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Oxidative stress, exercise, and antioxidant supplementation

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Abstract

Cells continuously produce free radicals and reactive oxygen species (ROS) as part of metabolic processes. These free radicals are neutralized by an elaborate antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and numerous non-enzymatic antioxidants, including vitamins A, E and C, glutathione, ubiquinone, and flavonoids. Exercise can produce an imbalance between ROS and antioxidants, which is referred to as oxidative stress. Dietary antioxidant supplements are marketed to and used by athletes as a means to counteract the oxidative stress of exercise. Whether strenuous exercise does, in fact, increase the need for additional antioxidants in the diet is not clear. This review examines the markers used to determine oxidative stress in blood and muscle samples (e.g. lipid peroxidation, expired pentane, malondialdehyde (MDA), F2-isoprostanes, congugated dienes, and 8-hydroxy-2′-deoxyguanosine (8-OhdG)), the changes in oxidative stress markers induced by exercise, and whether athletes require antioxidant supplements.

Keywords: Exercise; Oxidative stress; Antioxidants

1. Introduction

Cells continuously produce free radicals and reactive oxygen species (ROS) as part of metabolic processes. These free radicals are neutralized by an elaborate antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and numerous non-enzymatic antioxidants, including vitamins A, E and C, glutathione, ubiquinone, and flavonoids. Exercise can produce an imbalance between ROS and antioxidants, which is referred to as oxidative stress. Physical activity increases the generation of free radicals in several ways. Two to 5% of oxygen used in the mitochondria forms free radicals. As oxidative phosphorylation increases in response to exercise, there will be a concomitant increase in free radicals. Catecholamines that are released during exercise can lead to free radical production. Other sources of free radical increase with exercise include prostanoid metabolism, xanthine oxidase, NAD(P)H oxidase, and several secondary sources, such as the release of radicals by macrophages recruited to repair damaged tissue (Jackson, 2000).

Antioxidant supplements are marketed to and used by athletes as a means to counteract the oxidative stress of exercise. Whether strenuous

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exercise does, in fact, increase the need for additional antioxidants in the diet is not clear. If the increase in free radicals is greater than the ability to neutralize them, the radicals will attack cellular components, especially lipids. The attack on lipids initiates a chain reaction called lipid peroxidation, which leads to generation of more radicals and ROS that can harm other cellular components. The body appears able to withstand a limited increase in free radicals, and in fact, data suggest that an increase in ROS is necessary for muscle adaptation to occur (Jackson, 1999).

This chapter will review the markers used to determine oxidative stress, changes induced by exercise, and effects of antioxidant supplementation. The focus of the chapter will be on human response to exercise and supplementation.

2. Measurement of oxidative stress in humans

To examine acute oxidative stress in response to exercise, most researchers have assessed various stress makers in blood and urine. Few studies have examined oxidative stress in muscle tissue of humans in response to exercise (Child et al., 1999; Hellsten et al., 1996). Most commonly measured are by-products of lipid peroxidation, but changes in status of antioxidant compounds such as glutathione, protein and DNA oxidation products, and antioxidant enzyme activities have also been used. These are all indirect measures of free radical activity. Electron spin resonance, a direct measure of free radicals, has been used predominantly in in vitro studies, but recently used to detect free radicals in blood. This section will discuss various markers of oxidative stress and how they are altered by exercise. For other reviews and critiques see: Di Meo and Venditti, 2001; Fielding and Meydani, 1997; Goldfarb, 1999; Han et al., 2000; Jenkins, 2000; Ji, 1999; Ji et al., 1993; Leeuwenburgh and Heinecke, 2001; McArdle and Jackson, 2000; Niess et al., 1999; Powers et al., 1999; Reid, 2001; Sen and Packer, 2000; Sen, 2001, 1999; Sen and Roy, 2001; Tiidus, 1998.

3. Lipid peroxidation

Measures of lipid peroxidation include expired pentane, malondialdehydes (MDA), lipid hydroperoxides, isoprostanes, and conjugated dienes. Most studies have used MDA as a measure of oxidative stress imposed by exercise. When free radicals are generated they can attack polyunsaturated fatty acids in the cell membrane leading to a chain of chemical reactions called lipid peroxidation. As the fatty acid is broken down, hydrocarbon gases (ethane or pentane) and aldehydes are formed.

3.1. Expired pentane

Pentane can be measured in the expired air (Mendis et al., 1994), however few studies have used this as a marker of exercise stress. Expired pentane can be measured by gas chromatographic techniques. Dillard et al. (1978) found that expired pentane increased in response to exercise but not to inspired ozone. Other studies have confirmed that aerobic forms of exercise produce increased levels of expired pentane during and immediately after exercise (Leaf et al., 1997, 1999; Pincemail et al., 1990), and expired pentane increases proportionately with increasing exercise intensity (Kanter et al., 1993). This method is considered highly sensitive and is non-invasive (Han et al., 2000). However, this is a difficult technique, which explains its infrequent use.

3.2. Malondialdehyde

Aldehydes, especially MDA, have been frequently used as markers of oxidative stress in response to exercise. Fig. 1 presents the chain of chemical reactions leading to MDA, which can be measured by HPLC, spectrophotometry or spectrofluorescence (Halliwell and Chirico, 1993; Han et al., 2000). The most common method used to assess changes in MDA with exercise is the thiobarbituric acid (TBARS) assay. This method works well when used on defined membrane systems such as microsomes in vitro (Halliwell and Chirico, 1993), but the method has been criticized for use in human studies of oxidative

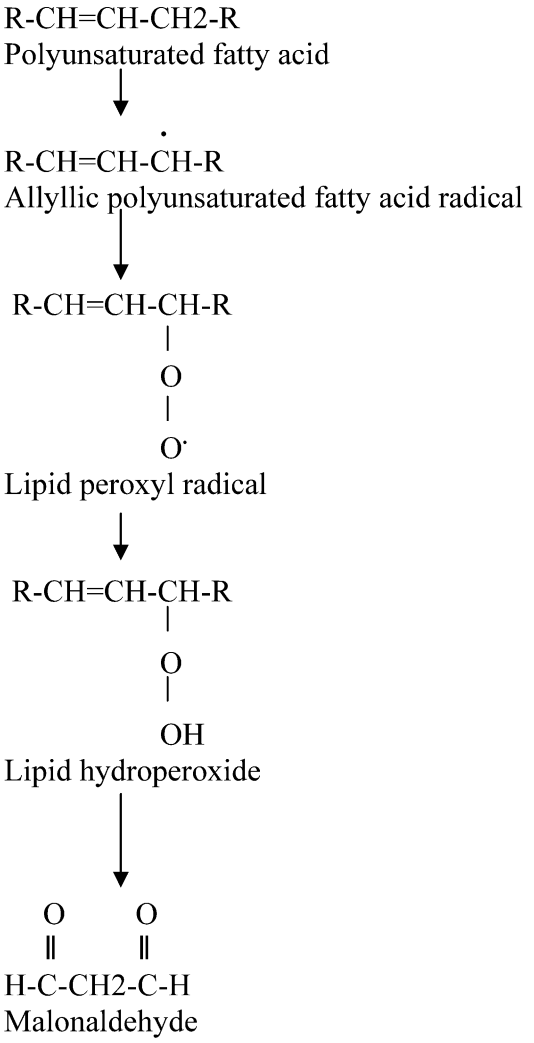

Fig. 1. Steps of lipid peroxidation (Alessio, 2000).

stress because TBARS lacks specificity. The assay also reacts with saturated and unsaturated non-functional aldehydes, carbohydrates and prostaglandins (Alessio, 2000).

Resting plasma MDA was found to be higher in sprint trained athletes and marathon runners compared with control subjects (Marzatico et al., 1997). Santos-Silva et al. (2001) also found elevated resting MDA levels in trained adolescent swimmers compared with control subjects. In contrast, Niess et al. (1996) reported higher plasma MDA in untrained subjects compared with trained subjects, and Miyazaki et al. (2001) observed no change in erythrocyte MDA after a 12-week training program.

Several studies reported that single bouts of exercise increase blood levels of MDA (Davies et al., 1982; Hartmann et al., 1995; Koska et al., 2000; Miyazaki et al., 2001). Marzatico et al. (1997) found plasma MDA increased over 48h post-sprint type exercise in sprinters and immediately post-endurance exercise in marathon runners. Kanter et al. (1988) reported increases in plasma MDA ($\sim$70%) following an extreme endurance event (50 m run) in elite athletes. Further, these measures correlated with plasma increases in CK and LDH, markers of muscle damage. Similarly, Child et al. (2000) found an increase in MDA of about 40% immediately after a half marathon.

Not all studies reported increases in MDA in response to exercise (Viinikka et al., 1984). Niess et al. (1996) measured plasma levels of MDA in trained and untrained individuals at rest, before and after an exhaustive bout of exercise. They found no significant increases in MDA in either group following a treadmill test to exhaustion, either at 15 min post-exercise or 24 h post-exercise. Moderately trained subjects who ran for 2.5 h on a treadmill showed no change in plasma MDA (Dufaux et al., 1997; Duthie et al., 1990). Similarly, there were no documented changes at rest, before or after 4 weeks of high intensity rowing training in plasma MDA levels (Dernbach et al., 1993) in athletes, and Alessio et al. (2000) found no change in plasma MDA after repeated isometric contractions.

Strenuous endurance training was shown to reduce indices of oxidative stress following exhausting exercise (Miyazaki et al., 2001). Untrained male subjects performed an acute period of exercise on a cycle ergometer before and after a 12-week strenuous endurance training program. There was a smaller increase in erythrocyte MDA in response to the exercise bout post-training compared to pre-training. Moreover, decreased levels of MDA in response to exercise have also been reported in highly trained skiers and runners immediately following exercise to exhaustion (Hubner-Wozniak et al., 1994; Rokitzki et al., 1994a).

Eccentric exercise, which is known to cause muscle inflammation, has been hypothesized to contribute to increased levels of lipid peroxidation presumably due to macrophage reactions in tissue. Maughan et al. (1989) found increases in MDA 6 h post downhill-running (biased toward eccentric contractions), with these levels returning to baseline levels at 72 h post exercise. Those subjects with the greatest increase in markers of muscle damage, (i.e. CK, lactate dehydrogenase (LDH)) experienced the greatest increases in serum MDA concentrations. However, muscle biopsies taken after a single bout of maximal eccentric exercise failed to show any change in MDA levels (Saxton et al., 1994). Furthermore, Child et al. (1999) reported no change in both plasma and muscle MDA levels following a single bout of eccentric exercise, despite the increase in inflammatory cell invasion into the tissue.

3.3. *Lipid hydroperoxides, isoprostanes, and conjugated dienes*

Lipid hydroperoxides (LOOH) are formed earlier in the pathway leading to MDA (Fig. 1). HPLC (chemiluminescence) and enzymatic methods are used to detect LOOH in blood and tissue (Han et al., 2000). Increases in blood levels of LOOH have been reported after exercise (Alessio et al., 2000; Bailey et al., 2001; Childs et al., 2001), but not many studies have used LOOH as a marker of oxidative stress with exercise. Alessio et al. reported that lipid hydroperoxides increased after repetitive isometric exercise but not after aerobic exercise.

Another measure that has been used to detect oxidative stress is F2-isoprotanes, prostaglandin-like compounds. F2-isoprostanes are produced by non-cyclooxygenase dependent peroxidation of arachidonic acid and measured using gas chromatography mass spectroscopy (GC-MS) (Roberts and Morrow, 1994). Few studies have used this marker of oxidative stress after exercise (Child et al., 1999; Mastaloudis et al., 2001). Mastaloudis et al. reported a 43% increase in F2-isoprostanes after a 50 km ultramarathon. These levels returned to baseline by 24 h. Child et al. (1999) found a similar increase after a strenuous elbow flexion exercise designed to produce muscle damage.

Conjugated dienes are a biomarker of lipid peroxidation because LOOH contain a conjugated diene structure (Han et al., 2000). They are measured by spectrophotometry or HPLC methods. Conjugated dienes have been used to assess low-density lipoprotein oxidation in vitro. Exercise has been shown to increase plasma conjugated dienes (Balakrishnan and Anuradha, 1998; Marzatico et al., 1997), and to increase conjugated dienes in an in vitro determination of low density lipoprotein susceptibility to oxidation (Liu et al., 1999; Sanchez-Quesada et al., 1998). For example, Liu et al. found that LDL oxidation was increased as determined from a reduction in the lag time for formation of conjugated dienes immediately and 4 days after a marathon. Marzatico et al. found that plasma conjugated dienes increased 6 h after a sprint exercise in sprinters but not after an endurance exercise in marathon runners. However, not all studies found that conjugated dienes increased in response to exercise. Duthie et al. (1990) reported no change in plasma conjugated dienes in subjects who performed a half-marathon.

4. Protein and DNA oxidation

An increase in blood protein carbonyls has recently been reported after aerobic exercise, but not after repetitive isometric exercise (Alessio et al., 2000). When ROS attack amino acids, carbonyl groups are produced and these are most accurately measured by HPLC or ELISA procedures (Griffiths, 2000; Han et al., 2000). Most studies of in vivo protein oxidation have been limited to animal studies (Griffiths, 2000). The methods have been criticized as being non-specific and unreliable, especially in human studies. Furthermore, whether carbonyls represent a good marker of protein oxidation in vivo is controversial.

Urinary 8-hydroxy-deoxyguanosine (8-OhdG) excretion has been found to increase after exercise (Okamura et al., 1997), and is considered a measure of DNA oxidation in response to free radicals (Han et al., 2000). Several techniques have

been used to assess 8-OhdG including HPLC, GC-MS, and enzymatic assays (Han et al., 2000). Okamura et al. (1997) examined changes in urinary excretion 8-OhdG in long distance runners who participated in 8 days of training. Twenty-four hour urine measures showed a significant increase from pre-training to the 8th day of training. In contrast, trained distance runners and sedentary subjects who performed a bout of treadmill exercise did not have elevated 8-OhdG immediately post-exercise. Whether these results suggest that repeated bouts of endurance exercise are necessary for accumulation of DNA oxidation products cannot be ascertained from only these few studies.

5. Glutathione

Glutathione is an antioxidant that has been used as a measure of oxidative stress. Specifically the GSH-GSSG ratio decreases under oxidative conditions. GSH and GSSG detection methods include HPLC and spectrophotometric techniques. Several studies have reported that blood oxidized glutathione (GSSG) and thus the GSH-GSSG ratio decreases in response to exercise (Laaksonen et al., 1999; Sastre et al., 1992; Sen, 1999). For example, Laaksonen et al. found that blood GSSG increased by 50% after 40 min of cycling at 60% VO2 max. Sastre et al. measured serum GSSG and GSH levels following an acute bout of high intensity treadmill running and found a 72% increase in GSSG levels post-exercise. One hour following testing, GSSG levels had returned to baseline values. In addition, a linear relationship between GSSG–GSH and lactate to pyruvate ratios were observed before, during, and after exercise. Tiidus et al. (1996), however, observed that muscle glutathione status was unaffected by 8 weeks of 35 min of aerobic cycle training (3 times/week).

Ji et al. (1993) examined the mechanism of the change in glutathione in the blood in response to exercise. They found that total GSH (GSH + GSSG) and GSH increased throughout a cycling exercise at 70% VO2 max to exhaustion. However, when subjects performed the same test but ingested carbohydrate, sufficient to increase blood glucose and insulin over the control condition, there was no increase in total glutathione or GSH. It was suggested that glucagon may be involved in hepatic GSH efflux during prolonged exercise.

6. Total antioxidant capacity

Also used as an indicator of oxidative stress is total antioxidant capacity (Cao et al., 1993). Many assays are available, but changes observed from these assays do not always correlate well with other assays (Han et al., 2000). Typically a tissue or blood sample is added in vitro to a chemical free-radical generating system, and the ability of the tissue or blood to resist oxidative stress (e.g. resistance to lipid peroxidation) is then measured. Several studies have used this method to detect increases in oxidative stress after exercise (Child et al., 1999; Ginsburg et al., 2001; Santos-Silva et al., 2001). However, Alessio et al. (1997) found that plasma total antioxidant capacity did not increase in response to a 30 min exercise, despite an increase in MDA.

7. Antioxidant enzymes

Changes in antioxidant enzyme activity in erythrocytes have been used to document oxidative stress. The enzymes that have been most commonly examined after exercise stress are superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase.

SOD functions in the cell as one of the primary enzymatic antioxidant defenses against superoxide radicals (Powers and Lennon, 1999). Increases in SOD enzyme activity corresponds with enhanced resistance to oxidative stress (Fielding and Meydani, 1997). For instance, resting muscle total and mitochondrial SOD activity were higher in volleyball trained athletes when compared to untrained individuals also at rest (Ortenblad et al., 1997). Brites et al. (1999) observed a similar relationship, with higher resting plasma SOD activity in soccer players compared to untrained subjects (Brites et al., 1999), and Marzatico et al. (1997) reported

higher erythrocyte SOD activity in sprinters and marathon runners. When the sprinters performed a sprint exercise and the runners performed a half marathon, there was a significant increase in SOD activity immediately post-exercise.

The results of the above studies suggest that increased levels of SOD activity in blood and muscle at rest are more common in trained individuals, and SOD activity is increased in response to exercise interventions in a trained population. However, not all studies are consistent with these conclusions. Untrained individuals did not show an increase in muscle SOD activity after completing an 8-week moderate-intensity cycling exercise program (Tiidus et al., 1996). Tauler et al. (1999) found no change in erythrocyte SOD activity following a moderate intensity duathlon in trained individuals. Furthermore, trained long distance skiers participating in a graded treadmill test to exhaustion revealed a decrease in erythrocyte SOD levels in the blood following an acute bout of exercise (Hubner-Wozniak et al., 1994).

The decomposition of hydrogen peroxide to form water and oxygen is accomplished in the cell by catalase. This antioxidative enzyme is widely distributed in the cell, with the majority of the activity occurring in the mitochondria and peroxisomes (Powers and Lennon, 1999). Positive relationships have been documented in runners between weekly training distance and resting levels of erythrocyte catalase activity (Ohno et al., 1988; Robertson et al., 1991). At higher training volumes, the increased production of hydrogen peroxide exceeds the capabilities of glutathione peroxidase (GPx). GPx is another antioxidant enzyme with a much greater affinity for hydrogen peroxide than catalase. Therefore, catalase production would be expected to increase in response to the demands of training volume, to compensate for the inability of GPx to scavenge hydrogen peroxide. However, 8 weeks of aerobic training did not change muscle catalase activity (Tiidus et al., 1996).

Catalase activity in response to a single bout of exercise is variable. For instance, Rokitzki et al. (1994a) found no difference in erythrocyte catalase activity levels following marathon running. However, in a population of trained cyclists, following a bout of submaximal exercise for 90 min, Aguilo et al. (2000) described a decrease in erythrocyte catalase activity of nearly 20%. Moreover, Marzatico et al. (1997) reported that sprinters who performed a sprint-type exercise did not have altered erythrocyte catalase activity, but distance runners who performed an endurance exercise showed increases in catalase activity at 24 and 48 h post-exercise.

An increase in oxygen consumption during exercise activates the enzyme GPx to remove hydrogen peroxide and organic hyperperoxides from the cell (Tiidus et al., 1996). Similar to SOD and catalase, GPx is located in both the mitochondria and the cytosol where it serves as an important cellular protectant against free radical induced damage to membrane lipids, proteins and nucleic acids (Powers and Lennon, 1999). During normal function of the antioxidant defense system, reduced glutathione (GSH) is used by GPx peroxidase to detoxify hydrogen peroxide. Additionally, glutathione reductase is necessary to convert hydrogen peroxide to GSH, which will also contribute to the detoxification of hydrogen peroxide (see Fig. 2) (Fowkes, 1996).

Marzatico et al. (1997) reported higher resting activity in erythrocyte GPx activity in trained sprinters and marathon runners compared to untrained individuals, and Robertson et al. (1991) found higher erythrocyte total glutathione, and GSH in low training runners (17–43 km/week) than sedentary subjects. High training runners (80–147 km/week) had higher total gluthathione, GSH and GSSG compared with sedentary subjects. Although Ortenblad et al. (1997) observed no difference in erythrocyte GPx or glutathione reductase activity between trained and untrained subjects, muscle activities of these enzymes were higher in the trained subjects. A 40-week training program tailored for half-marathon conditioning resulted in a significant increase in erythrocyte glutathione reductase activity (Evelo et al., 1992). A more modest approach (5 km, 6 times/week for 10 weeks) resulted in similar significant increases (Ohno et al., 1988). Additionally, Robertson et al. (1991) reported a significant correlation of GPx activity and weekly training distance in runners.

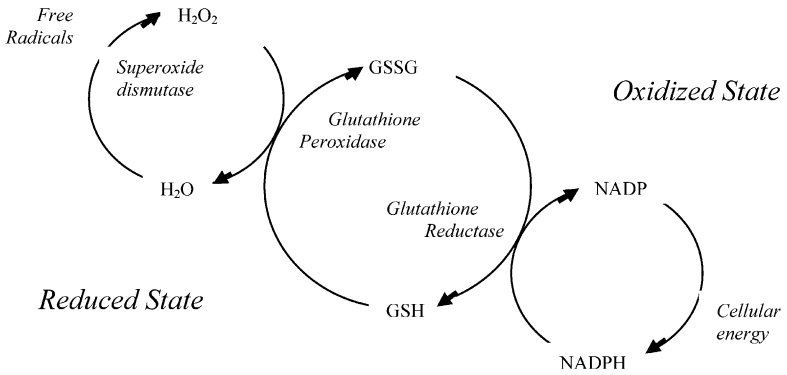

Fig. 2. The recycling of glutathione. Hydrogen peroxide (H_2O_2) is reduced to water (H_2O), while glutathione (GSH) is oxidized to glutathione disulfide (GSSG). Each cell has a limited amount of GSH, so GSSG must be recycled to the reduced state (GSH) to maintain protection against H_2O_2. Glutathione reductase (GR) uses electrons from the oxidation of NADH to convert GSSG into GSH. To continue to detoxify H_2O_2, a constant supply of NADPH must be available (Fowkes, 1996).

In response to an acute bout of high intensity exercise, Hubner-Wozniak et al. (1994) found an increase in plasma glutathione reductase activity. Marzatico et al. (1997) found an increase in erythrocyte GPx activity after a sprint exercise but no change when runners performed an endurance exercise. In contrast, marathon running and cycling in athletes showed little to no difference in pre- versus post race measures of erythrocyte GPx activity (Rokitzki et al., 1994b; Tauler et al., 1999). Moderately trained athletes had decreased glutathione peroxidase immediately after marathon running (Dufaux et al., 1997; Duthie et al., 1990) and these levels returned to baseline at 1 h post-exercise (Dufaux et al., 1997). The above studies used a number of different methods to identify GPx and glutathione reductase activity, which makes it difficult to compare study results (Jenkins, 2000).

Miyazaki et al. (2001) examined SOD, catalase, and GPx in untrained men who participated in the 12-week endurance training program. Before and after the training, subjects performed an incremental cycle ergometer test until exhaustion. They found an increase in resting erythrocyte activity of SOD and GPx, but not catalase. None of the three enzyme activities increased in response to the acute exercise bout either before or after the training. There was a reduced increase in erythrocyte MDA and neutrophil super anions in response to acute exercise after training. This study serves to illustrate how the various makers of oxidative stress are not consistent in their response to exercise and training.

8. Electron spin resonance

Recently, Ashton et al. (1999) used electron spin resonance spectroscopy in conjunction with the spin trapping techniques to directly measure free radical species in the blood in response to exercise stress. While this technique is considered the most sensitive direct measure of free radicals, it has been predominantly used with animal models. In the only studies to use this technique to examine human blood, Ashton et al. (1998, 1999) reported an increase in the concentration of the α-phenyl-tert-butylnitrone (PBN) adduct in blood of subjects who exercised to exhaustion on a cycle ergometer using a progressive and incremental exercise protocol. LOOH and MDA were also increased. Moreover, upon supplementation with vitamin C 2 h before the exercise, there was no increase in these measures in response to a repeated bout of the same exercise.

9. Effects of antioxidant supplementation

Studies that have examined the effects of antioxidant supplements have used exercise perfor-

mance and/or changes in oxidative stress as outcome measures. This section will briefly describe results of those studies. It is difficult, in some cases to draw definite conclusions due to the differences in amount or type of the supplement, the length of supplementation, and the various outcome measures used.

10. Vitamin C

In the 1970s some of the first studies to examine vitamin C supplementation and exercise appeared. These studies examined the effects of supplementation on exercise performance, although the mechanism of how vitamin C was purported to exert an effect was unclear. Gey et al. (1970) showed that 1000 mg/day for 12 weeks during training in Air Force officers did not enhance performance of a walk/run field test compared to a placebo. On the other hand, in a more well-controlled laboratory exercise test to exhaustion, Howald et al. (1975) found that work capacity at a heart rate of 170 was significantly greater for subjects who ingested 100 mg/day compared to a placebo, although there was no difference between groups in total work performed. In the 1980s, two studies reported no benefit of vitamin C supplementation on anaerobic performance (Keith and Merrill, 1983; Keren and Epstein, 1980).

Buzina and Suboticanec (1985) provided a clue as to why there may be differing effects of vitamin C on performance. While their study indicated that vitamin C was related to aerobic capacity, they noted that the association was strongest in subjects whose plasma vitamin C levels were low. These data suggested that vitamin C supplementation may only enhance exercise performance in those with a deficiency. Although Van der Beek et al. (1990) found no decrement in aerobic power when they restricted vitamin C intake for 7 weeks, they did find an increased heart rate at the onset of blood lactic acid accumulation in the subjects with a mild deficiency.

Additional research has focused on the effects of vitamin C supplementation on immune response to exercise and on countering exercise-induced muscle damage. The concentration of vitamin C is high in neutrophils and likely necessary for their function in the immune response. Peters (1997) reported that vitamin C supplementation reduced the incidence of post-race upper respiratory tract infection. Nieman et al. (1997) and Krause et al. (2001) found no benefit of vitamin C supplementation on neutrophil function after exercise, suggesting that the mechanism of reduced URTI symptoms with vitamin C supplementation was not due to enhanced neutrophil function. Peters et al. (2001a,b) reported that vitamin C supplementation attenuated the immunosuppressive adrenal hormones, cortisol and adrenaline, response to exercise. For a more detailed review on exercise immunology and vitamin C see Nieman (2000) and Mackinnon (2000).

In 1952, Staton reported that 30 days of 100 mg vitamin C supplementation resulted in less muscle soreness compared to a placebo (Staton, 1952). The measure of 'muscle soreness' was the number of sit-ups that could be performed after the 30 days compared to pre-treatment. Kaminski and Boal (1992) found that 3 days of 3000 g vitamin C/day prior to exercise and 4 days post exercise, resulted in less delayed onset muscle soreness compared to a placebo. In contrast, Thompson et al. (2001) found that one dose of 1000 mg of vitamin C given 2 h before a 90 min shuttle run, increased plasma vitamin C but did not alter the development of muscle soreness after exercise. It is likely that one dose would not be sufficient to exert an effect. So few studies have examined effects of only vitamin C on muscle soreness, it is difficult to draw any firm conclusions. There is a theoretical basis to propose that vitamin C will reduce the development of muscle soreness. Muscle soreness is a manifestation of muscle damage caused by a strenuous exercise. Part of the body's response to damage is an infiltration of macrophages to the damaged tissue. These macrophages release free radicals leading to further damage. Increased antioxidants could theoretically neutralize these radicals and thereby reduce muscle damage, and hence muscle soreness. Jakeman and Maxwell (1993) reported that subjects supplemented with 400 mg vitamin C for 21 days recovered strength and contractile function faster after a muscle damaging exercise compared subjects taking 400

mg vitamin E or a placebo. The authors suggested that the vitamin C might have protected cell structures, such as the sarcoplasmic reticulum, from oxidative stress and free radical injury.

11. Vitamin E

As with vitamin C, the early studies of exercise and vitamin E supplementation centered around its effect on performance. With the exception of exercise performed at a high altitude (where oxidative stress would be exacerbated) (Simon-Schnass and Pabst, 1988), other studies reported no ergogenic effect of vitamin E supplements (Lawrence et al., 1975a,b; Rokitzki et al., 1994b; Sharman et al., 1971, 1976; Shephard et al., 1974; Talbot and Jameson, 1977). One study (Bunnell et al., 1975) had men ingest a diet low in vitamin E for 13 months and found significantly lower vitamin E in the blood, leveling off at the lowest end of the normal range. However, there were no self-reported alterations in muscle weakness or other physical symptoms, although quantitative performance measures were not assessed.

Since vitamin E has been found to protect cellular membranes from lipid peroxidation, studies have focused on the ability of vitamin E supplementation to reduce the increase in oxidative stress or muscle damage caused by exercise. In 1979, Helgheim et al. reported that subjects who ingested 300 mg vitamin E/day for 6 weeks had similar levels of muscle proteins in the blood after exercise, as did the subjects who took the placebo (Helgheim et al., 1979). They concluded that vitamin E did not alter muscle disruption from the exercise. In contrast, Itoh et al. (2000), who imposed a more stressful exercise challenge over 6 days, found that 4 weeks of 1200 IU vitamin E/day reduced the leakage of muscle enzymes in response to the exercise. Vitamin E supplementation of 300 mg/day for 4 weeks was shown to reduce the increase in MDA in response to strenuous exercise (Sumida et al., 1989).

Cannon et al. (1991) found that vitamin E supplementation of 800 IU/day for 48 days resulted in a reduction in the plasma cytokine (IL-1β and IL-6) response to muscle damage-inducing eccentric exercise. The lower IL-1β was associated with lower 3-methylhistidine excretion, a marker of proteolysis. Thus, vitamin E appeared to exert a protective effect against muscle breakdown. Niess et al. (2000) examined the effect of 28 days of 500 IU vitamin E/day in a double blind, placebo controlled, cross-over study on exercise-induced cytoplasmic expression of inducible nitric oxide synthase (iNOS) and antioxidant stress protein heme oxygenase-1 (HO-1) in leukocytes. Induction of these proteins may depend on increases in free radicals and cytokines, which are both altered by exercise. The results showed that a 30 min exhaustive treadmill exercise induced expression of iNOS and HO-1 but this change was not altered by vitamin E supplementation. At present, the effect of vitamin E supplementation on the inflammatory response to exercise is unclear.

12. Antioxidant combinations

Vitamin C and vitamin E have been used together to determine the effects of antioxidant supplementation on exercise. This combination is thought to be more effective than either vitamin alone since vitamin C can regenerate vitamin E. Rokitzki et al. (1994a) had long distance runners ingest 400 IU vitamin E and 200 mg vitamin C (or a placebo) for 4.5 weeks prior to a marathon. The increase in creatine kinase (CK) was significantly lower in the supplemented group compared with the placebo group after the marathon, indicating less muscle damage in subjects taking the antioxidant supplement. However, Petersen et al. (2001) found no benefit of a combination of 500 mg vitamin C and 400 mg vitamin E for 14 days prior to a downhill treadmill run compared to a placebo. The cytokine (IL-6 and IL-1) increase, lymphocyte response, and increases in blood CK activity were similar between groups. Whether differences in the exercises used between these studies can explain the different findings is not known, however, at 24 h after the exercise, the peak increase in CK was about 900 IU/l in the Petersen et al. study, whereas in the Rokitzki et al. study the increase was about 400 IU/l.

Kanter et al. (1993) examined the effect of 592 mg vitamin E, 1000 mg vitamin C and 30 mg beta carotene or a placebo for 6 weeks on expired pentane and serum MDA levels in response to a 30 min treadmill exercise. The response to exercise pre and post supplementation was similar. However, the supplemented group had a lower baseline for expired pentane and for serum MDA, so that the peak increase was lower, thereby reflecting an overall lower level of oxidative stress. Schroder et al. (2000) reported that a similar supplementation regimen for 32 days during a competitive season in professional basketball players resulted in a decreased ratio of lipoperoxides to total antioxidant capacity in the supplemented group, which the researchers suggested reflected a reduction in oxidative stress. They also noted that in the placebo group, plasma vitamin C levels dropped dramatically to levels at the low end of normal.

A combination of 270 mg vitamin E, 600 mg vitamin C, and 100 mg ubiquinone (Co-enzyme Q10) or placebo was given daily for 6 weeks to triathletes (Nielson et al., 1999). The effects of this supplementation regimen on local muscular fatigue measures and maximal oxygen uptake were then assessed. The supplement did not affect aerobic capacity, nor measures of muscle fatigue (electrically stimulated contractions or energy depletion detected via magnetic resonance spectroscopy) after repeated isometric contractions at 45% MVC (approximately 9–10 min of exercise). It seems unlikely that antioxidant supplementation would be expected to alter VO2 max, and the isometric contractions may not be sufficiently stressful to induce free radical increases that would negatively affect the measurements. Ubiquinone supplementation alone has been tested for its effects on short supramaximal exercise performance. Faff et al. (1997) reported that subjects taking 100 mg/day for 30 days produced significantly higher power and work output for three all-out cycling bouts after, as compared to before, the supplementation. There was no difference for the placebo group. The mechanism to explain this performance benefit is unclear.

In 1994, Sen et al. examined the effect of n-acetylcysteine (NAC) supplementation on changes in blood glutathione levels in response to exercise (Sen et al., 1994). Because glutathione (GSH) is an important antioxidant, it was hypothesized that n-acetylcysteine would preserve GSH and maintain antioxidant capacity. Subjects took 800 mg of NAC for 2 days prior to and 800 mg on the test day when they performed a maximal cycling test. Compared to a max test performed at least 7 days earlier, the max test after NAC supplementation produced significantly less GSSG and TBARS. The supplementation also produced higher resting values of peroxyl radical scavenging capacity.

Recently, Childs et al. (2001) gave subjects 12.5 mg/kg body weight of vitamin C and 10 mg/kg body weight NAC or a placebo immediately after they performed an eccentric exercise with the elbow flexors, which was designed to produce muscle damage. The supplemented group showed higher levels of lipid hydroperoxides and 8-iso prostaglandin F2α. The researchers concluded that vitamin C and NAC supplementation given immediately post-injury, increased oxidative stress. This study points out that this antioxidant supplement could have a negative effect on recovery from muscle damaging exercise. However, muscle proteins that leak out of damaged muscle (CK, lactate dehydrogenase, and myoglobin) did not differ between the supplemented and the placebo groups. These findings are somewhat contrary to those of Jakeman and Maxwell (1993) who used a similar exercise stress and found that vitamin C supplementation prior to the exercise resulted in a faster recovery of muscle strength.

13. Summary

This chapter began by raising the question regarding whether athletes need antioxidant supplements to counter increases in oxidative stress from exercise. To definitively answer this question, we would need to document that indeed oxidative stress increased with exercise to a level that would cause more harm than good. However, the results of studies that addressed whether exercise increases oxidative stress are not consistent, perhaps because of the different levels of training of the subjects, the different exercises and intensities used, and the various measures of oxidative stress

employed. The lack of consistency of results precludes a definitive answer to the question that we posed. Basically, we can say that most, but not all, studies found some increase in oxidative stress of selected outcome measures in response to some types of exercise.

Despite this lack of consistency in results regarding whether exercise increases oxidative stress, many studies have sought to determine whether antioxidant supplements would benefit those who exercise regularly. Earlier studies found no advantage of antioxidant supplements on exercise performance, but there is little theoretical basis to believe that they would have an effect. Moreover, several factors govern human performance, thus making it difficult to detect effects of a supplement intervention (Sen, 2001). Other studies examined whether supplements reduced measures of oxidative stress. And, just as the studies to examine exercise-induced oxidative stress produced varied results, so did these studies regarding supplementation. The type of supplement, timing of the supplement, and the outcome measures were different among the studies, making any overall interpretation difficult.

At this time, the only statement that can be made is that exercise may or may not result in harmful oxidative stress, and antioxidants may or may not reduce oxidative stress if it occurred at all. We are uncertain whether an increase in oxidative stress that occurs with exercise is necessary for muscle adaptation to occur, or whether it is harmful, causing muscle damage that impairs the ability to perform or train. There is growing evidence that free radicals can serve as signals that stimulate adaptive processes (Jackson, 2000). We do not know at what level of increased oxidative stress the potential benefits will outweigh the risks. A prudent recommendation for athletes is to ingest a diet rich in antioxidants rather than taking supplements.

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