Consequently, there was probably minimal uptake of ascorbic acid into leukocytes and muscle tissue where it might be expected to modify cytokine production. Furthermore, addition of vitamin C to the sports drink containing carbohydrate did not have any additional effect

Table 2
Effects of antioxidant supplementation on changes in antioxidant status, cytokines, cortisol, oxidative stress markers and C-reactive protein after 1.5 to 2.5 h of running

Reference	Subjects	Exercise	Design	Supplement	Resting vitamin status[a]	Parameters	Maximum change from preexercise
Thompson et al. [69]	Physically active	1.5 h high-intensity intermittent shuttle running	Placebo controlled ($n=16$)	Placebo	Plasma (vitamin C) 50 μmol/L	Vitamin C	+1.4×*
						IL-6	+11×*
						Cortisol	+1.8×*
						CRP	+2.2×*
						MDA	+1.6×*
				400 mg vitamin C per day for 14 days before exercise	Plasma (vitamin C) 61 μmol/L†	Vitamin C	+1.2×*
						IL-6	+8×*
						Cortisol	+2×*
						CRP	+3×*
						MDA	+1.3×*
Thompson et al. [71]	Physically active	1.5 h high-intensity intermittent shuttle running	Placebo controlled ($n=16$)	Placebo	Plasma (vitamin C) 52 μmol/L	Vitamin C	+1.4×*
						IL-6	+4.5×*
						MDA	+1.3×*
				400 mg vitamin C per day for 3 days after exercise	Plasma (vitamin C) 50 μmol/L	Vitamin C	+1.9×*,†
						IL-6	+6.2×*
						MDA	+1.3×*
Nieman et al. [70]	Trained runners	2.5 h running at 70% VO_{2max}	Placebo controlled ($n=12$)	Placebo	No data available	IL-6	+5.2×*
						Cortisol	+1.1×
				1000 mg vitamin C per day for 7 days before exercise and on the day of exercise		IL-6	+7×*
						Cortisol	+1.2×
Singh et al. [72]	Trained runners	Run to exhaustion at 65–70% VO_{2max}	Crossover ($n=10$)	Placebo	Plasma (vitamin E) 27 μmol/L	IL-6	+11×*
						Cortisol	+1.8×*
				400 IU vitamin E per day for 3 days before exercise and on the day of exercise	Plasma (vitamin E) 44 μmol/L†	IL-6	+11×*
						Cortisol	+1.8×*

Abbreviations: CRP, C-reactive protein; MDA, malondialdehyde.
[a] Data are means.
* Significantly different from preexercise values, $P<.05$.
† Significantly different from placebo group, $P<.05$.

on the alterations in plasma IL-6 and cortisol concentrations after exercise [58]. One explanation for this finding is that blood glucose levels regulate changes in IL-6 and cortisol during exercise [64], while vitamin C has less effect.

Hagobian et al. [62] suggested that the negative findings in their study could have been related to the fitness level or training status of their participants. It is possible that untrained individuals may be more responsive to antioxidant supplementation than endurance-trained athletes. Some [65,66] but not all studies [67,68] indicate that endurance training improves endogenous antioxidant defenses. With the exception of the participants in the study by Vassilakopoulos et al. [60], the participants in the other studies described above were all physically active or well trained. Therefore, training status may not account for all of the variation in responsiveness to antioxidant supplementation. The magnitude of exercise-induced changes in cytokines also did not seem to influence the efficacy of antioxidant supplements.

6. Antioxidant supplements and cytokine responses following moderate- to high-intensity running of 1.5 to 2.5 h in duration

Several studies have investigated whether supplementation with vitamins C and E modifies cytokine responses to running. The effectiveness of antioxidants may be different during running because in addition to mitochondria and xanthine oxidase, the greater level of muscle damage associated with running is also likely to promote the release of RONS from phagocytic cells and iron-catalyzed reactions in muscle.

Three groups have examined the influence of supplementation with vitamin C both before and after prolonged running on changes in IL-6, C-reactive protein, cortisol and malondialdehyde (Table 2). Data from these studies are equivocal. Thompson et al. [69] reported that supplementation with vitamin C (400 mg/day) for 2 weeks before exercise significantly attenuated serum IL-6 concentration

2 h after exercise, whereas it did not influence changes in the serum concentrations of C-reactive protein and cortisol or plasma malondialdehyde concentration. In contrast, vitamin C supplementation for 1 week before (1000 mg/day) [70] or 3 days after exercise (400 mg/day) [71] did not affect alterations in the plasma concentrations of IL-6, cortisol or malondialdehyde. These studies also reported no significant effect of vitamin C on muscle damage as indicated by changes in muscular strength, plasma creatine kinase activity and myoglobin concentration after exercise [69,71]. Another group examined the effect of supplementation with vitamin E (400 IU/day) for 3 days before prolonged running (Table 2). Although the plasma concentrations of IL-6 and cortisol increased significantly during exercise, there were no significant differences between the supplement and placebo groups [72].

6.1. Discussion

As highlighted above, there are variable effects of antioxidant supplements on cytokine and cortisol responses to moderate- to high-intensity running lasting up to 2.5 h. Several factors may account for this variation, including the period of supplementation before exercise, the bioavailability of vitamins during exercise and endogenous antioxidant defenses related to the training status of participants.

The one study described above demonstrating that antioxidants attenuate the IL-6 and cortisol responses to exercise involved 2 weeks of supplementation with vitamin C [69]. In contrast, supplementation with either vitamin C or vitamin E for 1 week or less was not effective [70,72]. Clinical studies have reported that 2 weeks of supplementation with 200 mg vitamin C is the minimum period

Table 3
Effects of antioxidant supplementation on changes in antioxidant status, cytokines, oxidative stress markers and C-reactive protein after downhill running and local eccentric muscle contractions

Reference	Subjects	Exercise	Design	Supplement	Resting vitamin status[a]	Parameters	Maximum change from preexercise
Thompson et al. [81]	Physically active	30 min downhill running at 60% VO_{2max}	Placebo controlled ($n=14$)	Placebo	Plasma (vitamin C) 51 μmol/L	IL-6	+3.3×*
				400 mg vitamin C per day for 2 weeks before and 3 days after exercise	Plasma (vitamin C) 68 μmol/L	IL-6	+2.4×*
Petersen et al. [82]	Trained runners	1.5 h downhill running at 75% VO_{2max}	Placebo controlled ($n=20$)	Placebo	Plasma (vitamin E) 33 μmol/L	IL-1ra	+4.2×*
					Plasma (vitamin C) 51 μmol/L	IL-6	+20×*
				500 mg vitamin C and 400 mg vitamin E per day for 2 weeks before and 1 week after exercise	Plasma (vitamin E) 68 μmol/L	IL-1ra	+2.7×*
					Plasma (vitamin C) 95 μmol/L	IL-6	+19×*
Childs et al. [32]	Untrained	3 sets of 10 eccentric contractions of the elbow flexors at 80% one repetition maximum	Placebo controlled ($n=14$)	Placebo	No data available	STAS	−1.4×
						IL-6	+4×*
						F_2-IsoP	+2.3×*
						LOOH	+1.6×*
				12.5 mg vitamin C and 10 mg NAC per kg body weight per day for 7 days after exercise		STAS	+1.9×†
						IL-6	+2.9×
						F_2-IsoP	+2.8×*
						LOOH	+1.9×*,†
Phillips et al. [90]	Untrained	3 sets of 10 eccentric contractions of t he elbow flexors at 80% one repetition maximum	Placebo controlled ($n=35$)	Placebo	No data available	IL-6	+2×
						CRP	+1.3×
				300 mg mixed tocopherols, 300 mg flavonoids and 800 mg docosahexaenoate for 7 days before and after exercise		IL-6	+1.2×†
						CRP	+1.7×

Abbreviations: STAS, serum total antioxidant status; F_2-isoP, F_2-isoprostanes; LOOH, lipid hydroperoxides; CRP, C-reactive protein.

[a] Data are means.

* Significantly different from preexercise values, $P<.05$.

† Significantly different from placebo group, $P<.05$.

Table 4
Effects of antioxidant supplementation on changes in antioxidant status, cytokines, cortisol and oxidative stress markers after ultraendurance exercise

Reference	Subjects	Exercise	Design	Supplement	Resting vitamin status[a]	Parameters	Maximum change from preexercise
Nieman et al. [84]	Trained runners (90 km)	Ultramarathon	Placebo controlled ($n=29$)	Placebo	Serum (vitamin C) 83 μmol/L	Vitamin C	+1.6×
						TNF-α	+1.1×*
						IL-1β	+100%*
						IL-1ra	+16×*
						IL-6	+31×*
						IL-8	+6×*
						IL-10	+181×*
						Cortisol	+3.4×*
				500 mg vitamin C per day for 7 days before the race, on race day and 2 days after the race	Serum (vitamin C) 128 μmol/L†	Vitamin C	+1.2×
						TNF-α	+1.4×*
						IL-1β	+4.2×*
						IL-1ra	+23×*
						IL-6	+35×*
						IL-8	+7.5×*
						IL-10	+121×*
						Cortisol	+4.6×*
				1500 mg vitamin C per day for 7 days before the race, on race day and 2 days after the race	Serum (vitamin C) 153 μmol/L†	Vitamin C	+1.1×
						TNF-α	+1.5×*
						IL-1β	+7.3×*
						IL-1ra	+8×*,†
						IL-6	+31×*
						IL-8	+5.2×*
						IL-10	+90×*,†
						Cortisol	+3.1×*
Peters et al. [86]	Trained runners	Ultramarathon (90 km)	Placebo controlled ($n=16$)	Placebo	Serum (vitamin C) 86 μmol/L	Vitamin C	+1.3×
						IL-6	+6.3×*
						Cortisol	+4.4×*
						CRP	+11×*
				1000 mg vitamin C per day for 7 days before the race, on race day and 2 days after the race	Serum (vitamin C) 118 μmol/L†	Vitamin C	No change*
						IL-6	+6×*
						Cortisol	+3.4×*,†
						CRP	+19×*,†
Nieman et al. [85]	Trained runners	Ultramarathon (67 km)	Placebo controlled ($n=28$)	Placebo	Plasma (vitamin C) 40 μmol/L	Vitamin C	+1.9×
						IL-6	+28×*
						IL-1ra	+5.8×*
						IL-8	+3.2×*
						Cortisol	+1.5×*
						LOOH	+1.1×*
						F_2-IsoP	+1.4×*
				1000 mg vitamin C per day for 7 days before the race and on race day	Plasma (vitamin C) 70 μmol/L†	Vitamin C	+2.6×*,†
						IL-6	+36×*
						IL-1ra	+5.6×*
						IL-8	+3.6×*
						Cortisol	+2.2×*
						LOOH	+1.3×*
						F_2-IsoP	+1.3×*
Mastaloudis et al. [88]	Trained runners	Ultramarathon (50 km)	Placebo controlled ($n=28$)	Placebo	Plasma (vitamin E) 26 μmol/L	Vitamin E	−1.2×*
						Vitamin C	+1.2×*
					Plasma (vitamin C) 73 μmol/L	IL-6	+8×*
						F_2-IsoP	+1.5×*
				300 mg *RRR*-α-tocopherol and 1000 mg vitamin C for 6 weeks before the race	Plasma (vitamin E) 46 μmol/L†	Vitamin E	+1.3×*
						Vitamin C	+1.2×*
					Plasma (vitamin C) 127 μmol/L†	IL-6	+7×*
						F_2-IsoP	+1.2×†

Table 4 (*continued*)

Reference	Subjects	Exercise	Design	Supplement	Resting vitamin status[a]	Parameters	Maximum change from preexercise
Nieman et al. [87]	Triathletes	Ironman triathlon (3.8-km swim, 180-km cycle, 42-km marathon run)	Placebo controlled ($n=36$)	Placebo	(α-Tocopherol) 14 μmol/L (γ-Tocopherol) 1.3 μmol/L	α-Tocopherol	+1.1×
						γ-Tocopherol	−1.3×
						F_2-IsoP	+1.9×*
						IL-1ra	+16×*
						IL-6	+46×*
						IL-8	+11×*
						IL-10	+9×*
						Cortisol	+8×*
				800 IU D-α-tocopherol per day for 2 months before the race and on race day	(α-Tocopherol) 24 μmol/L† (γ-Tocopherol) 0.6 μmol/L†	α-Tocopherol	+1.2×†
						γ-Tocopherol	−1.4×†
						F_2-IsoP	+2.8×*
						IL-1ra	+37×*
						IL-6	+83×*,†
						IL-8	+8×*
						IL-10	+15×*
						Cortisol	+2.3×*

Abbreviations: CRP, C-reactive protein; LOOH, lipid hydroperoxides; F_2-isoP, F_2-isoprostanes.

[a] Data are means.

* Significantly different from preexercise values, $P<.05$.

† Significantly different from placebo group, $P<.05$.

required to saturate plasma concentrations of ascorbic acid [73]. With respect to supplementation with vitamin E, in one study, plasma levels of α-tocopherol reached steady state after 4 to 5 days of supplementation with up to 1200 IU of DL-α-tocopherol [74]. Another study indicated that plasma α-tocopherol concentration plateaued after 2 weeks of supplementation with 800 IU DL-α-tocopherol-acetate [75]. The plasma concentrations of ascorbic acid alone and α-tocopherol and ascorbic acid may not be the most valid measure of tissue stores of these vitamins. However, the plasma concentrations of these vitamins correlate with tissue stores, as indicated by the correlation with leukocyte ascorbic acid content [76] and muscle α-tocopherol content [75]. Therefore, antioxidants may only alter inflammatory responses to exercise after a minimum period of 2 weeks. Future studies are warranted to investigate this issue.

The bioavailability of vitamins is likely affected by the dose, timing and dietary cofactors enhancing or inhibiting absorption. The bioavailability of vitamin C is significantly reduced at doses in excess of 200 mg vitamin C per day [73], and this may partially explain why supplementation with 1000 mg vitamin C did not alter the IL-6 and cortisol responses to exercise in the study by Nieman et al. [70]. Regular dietary intake of vitamin C most likely did not influence the results of the vitamin C studies above [69–71] because in each study, dietary vitamin C intake during the supplementation period was similar between the supplement and placebo groups.

The bioavailability of vitamin E following supplementation depends on a variety of factors, including the dose, type of supplement (i.e., natural versus synthetic), half-life and dietary fat intake, all of which, in turn, influence pathways related to absorption and transport into the tissues [77,78]. Singh et al. [72] did not state the type of vitamin E that was given to the runners in their study. This factor could have influenced the effectiveness of the supplement because data from clinical trials indicate that the natural form of vitamin E, *RRR*-α-tocopherol, is more effective in reducing markers of inflammation than the synthetic form, all-*rac*-α-tocopherol [79]. Furthermore, the dose of 400 IU vitamin E used by Singh et al. [72] may have been insufficient. Clinical findings suggest that a threshold dose of ≥600–800 IU *RRR*-α-tocopherol appears to be most effective in reducing inflammation [79]. Lastly, the absorption of vitamin E is enhanced with higher intakes of dietary fat [74,78,80]. It is therefore possible that the traditionally low-fat intake of endurance athletes may restrict the absorption of vitamin E, which means that less vitamin E is available to counteract the production of RONS during exercise.

As mentioned previously, it is possible that training adaptation in endogenous antioxidant defenses may explain some of the differences between these studies. Thompson et al. [69] used physically active but not endurance-trained participants, whereas the other studies used endurance-trained athletes [70,72]. Finally, the greater muscle damage imposed by running compared to cycling may increase the production of RONS by phagocytic cells, thereby reducing the effectiveness of antioxidant supplements.

7. Antioxidant supplements and cytokine responses to downhill running

Two studies have investigated the influence of antioxidants on cytokine responses to downhill running. As explained in the introduction to the previous section, during

running, more muscle damage means that there is probably a greater contribution of RONS from sources such as phagocytic cells and iron-catalyzed reactions.

Thompson et al. [81] gave physically active males vitamin C (400 mg/day) for 2 weeks before and 3 days after 30 min downhill running at 60% VO_{2max}. Plasma IL-6 concentration increased significantly during exercise in both groups, but there were no significant differences between the groups (Table 3). Vitamin C supplementation also did not influence muscle damage. Petersen et al. [82] provided recreational runners with a combination of vitamins C (500 mg) and E (400 mg) for 2 weeks before and 1 week after downhill running for 1.5 h at 75% VO_{2max}. Although there was a greater IL-6 response in their study than that by Thompson et al. [81], the pattern of changes was similar between the supplement and placebo groups (Table 3). There were also no significant differences in plasma IL-1ra concentration and creatine kinase activity between the groups after exercise.

7.1. Discussion

These two studies involved 2 weeks of supplementation with vitamin C or a combination of vitamins C and E, which, as stated previously, appears to be the minimum period necessary to saturate plasma levels of ascorbic acid and α-tocopherol [73,75]. However, neither of these exercise studies reported any significant effects of antioxidant supplementation on cytokine responses and muscle damage after exercise [81,82]. Interestingly, the dose of vitamins C and E was similar in the studies by Petersen et al. [82] and Fischer et al. [61], but the period of supplementation was different between the two studies (2 weeks versus 4 weeks, respectively). It is possible that the longer period of supplementation can account for the greater attenuation of cytokines following exercise in the latter of these two studies. Petersen et al. [82] did not state what type of vitamin E was used in their supplement, while in their later study, the same group used *RRR*-α-tocopherol [61]. If different forms of vitamin E were used, as explained previously, this might account for the variable effects on cytokine responses after exercise. The natural form of vitamin E (*RRR*- or D-α-tocopherol) has twice the bioavailability of synthetic vitamin E (all-*rac* or DL-α-tocopherol) [83].

In explanation of their findings, Thompson et al. [81] suggested that vitamin C is less effective during exercise at lower intensity, which elicits smaller IL-6 responses. However, this concept seems unlikely because plasma IL-6 concentration increased to a greater extent after downhill running in the study by Petersen et al., yet supplementation with vitamins C and E did not alter the IL-6 or IL-1ra responses to exercise in that particular study [82]. Alternatively, as suggested previously, muscle damage may reduce the effectiveness of antioxidants. Further work is warranted to examine this concept.

8. Antioxidant supplements and cytokine responses to ultraendurance exercise

A number of studies have assessed the effects of antioxidants following ultramarathon and Ironman triathlon races. The cytokine response to ultraendurance exercise is considerably greater than any other form of exercise. This likely reflects not only the large metabolic demands of endurance exercise but also the substantial levels of oxidative stress and muscle damage resulting from such exercise.

8.1. Supplementation with vitamin C

Several studies have investigated the influence of vitamin C supplementation on alterations in IL-1β, TNF-α, IL-1ra, IL-6, IL-8, IL-10 and cortisol after ultramarathon running events [84–86]. All three studies involved supplementation with 500 to 1500 mg vitamin C for 7 days before exercise (Table 4). The data from these studies are equivocal. One study reported that supplementation with 1500 mg vitamin C significantly attenuated the plasma concentrations of IL-1ra, IL-10 and cortisol after ultramarathon running. In contrast, supplementation did not significantly influence changes in the plasma concentrations of IL-1β, TNF-α, IL-6 or IL-8 concentration [84]. In another study, supplementation with 1000 mg vitamin C did not influence plasma IL-6 concentration after ultramarathon running, whereas it significantly enhanced serum C-reactive protein concentration 24 h after exercise [86]. This effect may have occurred as a result of the reduced circulating levels of cortisol in response to supplementation [86]. Lastly, in another study, athletes were given 1500 mg vitamin C for 7 days before and 1500 mg vitamin C during an ultramarathon race [85]. After the race, plasma cytokine concentrations were elevated, but there were no significant differences between the groups. Plasma cortisol concentration after exercise was also similar between the supplement and placebo groups. Furthermore, vitamin C may have promoted rather than reduced oxidative stress after exercise, as indicated by a trend ($P=.051$) toward higher plasma levels of F_2-isoprostanes following exercise in the supplement group.

8.2. Supplementation with vitamin E

The findings from other studies involving supplementation with vitamin E on its own or in combination with vitamin C are also equivocal. In one study, athletes were given 800 IU *RRR*-α-tocopherol for 2 months before an Ironman triathlon [87]. Vitamin E supplementation tended to enhance exercise-induced increases in the plasma concentrations of IL-6, IL-1ra and F_2-isoprostanes as a marker of oxidative stress (Table 4). In contrast, vitamin E did not influence changes in plasma IL-8, IL-10 or cortisol concentrations. Another study provided athletes with a combination of 1000 mg vitamin C and 300 mg *RRR*-α-tocopherol for 6 weeks before an ultramarathon [88]. The plasma concentrations of TNF-α, IL-6 and C-reactive protein all increased significantly after the race, but there were no significant differences between the

supplement and placebo groups. Antioxidant supplementation significantly reduced the plasma concentration of F_2-isoprostanes after exercise.

8.3. Discussion

The findings reviewed above indicate that the effects of antioxidant supplements on inflammatory responses to ultraendurance exercise are highly variable. Some of these variations may be attributed to differences in the dose of supplementation, the timing of supplementation, the biological activity of vitamin C versus vitamin E and/or the exercise stimulus.

With respect to vitamin C, data from the three studies described previously suggest that over a short period of supplementation such as 7 days, only doses of vitamin C as high as 1500 mg/day alter the inflammatory response to exercise [84]. In contrast, doses of 500–1000 mg vitamin C per day over the same period seemed to be less effective [85,86]. However, it should be noted that in the study by Nieman et al. [84], the 1500-mg vitamin C supplement was consumed on the morning of exercise. Therefore, it is difficult to establish whether the attenuation of plasma cytokine concentrations after exercise reported in that study reflects (a) the influence of the supplementation period in the week before exercise or (b) the effects of a transient increase in circulating levels of ascorbic acid as a result of consuming the supplement on the morning of exercise. Evidence in favor of the latter of these two concepts is that the elimination half-life of vitamin C is 10 h [89] and that the bioavailability of vitamin C is significantly reduced at doses in excess of 200 mg vitamin C per day [73].

Vitamin E was also consumed on the morning of the Ironman triathlon in the study by Nieman et al. [87]. Furthermore, although the participants in this study were instructed to avoid race supplements high in vitamin E, vitamin E intake during the race was significantly higher in the supplement group compared with the placebo group [87]. α-Tocopherol is absorbed slowly into the bloodstream and has a long elimination half-life [78,89]. It is therefore possible that the vitamin E consumed before and during the race affected the results of this particular study.

In this vitamin E study [87], the greater cytokine response after exercise in the supplement versus the placebo group could be attributed to a possible pro-oxidant effect of α-tocopherol. Rietjens et al. [26] have proposed that under conditions of oxidative stress, increased levels of α-tocopherol can, in turn, generate elevated levels of α-tocopherol radicals and lipid peroxidation. When antioxidant systems are balanced, co-antioxidants inhibit the pro-oxidant action of α-tocopherol radicals, reducing them back to α-tocopherol. However, when α-tocopherol levels are elevated on their own during oxidative stress, the resultant levels of α-tocopherol radicals may overwhelm the capacity of co-antioxidants to reduce the α-tocopherol radicals [26]. If α-tocopherol radicals are formed during exercise in response to supplementation with large doses of vitamin E and oxidative stress, these radicals may act on NF-κB and/or calcineurin-NFAT pathways to enhance cytokine production. This concept would support the findings of Nieman et al. [87] that plasma cytokine (IL-6, IL-1ra) and oxidative stress (F_2-isoprostanes) concentrations were higher after exercise in the supplement versus the placebo group. The vitamin E pro-oxidant theory may explain why supplements containing small amounts of vitamin E with co-antioxidants can provide greater benefits than vitamin E supplements alone [63]. Evidence in support of this concept is that in two of the studies described in this review [61,88], moderate amounts of *RRR*-α-tocopherol (300–400 mg) combined with ascorbic acid (500−100 mg) effectively reduced plasma F_2-isoprostanes as a marker of oxidative stress after exercise. However, data from these two studies also indicated that reduced oxidative stress after exercise is not always associated with lower cytokine levels.

When comparing some of the studies above, changes in the anti-inflammatory cytokines IL-1ra and IL-10 and cortisol appeared to be greater after ultramarathon races with a distance of 90 km [84] than after 70-km races [85]. Interestingly, 1500 mg vitamin C attenuated the postexercise increase in anti-inflammatory cytokines and cortisol after the longer, but not the shorter, race. This trend suggests that the effects of vitamin C may depend on the duration of exercise and the resulting anti-inflammatory response. Plasma IL-6 concentration increased to a similar extent (~30×) after both races but was not influenced by vitamin C supplementation [84,85]. Therefore, vitamin C may influence some inflammatory variables but not others after ultraendurance exercise.

As suggested previously, the efficacy of antioxidant supplementation may also depend on the mode of exercise and the extent of muscle damage. Muscle damage may contribute to RONS production such that the capacity of antioxidant supplements to neutralize RONS is overwhelmed. Mastaloudis et al. [88] used a supplementation regimen (1000 mg vitamin C, 300 mg *RRR*-α-tocopherol for 6 weeks) similar to that of Fischer et al. [61] (500 mg vitamin C, 400 IU *RRR*-α-tocopherol for 4 weeks). However, Mastaloudis et al. found that the supplement did not significantly alter plasma levels of cytokines and C-reactive protein after exercise that involved muscle damage. In contrast, Fischer et al. reported attenuated plasma levels of cytokines, C-reactive protein and cortisol after exercise that did not cause appreciable muscle damage. It is possible that during ultraendurance exercise, the combined influence of metabolic demands, high levels of oxygen consumption and muscle damage exceeds the capacity of antioxidant supplements to attenuate cytokine production.

9. Antioxidant supplements and cytokine responses to local eccentric muscle contractions

Two studies have investigated the effects of antioxidant supplements on cytokine responses to local eccentric muscle

contractions involving the elbow flexors or biceps muscle group. Unlike aerobic exercise such as that described in the previous sections, local eccentric muscle contractions do not involve a large increase in mitochondrial respiration but result in muscle damage. Cytokine production following this form of exercise is likely stimulated by RONS generated from phagocytic cells and iron-catalyzed reactions in skeletal muscle.

In a study by Childs et al. [32], untrained individuals received a combination of vitamin C (12.5 mg/kg body weight) and NAC (10 mg/kg body weight) for 7 days after three sets of 10 eccentric contractions of the elbow flexors at 80% of one repetition maximum. Plasma IL-6 increased mildly after exercise but was not significantly different between the supplement and placebo groups (Table 3). There was evidence of greater oxidative stress in the supplement group after exercise, as indicated by higher plasma concentrations of lipid hydroperoxides and F_2-isoprostanes. These data suggest that vitamin C and NAC might have pro-oxidant effects under conditions of exercise-induced tissue damage. This effect may have occurred via a reaction between ascorbic acid and Fe^{3+} released from skeletal muscle, which can result in the formation of RONS [32].

Using the same exercise protocol, this group conducted another study to examine the efficacy of a combination of antioxidants (300 mg mixed tocopherols, 300 mg flavonoids and 800 mg docosahexaenoate) for 7 days before and 7 days after exercise [90]. Plasma IL-6 concentration was relatively low after exercise (Table 3); however, the antioxidant supplement significantly attenuated plasma IL-6 concentration by around 80% 3 days after exercise compared with the placebo group. The median increase in plasma C-reactive protein concentration was also significantly smaller in the supplement group 3 days after exercise [90].

Therefore, although similar exercise protocols were used in each study, the effects of supplementation were different. In the first study, the antioxidant supplement appeared to promote oxidative stress, while it slightly reduced inflammation. In the second study, the antioxidant supplement reduced inflammation to a greater extent. These differences may be due to the different composition of the two supplements that were used. Alternatively, the differences may be due to the fact that in the first study, the antioxidant supplement was ingested after exercise, whereas in the second study, the antioxidant supplement was ingested both before and after exercise.

10. Influence of regular dietary intake

When interpreting the data from some of the studies described in this review, the effect of regular dietary intake of antioxidants is also worth mentioning briefly. Some of the studies described recorded dietary intake of antioxidants during the supplementation period [69,81,86,87], and some studies instructed participants to avoid certain foodstuffs containing large amounts of antioxidant vitamins [69,87,88]. The uptake of antioxidant vitamins from supplements is likely to be influenced by regular dietary intake. Beyond a certain level of dietary intake, concentrations of antioxidants within the circulation and tissues are saturated [73,91]. Consequently, athletes with low dietary antioxidants are more likely to benefit from supplements [92]. The consumption of other foodstuffs in the diet may also affect the bioavailability of antioxidant vitamins. For example, the intestinal absorption of vitamin E is dependent on the lipid content of recently eaten food as supplements taken with high-fat meals are better absorbed than those consumed with low-fat meals [74,78,80]. Therefore, because not all studies monitored or attempted to regulate dietary intake of antioxidants during the supplementation period, these factors could have contributed to some of the variability between the studies.

11. Is antioxidant supplementation beneficial?

The equivocal nature of the research findings presented in this review precludes us from drawing any definitive conclusions regarding the effects of antioxidants on inflammatory responses to exercise. Further work is needed to improve our understanding of the relative contribution of the various stimuli and sources of RONS during different forms of exercise. Traditionally, sports science research has sought methods of reducing muscle damage and enhancing recovery, and this explains some of the interest in antioxidant supplements. However, data from recent studies indicate that the effects of antioxidant supplements on exercise-induced muscle damage are variable [69,81,93–96], which calls into question the role of RONS in the etiology of muscle damage. RONS may instead be a by-product of muscle damage. Importantly, RONS are now recognized as key mediators of cellular signaling and training adaptations [11]. Supplementation with large doses of antioxidants has been shown to attenuate some of the cellular signals that stimulate adaptation to exercise. For example, vitamin E reduces the beneficial effects of exercise training on cardiovascular risk factors such as progression of atherogenic lesions, plasma cholesterol concentration, aortic CAT activity and expression of endothelial nitric oxide synthase (eNOS) [6]. Furthermore, allopurinol blocks the exercise-induced oxidation of glutathione; the phosphorylation of p38, ERK1 and ERK2 MAPKs; the mRNA expression of Mn-SOD, inducible NOS and eNOS; and the activation of NF-κB in skeletal muscle [5]. Finally, large doses of antioxidants such as vitamins C and E may be ineffective [97–101] or even harmful to health in some situations [102,103]. Therefore, further work is warranted to investigate if athletes should use antioxidant supplements during training to enhance recovery and performance.

References

[1] Pedersen BK, Saltin B. Evidence for prescribing exercise as therapy in chronic disease. Scand J Med Sci Sports 2006;1(Suppl 1):3–63.

[2] Droge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47–95.
[3] Laufs U, Wassmann S, Czech T, Munzel T, Eisenhauer M, Bohm M, et al. Physical inactivity increases oxidative stress, endothelial dysfunction, and atherosclerosis. Arterioscler Thromb Vasc Biol 2005;25:809–14.
[4] Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol 2005;98:1154–62.
[5] Gomez-Cabrera M-C, Borras C, Pallardo FV, Sastre J, Ji LL, Vina J. Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. J Physiol (Lond) 2005;567:113–20.
[6] Meilhac O, Ramachandran S, Chiang K, Santanam N, Parthasarathy S. Role of arterial wall antioxidant defense in beneficial effects of exercise on atherosclerosis in mice. Arterioscler Thromb Vasc Biol 2001;21:1681–8.
[7] Thompson D. Supplemental ascorbate and exercise-induced IL-6 metabolism: focus on Fenton chemistry and redox-regulation of vascular homeostasis: reply. Eur J Appl Physiol 2005;94:490.
[8] Bailey DM. Supplemental ascorbate and exercise-induced IL-6 metabolism: focus on Fenton chemistry and redox-regulation of vascular homeostasis. Eur J Appl Physiol 2005;94:487–9.
[9] Vassilakopoulos T, Roussos C, Zakynthinos S. When are antioxidants effective in blunting the cytokine response to exercise? Med Sci Sports Exerc 2005;37:342–3.
[10] Nieman DC, McAnulty SR. When are antioxidants effective in blunting the cytokine response to exercise?: response. Med Sci Sports Exerc 2005;37:344.
[11] Ji LL, Gomez-Cabrera MC, Vina J. Exercise and hormesis: activation of cellular antioxidant signaling pathway. Ann NY Acad Sci 2006;1067:425–35.
[12] Fehrenbach E, Schneider ME. Trauma-induced systemic inflammatory response versus exercise-induced immunomodulatory effects. Sports Med 2006;36:373–84.
[13] Febbraio MA, Pedersen BK. Contraction-induced myokine production and release: is skeletal muscle an endocrine organ? Exerc Sport Sci Rev 2005;33:114–9.
[14] Nieman DC, Henson DA, Smith LL, Utter AC, Vinci DM, Davis JM, et al. Cytokine changes after a marathon race. J Appl Physiol 2001;91:109–14.
[15] Rhind SG, Gannon GA, Shek PN, Brenner IK, Severs Y, Zamecnik J, et al. Contribution of exertional hyperthermia to sympathoadrenal-mediated lymphocyte subset redistribution. J Appl Physiol 1999; 87:1178–85.
[16] Elenkov IJ, Chrousos GP, Wilder RL. Neuroendocrine regulation of IL-12 and TNF-alpha/IL-10 balance. Clinical implications. Ann NY Acad Sci 2000;917:94–105.
[17] Jeukendrup AE, Vet-Joop K, Sturk A, Stegen J, Senden J, Saris WHM, et al. Relationship between gastro-intestinal complaints and endotoxaemia, cytokine release and the acute-phase reaction during and after a long-distance triathlon in highly trained men. Clin Sci 2000;98:47–55.
[18] Konig D, Wagner K-H, Elmadfa I, Berg A. Exercise and oxidative stress: significance of antioxidants with reference to inflammatory, muscular, and systemic stress. Exerc Immunol Rev 2001;7:108–33.
[19] Haddad JJ. Pharmaco-redox regulation of cytokine-related pathways: from receptor signaling to pharmacogenomics. Free Radic Biol Med 2002;33:907–26.
[20] Aoi W, Naito Y, Takanami Y, Kawai Y, Sakuma K, Ichikawa H, et al. Oxidative stress and delayed-onset muscle damage after exercise. Free Radic Biol Med 2004;37:480–7.
[21] Kosmidou I, Vassilakopoulos T, Xagorari A, Zakynthinos S, Papapetropoulos A, Roussos C. Production of interleukin-6 by skeletal muscle myotubes. Role of reactive oxygen species. Am J Respir Cell Mol Biol 2002;26:587–93.
[22] Maziere C, Morliere P, Louandre C, Conte MA, Gomilla C, Santus R, et al. Low UVA doses activate the transcription factor NFAT in human fibroblasts by a calcium-calcineurin pathway. Free Radic Biol Med 2005;39:1629–37.
[23] Khassaf M, McArdle A, Esanu C, Vasilaki A, McArdle F, Griffiths RD, et al. Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. J Physiol 2003;549:645–6452.
[24] Ashton T, Young IS, Peters JR, Jones E, Jackson SK, Davies B, et al. Electron spin resonance spectroscopy, exercise, and oxidative stress: an ascorbic acid intervention study. J Appl Physiol 1999;87:2032–6.
[25] Li YP, Schwartz RJ, Waddell ID, Holloway BR, Reid MB. Skeletal muscle myocytes undergo protein loss and reactive oxygen-mediated NF-kappaB activation in response to tumor necrosis factor alpha. FASEB J 1998;12:871–80.
[26] Rietjens I, Boersma M, de Haan L, Spenkelink B, Awad H, Cnubben N, et al. The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. Environ Toxicol Pharmacol 2002;11:321–33.
[27] McArdle F, Pattwell DM, Vasilaki A, McArdle A, Jackson MJ. Intracellular generation of reactive oxygen species by contracting skeletal muscle cells. Free Radic Biol Med 2005;39:651–7.
[28] Pattwell DM, McArdle A, Morgan JE, Patridge TA, Jackson MJ. Release of reactive oxygen and nitrogen species from contracting skeletal muscle cells. Free Radic Biol Med 2004;37:1064–72.
[29] Silveira LR, Pereira-Da-Silva L, Juel C, Hellsten Y. Formation of hydrogen peroxide and nitric oxide in rat skeletal muscle cells during contractions. Free Radic Biol Med 2003;35:455–64.
[30] Close GL, Ashton T, Cable T, Doran D, MacLaren DP. Eccentric exercise, isokinetic muscle torque and delayed onset muscle soreness: the role of reactive oxygen species. Eur J Appl Physiol 2004;91:615–21.
[31] Bailey DM, Young IS, McEneny J, Lawrenson L, Kim J, Barden J, et al. Regulation of free radical outflow from an isolated muscle bed in exercising humans. Am J Physiol 2004;287:H1689–99.
[32] Childs A, Jacobs C, Kaminski T, Halliwell B, Leeuwenburgh C. Supplementation with vitamin C and *N*-acetyl-cysteine increases oxidative stress in humans after an acute muscle injury induced by eccentric exercise. Free Radic Biol Med 2001;31:745–53.
[33] Hellsten Y, Frandsen U, Orthenblad N, Sjodin B, Richter EA. Xanthine oxidase in human skeletal muscle following eccentric exercise: a role in inflammation. J Physiol 1997;498:239–48.
[34] Ji LL, Gomez-Cabrera MC, Steinhafel N, Vina J. Acute exercise activates nuclear factor (NF)-kappaB signaling pathway in rat skeletal muscle. FASEB J 2004;18:1499–506.
[35] Ho RC, Hirshman MF, Li Y, Cai D, Farmer JR, Aschenbach WG, et al. Regulation of IkappaB kinase and NF-kappaB in contracting adult rat skeletal muscle. Am J Physiol Cell Physiol 2005;289:C794–C801.
[36] Vider J, Laaksonen DE, Kilk A, Atalay M, Lehtmaa J, Zilmer M, et al. Physical exercise induces activation of NF-kappaB in human peripheral blood lymphocytes. Antioxid Redox Signal 2001;3:1131–7.
[37] Haddad JJ. Oxygen sensing and oxidant/redox-related pathways. Biochem Biophys Res Commun 2004;316:969–77.
[38] Korn SH, Wouters EF, Vos N, Janssen-Heininger YM. Cytokine-induced activation of nuclear factor-kappa B is inhibited by hydrogen peroxide through oxidative inactivation of IkappaB kinase. J Biol Chem 2001;276:35693–700.
[39] Bowie AG, O'Neill LA. Vitamin C inhibits NF-kappa B activation by TNF via the activation of p38 mitogen-activated protein kinase. J Immunol 2000;165:7180–8.
[40] Hayakawa M, Miyashita H, Sakamoto I, Kitagawa M, Tanaka H, Yasuda H, et al. Evidence that reactive oxygen species do not mediate NF-kappaB activation. EMBO J 2003;22:3356–66.
[41] Olson EN, Williams RS. Remodeling muscles with calcineurin. Bioessays 2000;22:510–9.
[42] Crabtree GR, Olson EN. NFAT signaling: choreographing the social lives of cells. Cell 2002;109(Suppl):S67–S79.
[43] Fruman DA, Bierer BE, Benes JE, Burakoff SJ, Austen KF, Katz HR. The complex of FK506-binding protein 12 and FK506 inhibits

calcineurin phosphatase activity and IgE activation-induced cytokine transcripts, but not exocytosis, in mouse mast cells. J Immunol 1995; 154:1846–51.
[44] Goldfeld AE, Tsai E, Kincaid R, Belshaw PJ, Schrieber SL, Strominger JL, et al. Calcineurin mediates human tumor necrosis factor alpha gene induction in stimulated T and B cells. J Exp Med 1994;180:763–8.
[45] Svensson U, Holst E, Sundler R. Cyclosporin-sensitive expression of cytokine mRNA in mouse macrophages responding to bacteria. Mol Immunol 1995;32:157–65.
[46] McCaffrey PG, Kim PK, Valge-Archer VE, Sen R, Rao A. Cyclosporin A sensitivity of the NF-kappa B site of the IL2R alpha promoter in untransformed murine T cells. Nucleic Acids Res 1994; 22:2134–42.
[47] Rao A, Luo C, Hogan PG. Transcription factors of the NFAT family: regulation and function. Annu Rev Immunol 1997;15:707–47.
[48] Morliere P, Moysan A, Santus R, Huppe G, Maziere JC, Dubertret L. UVA-induced lipid peroxidation in cultured human fibroblasts. Biochim Biophys Acta 1991;1084:261–8.
[49] Maziere C, Morliere P, Massy Z, Kamel S, Louandre C, Conte MA, et al. Oxidized low-density lipoprotein elicits an intracellular calcium rise and increases the binding activity of the transcription factor NFAT. Free Radic Biol Med 2005;38:472–80.
[50] Pahlavani MA, Harris MD. Effect of in vitro generation of oxygen free radicals on T cell function in young and old rats. Free Radic Biol Med 1998;25:903–13.
[51] Holmes AG, Watt MJ, Carey AL, Febbraio MA. Ionomycin, but not physiologic doses of epinephrine, stimulates skeletal muscle interleukin-6 mRNA expression and protein release. Metabolism 2004;53:1492–5.
[52] Asea A. Stress proteins and initiation of immune response: chaperokine activity of HSP72. Exerc Immunol Rev 2005;11:34–45.
[53] Gorman AM, Heavey B, Creagh E, Cotter TG, Samali A. Antioxidant-mediated inhibition of the heat shock response leads to apoptosis. FEBS Lett 1999;445:98–102.
[54] Wallen ES, Buettner GR, Moseley PL. Oxidants differentially regulate the heat shock response. Int J Hyperthermia 1997;13: 517–24.
[55] Martindale JL, Holbrook NJ. Cellular response to oxidative stress: signaling for suicide and survival. J Cell Physiol 2002;192:1–15.
[56] Fischer CP, Hiscock NJ, Basu S, Vessby B, Kallner A, Sjoberg LB, et al. Vitamin E isoform-specific inhibition of the exercise-induced heat shock protein 72 expression in humans. J Appl Physiol 2006; 100:1679–87.
[57] Jackson MJ, Khassaf M, Vasilaki A, McArdle F, McArdle A. Vitamin E and the oxidative stress of exercise. Ann NY Acad Sci 2004;1031:158–68.
[58] Davison G, Gleeson M. Influence of acute vitamin C and/or carbohydrate ingestion on hormonal, cytokine, and immune responses to prolonged exercise. Int J Sport Nutr Exerc Metab 2005;15:465–79.
[59] Davison G, Gleeson M. The effect of 2 weeks vitamin C supplementation on immunoendocrine responses to 2.5 h cycling exercise in man. Eur J Appl Physiol 2006;97:454–61.
[60] Vassilakopoulos T, Karatza M-H, Katsaounnou P, Kollintza A, Zakynthinos S, Roussos C. Antioxidants attenuate the plasma cytokine response to exercise in humans. J Appl Physiol 2003; 94:1025–32.
[61] Fischer C, Hiscock N, Penkowa M, Basu S, Vessby B, Kallner A, et al. Vitamin C and E supplementation inhibits the release of interleukin-6 from contracting human skeletal muscle. J Physiol 2004;558:633–45.
[62] Hagobian TA, Jacobs KA, Subudhi AW, Fattor JA, Rock PB, Muza SR, et al. Cytokine responses at high altitude: effects of exercise and antioxidants at 4300 m. Med Sci Sports Exerc 2006;38:276–85.
[63] Stocker R. The ambivalence of vitamin E in atherogenesis. Trends Biochem Sci 1999;24:219–23.
[64] Nieman DC, Davis JM, Henson DA, Gross SJ, Dumke CL, Utter AC, et al. Muscle cytokine mRNA changes after 2.5 h of cycling: influence of carbohydrate. Med Sci Sports Exerc 2005;37:1283–90.
[65] Fatouros IG, Jamurtas AZ, Villiotou V, Pouliopoulou S, Fotinakis P, Taxildaris K, et al. Oxidative stress responses in older men during endurance training and detraining. Med Sci Sports Exerc 2004; 36:2065–72.
[66] Miyazaki H, Oh-ishi S, Ookawara T, Kizaki T, Toshinai K, Ha S, et al. Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. Eur J Appl Physiol 2001;84: 1–6.
[67] Tonkonogi M, Walsh B, Svensson M, Sahlin K. Mitochondrial function and antioxidative defence in human muscle: effects of endurance training and oxidative stress. J Physiol 2000;528(Pt 2): 379–88.
[68] Tiidus PM, Pushkarenko J, Houston ME. Lack of antioxidant adaptation to short-term aerobic training in human muscle. Am J Physiol 1996;271:R832–6.
[69] Thompson D, Williams C, McGregor SJ, Nicholas CW, McArdle F, Jackson MJ, et al. Prolonged vitamin C supplementation and recovery from demanding exercise. Int J Sport Nutr Exerc Metab 2001;11:466–81.
[70] Nieman D, Henson D, Butterworth D, Warren B, Davis J, Fagoaga O, et al. Vitamin C supplementation does not alter the immune response to 2.5 hours of running. Int J Sport Nutr 1997;7:173–84.
[71] Thompson D, Williams C, Garcia-Roves P, McGregor SJ, McArdle MJ, Jackson MJ. Post-exercise vitamin C supplementation and recovery from demanding exercise. Eur J Appl Physiol 2003;89: 393–400.
[72] Singh A, Papanicolaou DA, Lawrence LL, Howell EA, Chrousos GP, Deuster PA. Neuroendocrine responses to running in women after zinc and vitamin E supplementation. Med Sci Sports Exerc 1999;31:536–42.
[73] Levine M, Conry-Cantilena C, Wang D, Welch R, Washko P, Dhariwal K, Park J, et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc Natl Acad Sci U S A 1996;93:3704–9.
[74] Dimitrov NV, Meyer C, Gilliland D, Ruppenthal M, Chenoweth W, Malone W. Plasma tocopherol concentrations in response to supplemental vitamin E. Am J Clin Nutr 1991;53:723–9.
[75] Meydani M, Fielding RA, Cannon JG, Blumberg JB, Evans WJ. Muscle uptake of vitamin E and its association with muscle fiber type. J Nutr Biochem 1997;8:74–8.
[76] Burr M, Elwood P, Hole D, Hurley R, Hughes R. Plasma and leucocyte ascorbic acid levels in the elderly. Am J Clin Nutr 1974;27:144–51.
[77] Blatt DH, Pryor WA, Mata JE, Rodriguez-Proteau R. Re-evaluation of the relative potency of synthetic and natural alpha-tocopherol: experimental and clinical observations. J Nutr Biochem 2004;15:380–95.
[78] Lodge JK, Hall WL, Jeanes YM, Proteggente AR. Physiological factors influencing vitamin E biokinetics. Ann NY Acad Sci 2004;1031:60–73.
[79] Singh U, Jialal I. Anti-inflammatory effects of alpha-tocopherol. Ann NY Acad Sci 2004;1031:195–203.
[80] Iuliano L, Micheletta F, Maranghi M, Frati G, Diczfalusy U, Violi F. Bioavailability of vitamin E as function of food intake in healthy subjects: effects on plasma peroxide-scavenging activity and cholesterol-oxidation products. Arterioscler Thromb Vasc Biol 2001;21:E34–7.
[81] Thompson D, Bailey DM, Hill J, Hurst T, Powell JR, Williams C. Prolonged vitamin C supplementation and recovery from eccentric exercise. Eur J Appl Physiol 2004;92:133–8.
[82] Petersen EW, Ostrowski K, Ibfelt T, Richelle M, Offord E, Halkjaer-Kristensen J, et al. Effect of vitamin supplementation on cytokine response and on muscle damage after strenuous exercise. Am J Physiol 2001;280:C1570–5.

[83] Burton GW, Traber MG, Acuff RV, Walters DN, Kayden H, Hughes L, et al. Human plasma and tissue alpha-tocopherol concentrations in response to supplementation with deuterated natural and synthetic vitamin E. Am J Clin Nutr 1998;67:669–84.
[84] Nieman D, Peters EM, Henson D, Nevines E, Thompson M. Influence of vitamin C supplementation on cytokine changes following an ultramarathon. J Interferon Cytokine Res 2000;20:1029–35.
[85] Nieman DC, Henson DA, McAnulty S, McAnulty L, Swick N, Utter A, et al. Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. J Appl Physiol 2002;92: 1970–7.
[86] Peters EM, Anderson R, Theron AJ. Attenuation of increase in circulating cortisol and enhancement of the acute phase protein response in vitamin C-supplemented ultramarathoners. Int J Sports Med 2001;22:120–6.
[87] Nieman DC, Henson DA, McAnulty SR, McAnulty L, JD M, Ahmed A, et al. Vitamin E and immunity after the Kona triathlon world championship. Med Sci Sports Exerc 2004;36:1328–35.
[88] Mastaloudis A, Morrow J, Hopkins D, Devaraj S, Traber M. Antioxidant supplementation prevents exercise-induced lipid peroxidation, but not inflammation, in ultramarathon runners. Free Radic Biol Med 2004;15:1329–41.
[89] Schwedhelm E, Maas R, Troost R, Boger RH. Clinical pharmacokinetics of antioxidants and their impact on systemic oxidative stress. Clin Pharmacokinet 2003;42:437–59.
[90] Phillips T, Childs AC, Dreon DM, Phinney S, Leeuwenburgh C. A dietary supplement attenuates IL-6 and CRP after eccentric exercise in untrained males. Med Sci Sports Exerc 2003;35:2032–7.
[91] Eichhorn JC, Lee R, Dunster C, Basu S, Kelly FJ. Alpha- and gamma-tocopherol plasma and urinary biokinetics following alpha-tocopherol supplementation. Ann NY Acad Sci 2004;1031:339–40.
[92] Peake J. Vitamin C: effects of exercise and requirements with training. Int J Sport Nutr Exerc Metab 2003;13:125–51.
[93] Thompson D, Williams C, Kingsley M, Nicholas CW, Lakomy HK, McArdle F, et al. Muscle soreness and damage parameters after prolonged intermittent shuttle-running following acute vitamin C supplementation. Int J Sports Med 2001;22:68–75.
[94] Beaton LJ, Allan DA, Tarnopolsky MA, Tiidus PM, Phillips SM. Contraction-induced muscle damage is unaffected by vitamin E supplementation. Med Sci Sports Exerc 2002;34:798–805.
[95] Bloomer RJ, Goldfarb AH, McKenzie MJ, You T, Nguyen L. Effects of antioxidant therapy in women exposed to eccentric exercise. Int J Sport Nutr Exerc Metab 2004;14:377–88.
[96] Mastaloudis A, Traber MG, Carstensen K, Widrick JJ. Antioxidants did not prevent muscle damage in response to an ultramarathon run. Med Sci Sports Exerc 2006;38:72–80.
[97] MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002;360:23–33.
[98] Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet 1999;354:447–55.
[99] Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med 2000;342:154–60.
[100] Bruunsgaard H, Poulsen HE, Pedersen BK, Nyyssonen K, Kaikkonen J, Salonen JT. Long-term combined supplementations with alpha-tocopherol and vitamin C have no detectable anti-inflammatory effects in healthy men. J Nutr 2003;133:1170–3.
[101] Kugelmas M, Hill DB, Vivian B, Marsano L, McClain CJ. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. Hepatology 2003;38:413–9.
[102] Lonn E, Bosch J, Yusuf S, Sheridan P, Pogue J, Arnold JM, et al. Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. JAMA 2005; 293:1338–47.
[103] Gleeson M, Nieman DC, Pedersen BK. Exercise, nutrition and immune function. J Sports Sci 2004;22:115–25.