Chapter II

Review of related literature
CHAPTER II

REVIEW OF LITERATURE

This chapter was provide a basic overview of current knowledge on oxidative stress and different types of enzymes which are important in the process of the stress and important antioxidant factors like green tea.

2.1. Exercise and Oxidative Stress

Exercise, has been associated with an acute increase in the production of reactive oxygen species (ROS) above resting values (McBride, 1999). However, it is not known how chronic exercise training affects resting and exercise ROS levels or if there are differences between varying types of training (i.e. aerobic vs. anaerobic).

Three basic mechanisms of exercise induced ROS production are commonly described:

1) mitochondrial leakage,
2) Ischemia-reperfusion injury,
3) Inflammation and immune response.

1) Under physiological conditions, mitochondrial leakage is the major source of. Oxygen is absorbed by the blood circulation which is transported via the cardiovascular system to the various tissues in the body where it is delivered to the mitochondria. An increase in the number of electrons present in the mitochondria that readily dissociate without a concurrent increase in molecular oxygen to bind results in more electrons than oxygen, leading to ROS production (Ji, 1995; Clanton, 2007). Specifically, the inner mitochondrial membrane has been implicated as a major site of oxygen free radical production, because ROS are formed during the reduction of oxygen that takes place as part of the electron transport chain.

2) The second mechanism of exercise induced ROS is ischemia-reperfusion injury caused by an over stimulation of oxygen following high intensity exercise (via excess post exercise oxygen consumption). When blood flow is decreased to tissue (ischemia) through arterial blockage or blood vessel
occlusion (often seen with resistance exercise), xanthine oxidase (XO) activity is increased. When normal blood flow is restored (reperfusion) and oxygen levels are increased within the tissue the increased XO activity leads to ROS production.

3) Lastly, exercise that is characterized by excessive eccentric activity or intense resistance has been shown to cause muscle damage. The damaged tissue becomes necrotic and causes an oxidative burst by a primary immune response (Quindry et al., 2003; Magalhaes et al., 2004; Hagobian et al., 2006).

There is a close relationship between ROS production and exercise high intensity. Previously, many studies have reported graded exercise test (GXT) protocols to produce ROS because high intensity aerobic exercise, particularly maximal effort, is associated with high levels of anaerobic metabolism and hypoxia. These factors are related to oxidative stress by creating a buildup of reducing agents in the mitochondria which stresses the electron transport chain (Clanton, 2007), or through ischemia-reperfusion injury caused by an over abundance of oxygen following high intensity aerobic exercise. Of the 12 studies revealed that analyzed TBARS following a GXT, 6 resulted in increased ROS activity (Lovlin et al., 1987; Kretzschmar et al., 1989; Rokitzki et al., 1994; Hartmann, 1995; Niess, 1996; Sen et al., 1994; Ashton et al., 1998; Szczesniak et al., 1998; Leaf et al., 1999; Sürmen-Gür et al., 1999; Jammes et al., 2005; Steinberg et al., 2006). Of the 11 studies showed that analyzed TBARS following sub maximal exercise for 30-90 minutes, 4 resulted in increased ROS activity (Lovlin et al., 1987; Maxwell et al., 1993; Sen et al., 1994; Alessio et al., 1997; Borsheim et al., 1999; Chung et al., 1999; Laaksonen et al., 1999; Wang et al., 2006). Therefore, the high intensity of the GXT may lead to OS in post exercise blood samples. Many studies support this concept that exercise intensity plays a major role in post-exercise blood Oxidative Stress. Kanter and Eddy (1993) found an increase in TBARS immediately following exercise in subjects that ran at 60% VO$_2$ max for 30 minutes followed by 5 min at 90% VO$_2$ max. Michailidis et al. (2007) found increased TBARS for several hours following exercise in subjects that ran for 45 minutes at 75% VO$_2$ max followed by running to exhaustion at 90% VO$_2$ max. On the other hand, continuous running at 10% above
lactate threshold for 45 minutes, or running at 75% VO\textsubscript{2} max to exhaustion was shown MDA or TBARS (Quindry et al., 2003; Gremillion and Sawyer, 2010).

![Schematic model](image)

**Figure 2.1.** Schematic model depicting the potential determinants and the consequences of exercise-related changes in the cellular redox balance in skeletal muscle. Increasing levels of ROS, generated in working muscle, lead to a change in the redox-balance towards a more pro-oxidant state. The extent of redox changes resulting from a given amount of generated ROS is mainly determined by the capacity of the antioxidant system. While moderate changes in the cellular redox state as reflected by the red arrow exert primarily more regulating properties, excessive ROS generation without adequate compensation result in damaging oxidative stress (For details see text). ROS: reactive oxygen species; CAT: Catalase; GPx: glutathione peroxidase; NADPH: nicotinamide adenine dinucleotide phosphate (reduced form); HO-1: heme oxygenase-1; HSP: heat shock proteins; SOD: superoxide dismutase.

### 2.2. Measurement of oxidative stress (OS).

Our review of literatures indicates that there are eight primary procedures used by researchers to measure Oxidative Stress in the body; the most common indices of ROS activity are measured either by enzyme activity or from byproducts. Enzymes which is measured are, Thiobarbituric acid reactive substances-malondialdehyde (TBARS-MDA) xanthine oxidase (XO), superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT). , conjugated dienes (DC), expired pentane, protein carbonyl (PC) out of these malondialdehyde (MDA), TBARS, and GSH appear to be somewhat equivocal, returning a mix of significant and non-significant differences in blood levels, even when similar groups are used (Lovlin et al., 1987; Sen et al., 1994; Ashton et al., 1998; Sürmen-Gür et al., 1999).
2.2.1. TBARS-Malondialdehyde (MDA)

Currently, MDA is one of the most commonly measured indirect markers of OS (McBride and Kraemer, 1999). Malondialdehyde is a decomposition product of lipid peroxidation that reacts with thiobarbituric acid (TBA) (Jenkins, 2000; McBride and Kraemer, 1999). TBA will combine with other substances besides MDA (Carbonneau, Peuchant, Sess, Canioni, and Clerc, 1991; Jenkins, 2000). Some aldehydes other than MDA can form chromagens with absorbance at 532 nm (Halliwell and Chirico, 1993).

Modification of the TBA assay can avoid many of the artifacts attributed to the TBARS-MDA assay (Halliwell and Chirico, 1993).

Increased levels of MDA have been observed immediately after a half marathon (Childs, Jacobs, Kamiski, Halliwell, and Leewenburgh, 2001). A downhill (eccentric) run did not show an increase in MDA until 48 hours and was not significant until 72 hours post-exercise (Close, Ashton, Cable, Doran, and MacLaren, 2004). In contrast, Bloomer, Goldfarb, and McKenzie (2006) found an increase in MDA immediately after a downhill run. These discrepancies in timing may be due to different exercise protocols, training status of subjects, or type of assay used.

2.2.2. Superoxide Dismutase (SOD)

The superoxide radical is converted to hydrogen peroxide and O₂ by the enzyme sod (JI, 1999). SOD exists in two different isoforms, one in which manganese is used as the metal (MN-SOD, mitochondria) and the other in which copper is used as the metal (CU-SOD, skeletal muscle) (Powers and Lennon, 1999). SOD distribution in skeletal muscle has been reported to vary by muscle fiber type (Laughlin, et al., 1990; Powers and Lennon, 1999). However, not all studies are in agreement with that finding (JI, FU, and Mitchell, 1992). An elevated level of sod may depict an enhanced antioxidant defense system (Hellsten, Apple, and Sjödin, 1996). As the half-life of SOD is only a few minutes, JI (1999) suggests that post-exercise detectable elevation of SOD may be due to de novo synthesis of new enzyme protein. Several enzymes possess the ability to create water from the hydrogen peroxide produced by SOD. The enzymes glutathione peroxidase (Gpx)
and glutathione reductase (GR) are the two enzymes most commonly responsible for this reaction (Hellsten, et al., 1996; Powers and Lennon, 1999).

### 2.2.3. Glutathione (GSH)

Thiols, such as glutathione are used as indicators of OS because of their ability to reduce radicals (Powers, et al., 2004). The ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) is reported to illustrate redox status in the body; therefore, indirectly indicating the degree of OS (Cooper, et al., 2002). The oxidized form of glutathione is formed as the result of the reduction of the ROS by GSH (Cooper, et al., 2002). Glutathione peroxidase (GPX) and gr are the two enzymes involved in these redox reactions (Hellsten, et al., 1996). Several factors can affect glutathione status. The amounts OF GPX, GR, and GSH may be relative to muscle fiber type (JI, FU, and Mitchell, 1992). Glutathione values return to baseline as quickly as 15 minutes post exercise (Sen and Packer, 2000). Glutathione status is subject to rapid change and may not be a reliable measure of antioxidant status (Goldfarb, Bloomer, and Mckenzie, 2005; Rossi, et al., 2002).

### 2.2.4. Catalase (cat)

Catalase is responsible for converting hydrogen peroxide into water (Laughlin, et al., 1990). An increase in cat levels has been correlated with an increased antioxidant defense system in elite endurance cyclists (Mena, et al., 1991). However, Laughlin, et al. (1990) did not find an increase in cat with training involving rats. Studies involving rats have also shown cat activity to vary by muscle fiber type (JI, et al., 1992; Laughlin, et al., 1990). Laughlin, et al. (1990) found cat activity was greater in oxidative skeletal muscle in both sedentary and trained rats.

### 2.3. Delayed Onset Muscle Soreness (DOMS)

Exercise-induced muscle soreness, clinically known as DOMS. Delayed onset muscle soreness is characterized by increased blood CK, muscle soreness, swelling/edema, reduced range of motion (ROM), and prolonged loss of force that typically appear 12-48 hours following exercise.

Typically, the response is more pronounced in individuals previously engage in new exercise programs (Pyne, 1994). Eccentric resistance training protocols or
downhill running lead to mechanical damage as well (Clarkson, 1997; Pyne, 1994). Figure 2 shows a proposed timeline of the DOMS and ROS response. The timing of the responses is approximate as the literature is ambiguous and events do overlap.

Figure 2.2 Proposed Timeline of DOMS and Secondary Oxidative Bursts
(adopted from Clarkson, 1997; Pyne, 1994).

The initial mechanical damage (the autogenic phase) is characterized by loss of membrane integrity (Clarkson, 1997; Pyne, 1994). The autogenic phase begins with the mechanical damage and can last up to several hours post exercise (Ji, 1999). Membrane integrity is commonly measured by CK (Clarkson, 1997; Clarkson and Hubal, 2002; Pyne, 1994). The timing for peak CK response values differs based on the mode of exercise. High-force eccentrically biased resistance
training does not yield increased CK values until approximately 48 hours post exercise. In contrast, downhill running results in increased CK values after exercise and peaking at 12 to 24 hours (Clarkson and Hubal, 2002).

In response to this efflux of enzymes, polymorphonucleotrophils (PMNS) migrate to the damaged site via chemotaxis (Ji, 1999). Polymorphonucleotrophils are a type of white the superoxide radical may be converted into hydrogen peroxide by SOD, then into the hydroxyl radical, or hypochlorous acid (HOCL) (Ji, 1999; Pyne, 1994).

The ‘burst’ of ROS generated by the PMNS and macrophages is uncontrolled, as they cannot distinguish between healthy cells and debris from damaged cells (Close, Ashton, McArdle, and MacLaren, 2005; Kendall and Eston, 2002).

Inflammation/edema is associated with fluid and plasma protein migration to the damaged tissue (Cheung, Hume, and Maxwell, 2003; Clarkson and Hubal, 2002). Fluid accumulates at the site of injury resulting in increased pressure (Cheung, Hume, and Maxwell, 2003; Clarkson, 1997; Clarkson and Hubal, 2002). Typically, decreased ROM is displayed along with muscle soreness (Clarkson, 1997). The decreased ROM may be a result of the soreness, sensation of the stiffness/pain, or caused by the edema. Additionally, the decreased ROM may be necessary for optimal healing from the muscle damage (Smith, 1991).

2.4. Mechanisms of DOMS

Warren et al (1993) showed that eccentric exercise results in damage to the cell membrane of the active muscle fibers, with the injury itself a mechanical disruption to sarcomeres, according to This is further supported in (McHugh et al. 1999) study, which identifies three prominent theories surrounding exercise-induced muscle damage; The ‘neural theory’, the ‘connective tissue theory’, and the ‘cellular theory’. Each theory explored is grounded on the basis of “sarcomere strain as the initial stage of damage”, which can be related to the model devised by (Connolly et al. 2003) in figure3. Both studies indentify the first stage in injury to the muscle membrane which then subsequently stimulates a secondary response, or stage two.
Kuipers (1994) mentioned that structural damage of the contractile elements of the muscle are reflected in delayed onset muscle soreness (DOMS), and is considered as the major contributing factor for inducing muscle damage. However, Edwards et al (1996) distinguish between the injury patterns associated with DOMS compared to muscle strain. (DOMS) presents injury pattern throughout the muscle, and with strong supporting evidence from (Libber et al. 1991) in conjunction, sarcoma disruption is adjudged not to extend the length of a myofibril and usually also not extending across a whole muscle fiber. In contrast, muscle strains usually present an injury pattern that is isolated to the muscle-tendon junction, and extended across the fibres. Stage two is evoked from these cellular and subcellular disturbances seen in stage one, according to (Clarkson & Hubal 2002), invoking an inflammatory response. This response leads to prostaglandin (PGE2) and leukotriene synthesis that stimulates the authority for the second stage of DOMS to progress further, consistent with the findings of Connolly et al (2003), In line with pain generation, swelling also contributes here with Warren et al (1993) finding a movement of cells and fluid from the bloodstream into the interstitial spaces and concluding therefore, a contribution towards the sensation of pain accompanying with inflammation. The process is initiated by the blood vessels local to the injured tissue, which alter to allow the exudation of plasma proteins and leukocytes into the surrounding tissue, in accordance with Serhan & Savill (2005). The increased flow of fluid into the tissue causes the characteristic swelling associated with inflammation since the lymphatic system doesn't have the capacity to compensate
for it. Leukotriene presence however, initiates another response further increasing vascular permeability and attracting neutrophils to the site of damage, which follows the model in figure 3.

The oxidation process during the generation of reactive oxygen species (ROS) has been termed the respiratory, or oxidative, burst. ROS are molecules associated to free radicals, oxygen ions and peroxides, with Connolly et al (2003) concluding that the respiratory burst of the neutrophils generates free radicals, which can exacerbate damage to the cell membrane; moving into the third stage of the DOMS model. Free redical proliferation is not only a consequence of the initial muscle damage, but a key factor in further damage to the muscle membrane.

Harmful effects of ROS mentioned below:
1. Damage of DNA
2. Oxidations of polydesaturated fatty acids in lipids
3. Oxidations of amino acids in proteins
4. Oxidative inactivate specific enzymes by oxidation of co-factors.

Pyne (1994) strongly suggests that free radical proliferation, as a product of ROS production, is a definitive mechanism in the damage response to exercise occurring mainly by way of phagocytosis and activation of the respiratory burst by neutrophils generated during the inflammatory response. Other chemical factors have also been considered within the muscle which could add to the damaging process. Hellsten et al (1997) found elevated levels of XO after an eccentric exercise programme. XO is believed to be capable of generating the superoxide radical, which leads to potential for proliferation of more dangerous free radicals, and hence, increased muscle damage. In healthy tissue XO mainly exists in a dehydrogenase form not capable of producing superoxide radicals, but the enzyme may be modulated to its superoxide form via oxidation. In further support, Wakabayashi et al (1995) demonstrated that activated neutrophils, induce an enhanced expression of XO, suggesting a two-directional interaction between XO and neutrophils.

2.5. DOMS and ROS

Several studies have assessed exercise-induced generation of ROS; likewise, there are several studies that have examined the occurrence of DOMS. However,
few studies have looked at the interaction of ROS and delayed onset muscle soreness (DOMS) concurrently. Unfortunately, each study uses different subject populations, exercise protocols, ROS assays, and DOMS measurements. Exercise protocols vary from bicep curls, knee extensions, and downhill runs. Most of these protocols involve eccentric contractions to induce muscle damage.

Lee, et al. (2002) examined the effects of markers of Oxidative Stress (OS) on delayed onset muscle soreness (DOMS). In this study eight men performed eccentric arm curls for 10 min. The load was approximately 150% of their maximal isometric force and they were encouraged to perform 60 repetitions in a 10 min span. Range of motion (ROM) was determined about the elbow. A likert scale was used to determine muscle soreness. Blood samples were analyzed spectrophotometrically for plasma CK, PC, and total glutathione. Samples were taken pre-exercise, 10 min post-exercise, and then at 24, 48, 72, and 96 hours post exercise. Results showed no change in total glutathione. Plasma CK was significantly elevated at 48 hours and peaked at 72 hours. Plasma PC was significantly elevated at 24 and 48 hours with the peak at 24 hours. Maximal isometric force and ROM was decreased in the exercised arm only, suggesting a localized effect of force loss and edema.

Lee et al. (2002) suggested that PC is a better marker of OS than glutathione. The authors showed that the glutathione status was unaffected due to the rapid conversion of GSSG back to GSH. The most significant finding of this study was the correlation of the timing of the PC response to DOMS at 24 and 48 hours post-exercise. A study demonstrated increased ROS production with downhill treadmill running for 30 minutes at 65% of VO$_2$ max on a -15% grade (Close, et al., 2004). This study also resulted in increased DOMS symptoms such as decreased strength, increased muscle soreness, and increased plasma CK levels. The results of this study indicate a time discrepancy between ROS production and symptoms of DOMS with CK levels peaking at 24 hrs and MDA becoming significant at 48 hrs. However, in a later review article, the authors stated that the results of the 2004 study did not involve antioxidant supplementation and therefore implied lack of relationship between DOMS and ROS production (Close, et al., 2005).
However, several studies have shown increased level of OS within 24-48 hours of exercise (Alessio, et al., 2002; Goldfarb, et al., 2005; Lee et. al., 2002; Nagasawa, et al., 2000), which correlates well with the time course for peak blood CK and DOMS (Clarkson, 1997).

2.6. Symptoms of DOMS

McHugh et al (1999) underlines five key symptoms associated with DOMS; strength loss, pain, muscle tenderness stiffness, and swelling. Strength loss usually peaks immediately after exercise within 2 to 48 hours, with full recovery taking up to five days. Pain and tenderness peak 1-3 days after exercise, subsiding within 7 days, with swelling and stiffness usually peaking 3-4 days and typically resolve within 10 days (Sayers et al, 2000, 2001; McHugh et al, 1999a, 1999b, 2000; Abrams, 1997; Vane, 1987). Further associations have been made in DOMS research, including discomfort at the site of injury and tendon insertion points (MacIntyre et al. 1995), edema (Clarkson and Sayers 1999), compromised muscular function, reduction in maximal force generating capacity of the affected muscle (Clarkson and Sayers 1999; Clarkson et al. 1992; Macintyre et al. 1995), and loss of range of motion across an affected joint (Lee et al. 2002; Rawson et al. 2001).

Summary of the more common measures presented in Table 2.1.

<table>
<thead>
<tr>
<th>Table 2.1. Indices evaluating muscle damage. Adapted from Connolly, Sayers &amp; McHugh (2003).</th>
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<tbody>
<tr>
<td><strong>Index</strong></td>
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<tr>
<td>Biopsy</td>
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<tr>
<td>Strength</td>
</tr>
<tr>
<td>Pain</td>
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<tr>
<td>Tenderness</td>
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<tr>
<td>Stiffness</td>
</tr>
<tr>
<td>Swelling</td>
</tr>
<tr>
<td>Creatine kinase</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
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</tbody>
</table>
2.7. Biochemical indices associated with muscle damage

Biochemical changes associated with muscle damage include elevations in creatine kinase; collagen-degrading enzymes, leukocyte and neutrophil concentrations, and myoglobin have also shown marked alterations in subsequent days following an eccentric loading period (Gleeson et al. 1995; Koskinen et al. 2001; Macintyre et al. 1996, 2001; Rodenburg et al. 1993; Sorichter et al. 1995). As in McHugh et al (1999, 2000) the onset of symptoms resulting from muscle damage is widely variable; however, some clear comparisons can be drawn with muscle soreness found to develop within the first 24 hours following the exercise session and may persist for up to 10 days (Clarkson et al. 1992). Loss of force generating power is repeatedly reported to be affected within 2 hours of the eccentric bout of exercise (MacIntyre et al. 1996; Rodenburg et al. 1993; Sorichter et al. 1995).

Clarkson and Sayers (1999), along with Warren et al (2001), showed evaluated proposed causative mechanisms for muscle damage and notably Harrison et al. (2001) has examined the efficacy of intervention strategies and analgesic treatments for helping to minimize the magnitude of such symptoms, along with some other key texts (Johansson et al. 1999; Lambert et al. 2002; Lecomte et al. 1998).
DOMS assessment tests

Table 2.2, shows summary of DOMS assessment test.

<table>
<thead>
<tr>
<th>Test</th>
<th>Author(s) and Text</th>
</tr>
</thead>
</table>
| Visual Analogue Scale     | Carlsson (1983) *Aspects of the reliability and validity of the visual analogue scale.*  
                            | Gallagher (2001) *Prospective validation of clinically important changes in pain severity measured on a visual analogue scale.* |
| Algometer (Pain)          | Reeves et al. (1986) *Reliability of the pressure algometer as a measure of myofascial trigger point sensitivity.*  
                            | Antonaci et al. (1992) *Pain threshold in humans. A study with the pressure algometer.*  
                            | Gallagher et al. (1989) *Design and construction of a pressure algometer.* |
                            | Schwane et al. (1987) *Effects of training on delayed muscle soreness and serum creatine kinase activity after running.*  
                            | Florence et al. (1992) *Intrarater reliability of manual muscle test (Medical Research Council scale) grades in Duchenne's muscular dystrophy.*  
| Range of Motion           | Bohanon & Gajdosik (1987) *Clinical measurement of range of motion. Review of goniometry emphasizing reliability and validity.*  
                            | Wiktorsson-Moller et al. (1983) *Effects of warming up, massage, and stretching on range of motion and muscle strength in the lower extremity.*  
2.8. Antioxidants

Cells continuously produce reactive oxygen species (ROS) as part of the metabolic processes. Among the earliest biochemical reactions found were hydrolysis of fatty acid from membrane phospholipids, production of biologically active eicosanoids, and peroxidation of lipids with formation of reactive oxygen species (ROS). The latter reaction will produce agents which are responsible for cellular damage. Superoxide ($O_2^-$), and hydroxyl anions are common reactive compounds that cause lipid peroxidation.

Evidence from both animal and human studies indicates that many cell types adapt to increased exposure to oxidants to reduce the risk of damage to the tissue (Niwa et al. 1993; Marini et al. 1996; Jones et al. 1999; McArdle et al. 2001). Lymphocytes increase their activity of SOD, catalase (CAT) and glutathione peroxidase in response to endogenous oxidants (Barnett et al. 1995) and an acute bout of exercise increases the activities of SOD, glutathione peroxidase, glutathione reductase and catalase in skeletal muscle of rats (Ji, 1993). Longer-term exercise training also appears to increase the activity of several antioxidant enzymes, such as SOD and CAT (Higuchi et al. 1985) or glutathione peroxidase (Ji, 1993) in muscle, although these are not consistent findings (Alessio & Goldfarb, 1988). In humans, exercise training has been reported to increase skeletal muscle SOD activities (Jenkins et al. 1984) and the activities of various protective enzymes in blood (Robertson et al. 1991).

Oxidative stress occurs due to an imbalance between oxidant production and the antioxidant capacity of the cell (Fig. 1). Cells are protected against oxidant injury by a complex network of antioxidants. Specifically, enzymatic and non-enzymatic antioxidants exist in both the intracellular and extracellular environments and work as complex units to remove different reactive oxygen species (ROS). To provide maximum intracellular protection, these scavengers are strategically compartmentalized throughout the cell. Illustrates the cellular locations of important antioxidants and Tables provide a brief overview of the antioxidant function of these molecules.
Table 2.3. Important enzymatic antioxidants

<table>
<thead>
<tr>
<th>Enzymatic antioxidants</th>
<th>Properties</th>
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<tbody>
<tr>
<td>Superoxide dismutase</td>
<td>Located in both mitochondria and cytosol; dismutates superoxide radicals</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>Located in both mitochondria and cytosol; removes hydrogen peroxide and organic hydroperoxides</td>
</tr>
<tr>
<td>Catalase</td>
<td>Located in cytosol (and in mitochondria of heart); removes hydrogen peroxide</td>
</tr>
</tbody>
</table>

2.9. Training and Antioxidant Status

Generally, endurance training studies demonstrate an enhanced antioxidant system with training (Laughlin, et al., 1990; Sen, 1995). However, there is a paucity of controlled studies involving exercise, reactive oxygen species (ROS) production, and antioxidants with recreational athletes.

Watson, et al. (2005) evaluated endurance-trained male athletes who followed either a restricted antioxidant diet (R-AO) or a high antioxidant diet (H-AO) for two weeks. Subjects were asked to refrain from consuming vitamins for three months prior to the study. A VO\textsubscript{2} test was performed on a treadmill in order to establish intensity for future testing. A subsequent test involved running at 60% of VO\textsubscript{2}max for 30 minutes followed by an incremental treadmill run to exhaustion.

They returned to baseline during the recovery period. Levels of GSSG rose significantly during the submaximal run. The ratio of GSH: GSSG was statistically significantly reduced due to the rise in GSSG compared to pre exercise values. The authors concluded that the H-AO group was able to defend the body against exercise generated ROS, while the R-AO group was not able to do so (Watson, et al., 2005).

Mena, et al. (1991) compared basal antioxidant status of healthy sedentary subjects to amateur and professional endurance cyclists. At rest, all levels of cyclists had higher levels of SOD and GPX. The professional cyclists had higher levels of
CAT. They also compared pre and post-race data for the amateur and professional cyclists. The cyclists performed either a 700 km race (6 days) or a 2800 km race (20 days). Blood samples were taken pre-race and within 30 minutes of finishing the race. An additional blood sample was taken after the first stage (approximately after 5 hours or 229 km of cycling) from the cyclists participating in the 2800 km race. All samples were analyzed for MDA-TBARS, CAT, SOD, and GPX.

It is concluded that endurance trained professional cyclists have increased basal levels of SOD, CAT, and GPX compared to sedentary subjects, indicating enhanced activity of antioxidant enzymes. Levels of MDA increased in endurance-trained subjects after an acute bout of exercise (i.e. after stage 1) and remained elevated after a multiple-stage endurance race. The authors acknowledged the lack of dietary control in their study as the cycling team would not divulge what supplements they might have been taking (Mena, et al., 1991).

In a study involving trained runners participating in a half marathon, Duthie, et al. (1990) examined the effect of endurance running on oxidative damage and antioxidant status. The subjects were seven male endurance-trained athletes with an average VO$_2$ max of 66 ml/kg/min and mean race completion time of 81 minutes. Blood samples were taken at 48 hours and one hour pre-race. Post-race samples were taken at 5 minutes, 24, 48, 72, and 120 hours.

Antioxidant enzymes CAT, SOD, and GSH were also measured. CK was elevated at 24 and 48 hours post-race and peaked at 24 hours post race. Levels returned to baseline by 120 hours. Vitamins A and C were significantly elevated immediately post-race and returned to baseline by 24 hours. Conversely, vitamin E was unchanged immediately post-race, but was significantly elevated at 24 hours and continued to be elevated up to 120 hours. The timing differences of vitamins C and E may be due to vitamin C reducing the vitamin E radical as discussed in the next section. Results revealed no differences between pre and post-race values for TBARS-MDA, DC, CAT, SOD, or total GSH. The authors offered two explanations. This run was not enough for the lipid peroxidation to overwhelm the antioxidant defense system of the trained runners or the assays were not specific enough. The assay for DC cannot discriminate between conjugated dienes formed.
from lipid peroxidation or dietary sources. This study did not include a control group for comparison and did not control for diet or supplementation (Duthie, et al., 1990).

A similar study using trained male runners reported no change in MDA after a half marathon run (Kelle, Diken, Sermet, Atmaca, and Kocyiğit, 1998). Ten endurance-trained males completed a half marathon run in an average time of 74 minutes. Blood samples were taken 5 minutes before and after the run. Previously documented methods were used to determine SOD, CAT, MDA-TBARS, GSH, and GSSG. Plasma CK was measured spectrophotometrically and vitamin E was measured by HPLC. SOD and CAT also remained unchanged. Total GSH was significantly reduced compared to pre-race values. While total glutathione and GSH were lower, GSSG was unchanged. Values for MDA-TBARS were not statistically different as was vitamin E. Plasma CK levels were significantly elevated. The change in glutathione status may reflect the body’s reliance on the natural antioxidant defense system in response to endurance exercise (Kelle, et al., 1998).

Hellsten, et al. (1996) examined the effects of sprint cycle training on antioxidant enzymes. Biopsied tissue was analyzed for GPX, GR, SOD, and CK. Results from six weeks of sprint training revealed no changes in GPX, GR, or SOD. Muscle CK levels were significantly increased after the first 6 weeks. Data from the additional week of intense sprint training yielded a significantly higher level of GPX and GR for the 24-hour sample only. No statistically significant relationship was found between SOD, GPX, and GR. The authors concluded that there was no apparent relationship between anaerobic training and ROS scavenging enzymes in the muscle.

2.10. Non-enzymatic and dietary antioxidants

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

2.10.1. Glutathione

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

Glutathione concentration in the cell is typically in the mill molar range, but there is wide variability in glutathione content across organs depending on their basal levels of radical production. For example, the two highest concentrations of glutathione in the body are found in the lens of the eye (10 mmol l⁻¹) and the liver (5–7 mmol l⁻¹) (Halliwell and Gutteridge, 1999). Other key organs such as the lung, kidney and heart contain about 2–3 mmol l⁻¹ of glutathione (Ji, 1995a). Glutathione serves several roles in the cellular antioxidant defense system. First, glutathione directly scavenges a variety of radicals, including hydroxyl and carbon centered radicals, by donating a hydrogen atom (Yu, 1994). A second key antioxidant function of glutathione is to remove both hydrogen and organic peroxides (e.g. lipid peroxide) during a reaction catalysed by the enzyme glutathione peroxidase. During this reaction, glutathione donates a pair of hydrogen atoms and two glutathione are oxidized to form glutathione disulphide. Glutathione has also been shown to be involved in reducing or ‘recycling’ a variety of antioxidants in the cell. For example, glutathione has been postulated to reduce vitamin E radicals that are formed in the chain-breaking reactions with alkoxy or lipid proxy radicals (Packer, 1991). Furthermore, glutathione can also reduce the semi-dehydroascorbate radical (vitamin C radical) derived in the recycling of vitamin E. This reaction has recently been hypothesized to play an important role in the recycling of ascorbic acid (Packer, 1991).

2.10.2. Vitamin E

The generic term vitamin E refers to at least eight structural isomers of tocopherols or tocotrienols. Among these, a-tocopherol potent antioxidant activity (Burton and Ingold, 1989; Janero, 1991). From an antioxidant perspective, vitamin E is the primary chain-breaking antioxidant in cell membranes (Burton and Ingold, 1989; Janero, 1991). Because of its high lipid solubility; vitamin E is associated with lipid-rich structures such as mitochondria, sarcoplasmic reticulum and the plasma membrane. Under most dietary conditions, the concentration of vitamin E in tissues is relatively low. For example, the ratio of vitamin E to lipids in the membrane may
range from 1:1000 in red blood cells to 1:3000 in other tissues and organelles (Janero, 1991; Packer, 1991). Note, however, that vitamin E concentrations in tissues and organelles can be elevated with dietary supplementation (Janero, 1991). As an antioxidant, vitamin E is particularly important because of its ability to convert superoxide, hydroxyl and lipid proxy radicals to less reactive forms. Vitamin E can also break lipid peroxidation chain reactions that occur during free radical reactions in biological membranes (Burton and Traber, 1990).

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

**Table 2.4 A list of selected dietary antioxidants**

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

a. Dietary reference intakes (DRI) are the most recent set of dietary recommendations established for Canadians and Americans by the Food and Nutrition Board of the Institute of Medicine, 1997–2001. The values shown are the highest DRI for each nutrient.

b. The tolerable upper intake level (UL) is the upper level of intake considered to be safe for adults in Canada and the USA. In some cases, lower ULs have been established for children. NE=DRI or UL not established.

Source: Council for Responsible Nutrition, 1828 L. Street NW, Suite 900, Washington, DC 20036-5114, USA.
2.10.3. Vitamin C

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

The role of vitamin C as an antioxidant is two-fold. Vitamin C can directly scavenge superoxide, hydroxyl and lipid hydro peroxide radicals. Additionally, vitamin C plays an important role in recycling the vitamin E radical back to its reduced state (Packer et al., 1979). In the process of recycling vitamin E, reduced vitamin C is converted to a vitamin C (semiascorbyl) radical (Packer et al., 1979). Recycling of the vitamin C radical can be achieved by NADH semiascorbyl reductase, or cellular thiols such as glutathione and dihydrolipoic acid (Sevanian et al., 1985).

Vitamin C can exert pro-oxidant effects in the presence of transition metals such as Fe3+ or Cu2+. The pro-oxidant action of vitamin C stems from its ability to reduce ferric iron (Fe3+) to the ferrous (Fe2+) state.

2.11. Vitamins C and E as Antioxidants

Vitamins C and E are the vitamin antioxidants that have been studied the most in relation to exercise and ROS. Vitamin E reduces ROS and, in turn, creates the vitamin E radical. Vitamin C reduces the vitamin E radical (and other radicals) back to vitamin E (Powers, et al., 2004). This reaction creates a vitamin C radical that is reduced by thiols and other antioxidants. After supplementation of 330 mg of α-tocopherol acetate, resting levels of MDA and CK were lower in trained cyclists than the placebo group of trained cyclists (5 authors) Rokitzki, Logemann, Huber, Keck, and Akeul, 1994). This implicates that supplementation with vitamin E can enhance the antioxidant defense system above the level that training alone can provide. Supplementing with high levels of vitamin C in order to reduce vitamin E may not be beneficial. High concentrations of vitamin C can create a pro-oxidant environment with transition metals such as Fe^{3+} and Cu^{2+} (Powers, et al., 2004).
2.12. Flavonoids

Flavonoids are a large family of diphenylpropanes (over 4000 members have been identified) that are commonly found in plants consumed by humans. Family members include, but are not limited to, flavones, are flavones, flavanones, anthocyanins and catechins (Das, 1994). Flavonoids have been reported to possess a wide variety of biological activities ranging from inhibition of inflammatory enzymes (e.g. lipoxygenase, cyclooxygenase, xanthine oxidase, NADH-oxidase, phospholipase A2) to anti-tumoral, anti-viral, anti-mutagen, anti-inflammatory, anti-ischaemic and anti-allergic activities (Cao et al., 1997). Many of these biological effects are thought to be a result of the antioxidant capacity of flavonoids (Bors et al., 1994; Saija et al., 1995; Scalbert et al., 2002). Radical scavenging activities of flavonoids appear to vary greatly among family members, but include quenching of proxy, hydroxyl and superoxide radicals, as well as hydrogen peroxide and a variety of chemically generated radicals not naturally found in the body (Cao et al., 1997).

Among the many flavonoids under investigation for their potential role in protecting against radical mediated disease processes is the poly phenol flavonoid family, the catechins, which are found in significant concentrations in green and black tea and red wine. Catechins are amphipathic and thus exert their antioxidant activities in both lipid and aqueous environments. They have been shown to be efficient scavengers of superoxide, hydroxyl and peroxyl radicals, to inhibit metal ion-mediated radical formation and to inhibit formation of lipid peroxyl radical species (Sichel et al., 1991; Salah et al., 1995; Lotito et al., 2002). In addition to inhibiting lipid peroxidation, catechins were reported to prevent the radical-mediated depletion of vitamin E and b-carotene in human plasma in a dose-dependent manner (Lotito and Fraga, 1999).

ANTIOXIDANTS AND EXERCISE PERFORMANCE

2.13. Antioxidant deficiencies and exercise performance

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

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

2.14. Antioxidant effects on muscle contraction and exercise performance

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

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

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

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

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

2.15. Exercise and antioxidant requirements

As discussed earlier, current opinion holds that exercise-induced oxidative stress may be deleterious to exercise performance. This notion is based on cellular (Alessio, 1993; Lawler et al., 1993) and extracellular (Mastaloudis et al., 2001) indices of oxidant damage to lipids and proteins after exercise. Empirical data usually demonstrate that dietary antioxidant supplementation diminishes blood (Sumida et al., 1989; Ashton et al., 1999) and cellular markers (Goldfarb et al., 1994) of radical-mediated damage during exercise. Excessive exposure to
environmental pollutants during training may further support the need for antioxidant supplementation (Papas, 1996). However, the current consensus on antioxidant supplementation for athletes remains equivocal because of a paucity of well-designed studies that clearly outline the need for dietary antioxidants in highly trained populations. The remainder of this review will address athletes’ risk for exercise-induced oxidative stress, existing evidence for supplementation efficacy and perspectives for future directions in antioxidant supplement research and athletics.

2.16. Efficacy of antioxidant supplementation to reduce oxidative stress.

Evidence addressing the efficacy of supplementation in athletic populations remains ambiguous. Existing studies generally report decreased oxidative damage after antioxidant supplementation. However, only limited data demonstrate the ability of antioxidant supplementation to prevent the exercise-associated rise in markers of oxidative stress. For example, Sumida et al. (1989) demonstrated that 4 weeks of vitamin E supplementation prevented the rise in plasma MDA and markers of muscle damage observed in non-supplemented controls after maximal-intensity cycle exercise.

In contrast to these findings, daily supplementation with an antioxidant mixture (30 mg b-carotene, 592 mg α-tocopherol and 1000 mg ascorbate) did not prevent the exercise-induced rise in plasma malondialdehyde after moderate- to high-intensity treadmill running (Kanter et al., 1993). Additionally, Helgheim et al. (1979) reported that vitamin E supplementation was ineffective in preventing a rise in blood markers of muscle damage. Collectively, these studies illustrate the mixed results that have been reported about the effectiveness of antioxidant supplementation in decreasing exercise-induced Oxidative Stress (OS).

It is understood that exercise appears to raise reactive oxygen species level, which can result in damage to cells. Clarkson & Thompson (2000) speculated as to whether our natural antioxidant defense system is adequate to counteract this rise with exercise, or if additional exogenous supplements are needed. A key conclusion made was that trained athletes who received antioxidant supplements did show evidence of reduced Oxidative Stress (OS).
Packer (1997) found that Vitamin E, an antioxidant in cell membranes, protects against lipid peroxidation by acting with oxygen radicals such as, lipid peroxides and the superoxide radical, to transform to a relatively harmless radical (tocopherol radical). Bieri (1990) was the foundation for the later study, which concluded that a nullifying affect of free radicals was evident within the cell through Vitamin E and other antioxidant presence. Coinciding with this, Sauberlich (1990) added that Vitamin C can interact directly with the produced tocopherol radical and its water soluble properties allows it to also react with superoxide and hydroxyl radicals. Glutathione peroxidase is an enzyme that utilises the removal hydrogen peroxide, and glutathione is acted on by this enzyme to produce its oxidized form, GSSG. The mineral selenium is an essential component of glutathione peroxidase, and along with other antioxidant enzymes Maxwell (1997) strongly states the importance to reduce lipid peroxidation and free radical damage, satisfied in the role and actions of antioxidants and other related factors.

Mastaloudis, et al. (2006) supplemented trained endurance runners with 300 mg of RRR-α-tocopherol acetate and 1000 mg of ascorbic acid or a placebo twice daily for 6 weeks. After the 6 weeks, the runners participated in an ultramarathon run. Subjects performed an MVC prior to supplementation, 1 day pre-race, 2 hours post-race, and 6 days post-race. Blood samples were also taken at 12 different times (prior to supplementation, 1 day pre-race, 1 hour pre-race, mid-race, immediately post-race, 2 hours post-race, and 6 days post-race). Plasma α-tocopherol was increased in the supplemented group and remained unchanged in the control group. Ascorbic acid levels followed a similar trend. Increases in CK were significant from baseline, but not between the treatments. Post-race MVC results were lower compared to baseline and no differences among treatments were found. In this case, supplementation with vitamins C and E did not attenuate the loss of muscle force or the increase in CK associated with ultramarathon running. The authors suggested that a larger dosage of vitamin E is needed to prevent the muscle damage found in this protocol.

Nieman, et al.(2001) supplemented experienced ultramarathon runners with vitamin C for 7 days prior to a 80 km run. Runners ingested either a placebo or 1500 mg of vitamin C daily for 7 days prior to the race. During the race, runners were
given coded bottles of carbohydrate beverages (150 mg/l) with or without vitamin C. Saliva and blood samples were taken pre race, mid-race (32 km), and 5 minutes post-race. Both measurements of oxidative stress were elevated, but failed to reach significance in either group. They were also positively correlated to each other (r=0.44). No significant group or interaction effects were found for neutrophil or monocyte counts. They rose in both groups, but failed to reach significance. The level of serum vitamin C was significantly higher in the supplemented group. These data indicate that 1500 mg supplementation of vitamin C over 7 days will not protect endurance athletes from Oxidative Stress (OS). This lack of statistical significance could also be due to the timing of post-race sampling or perhaps vitamins C and E working synergistically. The study only asked participants to avoid foods with large amounts of vitamin C and to follow a high carbohydrate diet. Participants were also allowed to continue vitamin supplements as long as they did not provide more than 100% of the recommended daily values. Plasma vitamin E levels were not measured (Nieman, et al., 2001).

In contrast to these studies, Shafat, et al. (2004) reported a reduction in the loss of isometric force following 37 days of supplementation of vitamins C and E following an acute bout of 300 maximal eccentric knee extensions. The supplemented group took 500 mg of vitamin C and 1200 IU of d-α-tocopherol daily for 30 days prior to the exercise bout and 7 days post-exercise. Isometric MVC knee extensions were performed prior to and after a bout of eccentric exercise. The eccentric exercise consisted of 30 sets of 10 maximal eccentric knee extensions. MVCs were also taken on days 1-7 post exercise. The extent of DOMS (soreness) was evaluated by a Likert scale. The vitamin-supplemented group exhibited a reduced loss of force during the last five eccentric contractions. Post-exercise decrease in MVC force was attenuated in the vitamin group (Shafat, et al., 2004). Results from Rokitzki, et al. (1994) showed decreased CK and MDA after 5 months of vitamin E supplementation and endurance training of elite cyclists. Thirty-six members of the men’s German national cycling team participated in this study. Vitamin E was administered as α-tocopherol-acetate with a dosage of 330 mg/day. Other antioxidant supplementation was forbidden during the study. The placebo group took a capsule of soybean oil. Subjects performed two incremental cycling tests to exhaustion: one after 1 week of supplementation and one at the end of 5
months of supplementation. Each test began at 100 W and was increased 50 W every five minutes until exhaustion. Pre and post-test blood samples were collected each time. Following 5 months of supplementation, pre- test and post-test values for CK and MDA-TBARS were both lower in the treatment group vs. the control group. Performance data from this study indicated no performance enhancement even though the measure of oxidative stress was lower in the supplemented group. The authors conclude that vitamin E supplementation was responsible for the reduction of CK, but the relationship between vitamin E, lipid peroxidation, and muscle damage warrants further investigation.

2.17. Supplementation for DOMS

Supplementation, via potent antioxidant method, targets the third stage of DOMS, as according to Connolly et al (2003). Stage three, Free Radical Proliferation, can rationally be supported through antioxidant supplementation according to several key texts (Connolly et al, 2003; Hellsten et al, 1997; Koskinen et al, 2001; MacIntyre et al, 1996, 2001; Clarkson & Sayers, 1999). Changes in blood amounts of vitamins C and E, as well as changes in glutathione in the blood, amongst others, have been used to indicate increased oxidative reaction across other studies and assessments and it is the understanding of Clarkson & Thompson (2000) that these antioxidants may be mobilized from tissue stores to combat oxidative stress in another parts of the body.

The presence of ascorbate (vitamin C) is essential for a range of metabolic reactions in all animals. It is made internally by almost all organisms, however with the exception of humans who must obtain it through their diet, utilised as an antioxidant, “protecting the body against OS”, as described by Padayattay et al. (2003). Recommendations for vitamin C intake have been set by various national agencies, with the United Kingdom's Food Standards Agency suggesting 40 milligrams per day and World Health Organization a slightly higher 45 milligrams per day. However, the recommended daily allowance in the USA ranges upto 90 milligrams per day, typifying the differing standard dietary requirements across the world. A tolerable upper intake level of 2000 milligrams per day is generally accepted according to the World Health organization (2007).
Other interventions for treatment and prevention of DOMS

2.18. Pharmacological Treatment of DOMS Using NSAIDs

One of the many treatment modalities advocated to facilitate recovery of muscle function and alleviate the symptoms of delayed onset muscle soreness (DOMS) is Non steroidal anti-inflammatory drugs (NSAIDs). The value of NSAIDs therapy in the treatment of DOMS is equivocal, with the majority of studies showing no effect despite a strong theoretical basis for efficacy. The following sections will address the inflammatory response of the tissue to mechanical damage, the effects of NSAIDs on this response, and the potential mechanism for alleviating DOMS. NSAIDs work after strenuous exercise by inhibiting the COX enzyme and thus PGE2 synthesis. Non steroidal anti-inflammatory drugs can be classified as single- or dual-action drugs. No steroidal anti-inflammatory drugs such as aspirin, naproxen, flurbiprofen, and ibuprofen are COX inhibitors only and are single-action drugs. Other NSAIDs such as diclofenac and ketoprofen are dual-action NSAIDs blocking both the COX and LIPOX pathways of arachidonic acid metabolism. The latter NSAIDs may therefore have a more powerful anti-inflammatory effect than the single-action NSAIDs. Dual-action NSAIDs may be more similar to steroid hormones in their effects on inflammation. Corticosteroids, such as glucocorticoids, inhibit phospholipase A2 and thus block the initial cleavage of arachidonic acid from the cell membranes, resulting in a more complete anti-inflammatory effect than most commonly used over-the-counter single-action NSAIDs. However, glucocorticoids have numerous side effects including facilitation of bone and muscle loss, edema,
and hypertension (Marieb, e 1992); thus, their role as an anti-inflammatory agent should be limited. The NSAIDs also have negative side effects such as gastrointestinal distress and renal and hypertensive effects (Brooks, P.M., and R.O. Day.1991); however, these side effects are less frequent and of less severity than those of the glucocorticoids. At lower doses NSAIDs tend to exert an analgesic effect, whereas at higher doses an anti-inflammatory effect is achieved (Hertel, J.1997). One possible explanation for this is that NSAIDs at higher doses disrupt the activity of certain white blood cells such as neutrophils and macrophages (Abramson, S.B., and G. Weissmann.1982). Thus, NSAIDs may have a direct inhibitory effect on inflammation, independent of the pain-reducing effects of PGE2 inhibition.

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>NSAID</th>
<th>Dose (mg)</th>
<th>Treatment period</th>
<th>DOMS (±/-)‡</th>
<th>Muscle function (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuipers et al. (42)</td>
<td>6</td>
<td>Flurbiprofen</td>
<td>150</td>
<td>-24 to 72 h Post</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Donnelly et al. (16)</td>
<td>16</td>
<td>Ibuprofen</td>
<td>2,400</td>
<td>-25 to 72 h Post</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Grossman et al. (29)</td>
<td>10</td>
<td>Ibuprofen</td>
<td>2,400</td>
<td>-24 to 86 h Post</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pizza et al. (67)</td>
<td>10</td>
<td>Ibuprofen</td>
<td>2,400</td>
<td>-5 to 10 d Post</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Howell et al. (33)</td>
<td>15</td>
<td>Flurbiprofen</td>
<td>300</td>
<td>-24 h to 14 d Post</td>
<td>-</td>
<td>-‡</td>
</tr>
<tr>
<td>Howell et al. (34)</td>
<td>16</td>
<td>Ibuprofen</td>
<td>1,600 or 3,200</td>
<td>-24 h to 6 d Post</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Semark et al. (75)</td>
<td>13</td>
<td>Flurbiprofen</td>
<td>40</td>
<td>-12 h to 72 h Post</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bourgeois et al. (5)</td>
<td>8</td>
<td>Naproxen</td>
<td>500</td>
<td>-4 h to 48 h Post</td>
<td>-</td>
<td>+/-§</td>
</tr>
<tr>
<td>Barlas et al. (4)</td>
<td>12</td>
<td>Aspirin</td>
<td>900</td>
<td>0 to 11 d Post</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* - indicates treatment began prior to the damage session.† DOMS = delayed onset muscle soreness.
‡ Impaired force recovery at 14 days.§ Study used both eccentric and concentric contractions.

2.19. The Efficacy of NSAIDs on DOMS

All studies used some form of eccentric exercise that standardized the injury protocol. However, the modes of eccentric exercise differed between studies and included isokinetic dynamometry (lecomte, j.m., v.j. lacroix, and d.l.
montgomery.1998), cycle ergometry, drop jumping (Semark, A., T.D. Noakes, A. ST.1999), high-force eccentric exercise (Howell, J.N., R.R. Conatser, G.S. Chleboun, d.l.1998), box stepping (Organdy, M., A.C. Hackney, k. 2000), and downhill running (Donnelly, A.E., R.J. Maughan, and P.H. Whiting. 1990). The NSAIDs therapy in these studies was either prophylactic, beginning before the eccentric exercise (as a prevention to exercise damage), or therapeutic, beginning after eccentric exercise (as a treatment to exercise damage). A summary of the results of studies demonstrating either no efficacy or some efficacy of NSAIDs on DOMS is shown in Tables 4 and 5, respectively.

2.20. Studies demonstrating no efficacy of NSAIDs on DOMS

Most of the NSAIDs studies show no effect on DOMS involved the use of ibuprofen or flurbiprofen. Both medications are from the same carboxylic acid classification and the same prop ionic acid sub classification (Barlas, P., J.A. Craig, J. Robinson, D.M. Walsh, 2000). The reader should note that direct comparison among study findings is not always straightforward because of variations in study design, most noticeably medication dosage and damage protocol.

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>NSAID</th>
<th>Dose (mg)</th>
<th>Treatment period*</th>
<th>DOMS (+/-)?</th>
<th>Muscle function (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Francis and Hoobler (24)</td>
<td>10</td>
<td>Aspirin</td>
<td>2,600</td>
<td>-4 to 48 h Post</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Donnelly et al. (17)</td>
<td>20</td>
<td>Diclofenac</td>
<td>150</td>
<td>-1.5 to 72 h Post</td>
<td>+/-</td>
<td>N/A†</td>
</tr>
<tr>
<td>Hasson et al. (30)</td>
<td>5</td>
<td>Ibuprofen</td>
<td>1,200</td>
<td>-4 to 24 h or 24 h Post</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dudley et al. (18)</td>
<td>8</td>
<td>Naprofen</td>
<td>660</td>
<td>0 to 10 d Post</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lecomte et al. (44)</td>
<td>20</td>
<td>Naprofen</td>
<td>1,000</td>
<td>0 to 7 d Post</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>O’Grady et al. (66)</td>
<td>27</td>
<td>Diclofenac</td>
<td>150</td>
<td>-13 to 14 d Post</td>
<td>+</td>
<td>+§</td>
</tr>
<tr>
<td>Sayers et al. (74)</td>
<td>12</td>
<td>Ketoprofen</td>
<td>25 or 100</td>
<td>36 h Post</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* - indicates treatment began prior to the damage session.
† DOMS = delayed onset muscle soreness.‡ NA = not applicable.§ Histological assessment of muscle.
Kippers et al (1985) were the first to address the question of whether anti-inflammatory medications showed efficacy in the treatment of DOMS. This study used 6 subjects in a crossover design. Subjects were treated with either 150 mg of flurbiprofen or a placebo 24 hours before eccentric cycling and then for 72 hours after exercise. Although the researchers reported no effect of flurbiprofen on DOMS, creatine kinase (CK), or muscle histology, there was significantly lower muscle soreness after the second eccentric exercise bout, which was administered 3 weeks after the first bout. This phenomenon has been described widely as the repeated bout effect (RBE) and has been shown by multiple investigators (Mchugh, m.p., d.a.j. Connolly, r.g. Eston, 1998). In light of the RBE, the exact effect of the flurbiprofen remains unclear. A similar methodological concern was also observed in a crossover design study by Donnelly et al using ibuprofen. Sixteen subjects were treated prophylactically with 2,400 mg of ibuprofen or a placebo 24 hours before and for 72 hours after a 45-minute downhill running protocol. Repeated bouts of this exercise were only 10 weeks apart, and although they report no efficacy of ibuprofen on delayed onset muscle soreness (DOMS), muscle strength, or endurance time, CK activity was actually higher in the ibuprofen group compared with the placebo after both eccentric exercise protocols. These results suggest that ibuprofen could contribute to greater levels of damage in the treated muscle. However, a study by Pizza et al. showed that treatment with 2,400 mg of ibuprofen for 5 days before and 10 days after high-force eccentric exercise of the elbow flexors reduced CK activity in 10 men. It is unclear why inconsistencies were observed when using the same NSAID; however, differences in the mode and intensity of exercise or the length of the treatment period may have contributed to the observed differences. Two studies, however, have reported impaired recovery of muscle function with NSAID treatment after eccentric contractions (Mishra, d.k., j. Friden, m.c. Schmidt, and r.l. Lieber.1997). Howell et al treated 15 subjects prophylactically with either 300 mg of flurbiprofen or a placebo 24 hours before and for 14 days after eccentric exercise of the elbow flexors. They reported no efficacy of flurbiprofen on DOMS, swelling, or stiffness; however, maximal force was significantly lower 14 days later in the flurbiprofen group. Mishra et al similarly reported impaired recovery of muscle function 28 days after a 6-day flurbiprofen treatment in the animal model. Because most human studies have not evaluated recovery after 14 days, it is not known whether NSAIDs may contribute to longterm negative effects on muscle function.
reported in the Mishra et al. study. Other studies have also demonstrated a lack of efficacy of NSAID treatment after eccentric exercise. Although the flurbiprofen and ibuprofen studies used dosages ranging from analgesic (34, 75) to anti-inflammatory dosages (33, 34), no efficacy was demonstrated consistently. Furthermore, the flurbiprofen and ibuprofen studies showed a lack of efficacy over both short and long dosing periods (Pizza, f.x., d. Cavender, a. Stockard, h.1999). Thus, it appears that lack of efficacy of these 2 NSAIDs is neither dose dependent nor time dependent. Also, all flurbiprofen and ibuprofen studies that showed no efficacy used prophylactic doses. The expectation might be that prophylactic NSAID treatment would maximize the anti-inflammatory effect by inhibiting the immediate response to the mechanical injury. However, this was not observed in any of the studies.

2.21. Therapeutic Treatment of DOMS Using Physical Modalities

Numerous therapeutic interventions aimed at alleviating DOMS have been proposed. Standard physical therapy modalities such as cryotherapy, ultrasound, and electric stimulation have been used (Chleboun, G.S., J.N. Howell, H.L.1995). In addition, massage, stretching, light exercise, immobilization, and simple rest have been examined (Viitasalo, J.T. K. Niemela, R. Kaappola, T.1995). Alternative treatments include hyperbaric oxygen therapy (HBOT) and electromagnetic shielding (Zhang, j., d. Clement, and j. Taunton. 2000). Despite the volume of work in this area, there is little consensus among practitioners as to the most effective way to manage the symptoms of damage. Reliance on anecdotal evidence or studies with poor experimental design may perpetuate ineffective treatments where, at best, placebo effects predominate.

2.22. Warm-up, Stretching, and Massage

Arguably the most commonly practiced treatments for DOMS are passive stretching and massage. Surprisingly, little scientific evidence to support the effectiveness of this treatment exists. Some studies have examined combinations of treatments, such as, warm-up, stretching, and massage (Rodenburg, j.b., d. Steenbeck, p.1994), warm underwater water jet massage (Viitasalo, j.t., k. Niemela, r.1995) and ice massage. Other studies have examined single interventions of massage and stretching (Lund, h., p. Vestergaard- Poulsen, i.l. 1998). In yet another
study, massage was compared with electric stimulation and light exercise. The combination of pre-exercise warm-up with stretching and post exercise massage (i.e., on subsequent days) had positive effects as did warm underwater water-jet massage. Rotenberg et al. randomly assigned 50 subjects to a treatment or control group. An eccentric elbow flexor exercise protocol was used as the treatment with pre-exercise warm-up and post exercise massage 15 minutes after exercise. There was evidence of less muscle tenderness, less strength loss, and greater elbow flexion ROM in the treatment group, but relaxed elbow extension, CK activity, and serum myoglobin were not different between groups. However, it is difficult to attribute these positive effects to massage. Pre-exercise warm-up has been shown to be effective in reducing delayed onset muscle soreness (DOMS), and such an effect alone could explain the results of rodenburg et al. As for the effectiveness of water jet massage in reducing DOMS, these results cannot be generalized to the more commonly practiced manual massage. There is a similar lack of evidence to support post exercise stretching for treating DOMS. It could be argued that no study has adequately examined the potential therapeutic effects of stretching or massage with proper experimental design and sufficient sample size. However, more importantly, a sound rationale for why either stretching or massage would alleviate DOMS has not been established.

2.23. Cryotherapy and Compression

In contrast to massage and stretching, there is a sound rationale for the use of cryotherapy and compression in the treatment of delayed onset muscle soreness (DOMS). Various modes of applying ice and compression are used routinely in clinical practice to provide pain relief, diminish inflammatory responses, and reduce swelling for numerous types of injuries (Swenson, c., l. Sward, and j. Karlsson.1996). With respect to DOMS, cold-water immersion (Eston, r., and d. Peters.1999), intermittent pneumatic compression, and compression sleeves have been shown to be effective in providing some relief of delayed onset muscle soreness (DOMS). A treatment of cold-water immersion for 15 minutes immediately after eccentric elbow flexor exercise and every 12 hours for a total of 7 treatments was effective in reducing stiffness, as measured by relaxed arm angle, and resulted in lower values for plasma CK activity. A treatment of intermittent pneumatic
compression for 20 minutes immediately after eccentric elbow flexor exercise and daily for the next 5 days was effective in reducing stiffness and swelling. However, these effects were only evident immediately after treatment. Longer duration effects were not investigated because a control group not receiving any treatment was not used recently; Kraemer et al demonstrated that wearing a compression sleeve garment for 5 days after a bout of eccentric elbow flexor exercise was effective in reducing the strength loss, soreness, swelling, and stiffness. In contrast to these effective treatments, ice massage was an ineffective treatment. However, this may have been due to the fact that only 1 treatment was applied for, 15 minutes, 24 hours, or 48 hours, after the exercise. Based on clinical practice and the encouraging results with ice and compression separately, this combination might prove to be the most efficacious treatment.

2.24. Rest vs. Therapeutic Exercise

The issue of whether it is better to exercise or rest when experiencing DOMS has spurred much interest. Recently, Sayers et al examined the potential benefits of light exercise or immobilization compared with those of simply resting. The elbow joints of 9 subjects were immobilized at 90° immediately after eccentric elbow flexor exercise. Light exercise was performed by 9 subjects (50 bicep curls with 5 lb), and 8 subjects simply rested their elbow flexors. Strength recovery was better after either light exercise or immobilization when compared with just rest. These results were encouraging because there is a natural tendency to perform light exercise to alleviate delayed onset muscle soreness (DOMS). The benefits of immobilization emphasize that interventions should be directed at enhancing the healing potential of the muscle tissue. Interestingly, immobilization with the muscle in a lengthened position is the recommended treatment for quadriceps contusions. This is thought to reduce scar tissue formation and facilitate sarcomere regeneration. Although a muscle contusion is a much more severe injury than exercise-induced muscle damage, the same principles of healing likely apply. Future work might examine whether immobilization at 0° (lengthened position) is more effective.
2.25. Green tea

Tea is one of the most popular beverages consumed worldwide. Tea, from the plant Camellia sinensis, is consumed in different parts of the world as green, black, or Oolong tea. Among all of these, however, the most significant effects on human health have been observed with the consumption of green tea (Cabrera et al. 2006). The first green tea was exported from India to Japan during the 17th century. It is estimated that about 2.5 million tons of tea leaves are produced each year throughout the world, with 20% produced as green tea, which is mainly consumed in Asia, some parts of North Africa, the United States, and Europe (Japanese Green Tea Online). The association between tea consumption, especially green tea, and human health has long been appreciated (Weinberger et al. 2000, Sato et al. 2000).

Green tea and black tea are processed differently during manufacturing. To produce green tea, freshly harvested leaves are immediately steamed to prevent fermentation, yielding a dry, stable product. This steaming process destroys the enzymes responsible for breaking down the color pigments in the leaves and allows the tea to maintain its green color during the subsequent rolling and drying processes. These processes preserve natural polyphenols with respect to the health-promoting properties. As green tea is fermented to Oolong and then to black tea, polyphenol compounds (catechins) in green tea are dimerized to form a variety of theaflavins, such that these teas may have different biological activities.

2.26. Green tea composition

The chemical composition of green tea is complex: proteins (15-20% dry weight), whose enzymes constitute an important fraction; amino acids (1-4% dry weight) such as theanine or 5-N-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, and lysine; carbohydrates (5-7% dry weight) such as cellulose, pectins, glucose, fructose, and sucrose; minerals and trace elements (5% dry weight) such as calcium, magnesium, chromium, manganese, iron, copper, zinc, molybdenum, selenium, sodium, phosphorus, cobalt, strontium, nickel, potassium, fluorine, and aluminum; and trace amounts of lipids (linoleic and a-linolenic acids), sterols (stigmasterol), vitamins (B, C, E), xanthic bases (caffeine, theophylline), pigments (chlorophyll, carotenoids),
and volatile compounds (aldehydes, alcohols, esters, lactones, hydrocarbons). Due to the great importance of the mineral presence in tea, many studies have determined their levels in tea leaves and their infusions (Table 1) (Belitz et al. 1997). Fresh leaves contain, on average, 3-4% of alkaloids known as methylxanthines, such as caffeine, theobromine, and theophylline (Graham, H.N. 1992). In addition, there are phenolic acids such as gallic acids and characteristic amino acid such as thiamine present (Graham.H.N 1992).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Green Tea</th>
<th>Black tea</th>
<th>Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>15</td>
<td>15</td>
<td>trace</td>
</tr>
<tr>
<td>Amino acids</td>
<td>4</td>
<td>4</td>
<td>3.5</td>
</tr>
<tr>
<td>Fiber</td>
<td>26</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>Others carbohydrates</td>
<td>7</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Lipids</td>
<td>7</td>
<td>7</td>
<td>trace</td>
</tr>
<tr>
<td>Pigments</td>
<td>2</td>
<td>2</td>
<td>trace</td>
</tr>
<tr>
<td>Minerals</td>
<td>5</td>
<td>5</td>
<td>4.5</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>30</td>
<td>5</td>
<td>4.5</td>
</tr>
<tr>
<td>Oxidized phenolic compounds</td>
<td>0</td>
<td>25</td>
<td>4.5</td>
</tr>
</tbody>
</table>

* Data refer to dry weight of tea leaves.
† Infusion time: 3 minutes
§ Especially flavonoids
¶ Especially thearubigins and theaflavins

Green tea contains polyphenols, which include flavones, flavonoids, flavonoids, and phenolic acids; these compounds may account for up to 30% of the dry weight. Most of the green tea polyphenols (GTPs) are flavonols, commonly known as catechins. Products derived from green tea are mainly extracts of green tea in liquid or powder form that vary in the proportion of polyphenols (45-90%) and caffeine content (0.4-10%). The major flavonoids of green tea are various catechins, which are found in greater amounts in green tea than in black or Oolong tea (Vinson.J.A 2000). There are four kinds of catechins mainly find in green tea: epicatechin, epigallocatechin, epicatechin-3-gallate, and EGCG (Sano et al.
The preparation methods influence the catechins both quantitatively and qualitatively; the amount of catechins also varies in the original tea leaves due to differences in variety, origin, and growing conditions (Khokhar. 2002). The preparation of fresh green tea cannot totally extract catechins from the leaves; therefore, the concentration found differs from the absolute values determined through the complete extraction of leaves (Fernandez et al. 2000). Moreover, catechins are relatively unstable and could be quantitatively and qualitatively modified during the time frame of an experiment (Chen et al. 1998, 2001). Thus, comparison of ingested doses in animal studies is not possible because the catechin quantification before administration is often not known.

### 2.27. Green Tea Antioxidants and Exercise

Alessio, et al. (2002) examined the effects of green tea consumption on biomarkers of exercise-induced oxidative stress in rats. The study consisted of four treatment groups: water, water + exercise, green tea, and green tea + exercise. Decaffeinated green tea was administered ad lib in the rats’ water bottles. They displayed no taste aversion to the tea. All rats were allowed mild physical activity for 30 minutes twice per week. After 6.5 weeks of tea consumption, the water only and tea only groups were sacrificed in a resting state. Rats from the water + exercise and tea + exercise groups ran for 30 minutes on a treadmill with an average speed of 25 m/min on a level grade. These rats were sacrificed immediately after exercise. Muscle tissue, liver, and kidney samples were taken to measure for biomarkers of OS. Samples were analyzed for MDA-TBARS and PC using spectrophotometry (Alessio, et al., 2002).

Results for PC levels were non-significant for all groups. Levels of MDA-TBARS in the white gastronomies were elevated in the tea + exercise group but failed to reach significance over the water only group. Rats in the water + exercise group displayed significantly elevated levels of kidney MDA (29%) over the water group. Levels of MDA-TBARS in the kidney and liver samples were unchanged in rats consuming tea and exercising. This implies that the green tea prevented lipid peroxidation in the kidney tissues (Alessio, et al., 2002). Polyphenol levels in plasma remained low even though animals consumed tea for 6.5 weeks. The authors suggested that the polyphenols are transported to the kidney, metabolized, and excreted by the body.
Nagasawa, et al. (2000) applied an electrical stimulus to the hindlimb muscles of rats and examined the effects of EGCG supplementation on MDA and PC. Rats in the control group consumed a standard diet and were given an electrical stimulus in one leg and non-electrical stimulus (needle only-no current) in the other leg. This was performed every second day for two weeks. Rats in the EGCG groups consumed the same diet with .01% EGCG added to it and received the same stimuli. All rats were sacrificed three hours after the last stimulation (Nagasawa, et al., 2000).

Supplementing the diet with EGCG showed no adverse effects with respect to food consumption. Electrical stimulation resulted in hypertrophy of the gastronomies and soleus in both groups. The amount of hypertrophy was unaffected by EGCG. A spectrophotometric method was used to determine MDA-TBARS. Although the levels of MDA-TBARS were lower in the supplemented group, the levels were not significantly different. Protein levels in the electrically stimulated legs of the EGCG group, also measured by HPLC, were significantly lower than the non-stimulated leg, indicating possible protection against the oxidation of the amino acids in the stimulated leg. The catechin had no effect on the activity levels of SOD and GPX. In the control group, stimulation did not result in antioxidant enzyme changes. The authors suggested that the lack of significance of MDA increase is due to the ROS generated in response to intense electrical stimuli acting directly on muscle proteins instead of generating lipid hydroperoxides or aldehydes (Nagasawa, et al., 2000).

There was a study to date evaluating tea consumption, ROS, and exercise in human subjects, (Tsai, et al. 2005) asked 24 male rugby players to consume three cups of oolong tea per day for 30 days. All subjects were trained rugby players currently engaged in training, which was continued throughout the study. Players consuming vitamins were excluded from the study and all participants kept dietary records. Drinks containing either 1 g of oolong powder and 150 cm$^3$ water or only 150 cm$^3$ of water were sealed in similar containers and given to the subjects.

The subjects performed a graded exercise test to exhaustion on a treadmill. The test started at 6 km/hr for 3 minutes, and increased at 2 minutes for each successive stage. Speed was increased to 9 km/hr, 11 km/hr, 13 km/hr, 14 km/hr,
etc. until subjects reached exhaustion. Blood samples for MDA and SOD were taken immediately post-exercise. Assays were performed using commercially available spectrophotometric kits. Subjects returned after 30 days of tea or water consumption and performed the same exercise test to exhaustion.

Resting MDA levels were significantly lower post-study in the supplemented group when compared to pre-study resting values. No difference existed for resting MDA post-study for the water only group. Post-exercise MDA values following 30 days of oolong tea consumption were significantly lower than pre-exercise values. SOD activity increased post-exercise in the supplement group following tea consumption. The authors explain this finding as being a result of a synergistic relationship between tea antioxidants and SOD to scavenge ROS. They further conclude that supplementation of oolong tea results in a significant reduction of plasma MDA and that tea catechins apparently act as a natural water-soluble antioxidant. The authors recommend tea consumption for rugby players in training as a method of maximizing biological antioxidant activity.

2.28. Health benefits of green tea in humans and animals

Studies using animal models show that green tea catechins provide some protection against degenerative diseases (Vanessa.C.Grey,W. 2004). Some studies indicated that green tea has an antiproliferative activity on hepatoma cells and a hypolipidemic activity in hepatoma-treated rats, as well as the prevention of hepatotoxicity (Vanessa.C. 2004). As a preventive agent against mammary cancer post-initiation (Vanessa. 2004). Green tea catechins could also act as antitumorigenic agents (Roomi et al. 2007) and as immune modulators in immune dysfunction caused by transplanted tumors or by carcinogen treatment (Vanessa.C. 2004). Moreover, green tea, its extract, and its isolated constituents were also found to be effective in preventing oxidative stress (Babu et al. 2006) and neurological problems (Unno.K. et al. 2007).

Green tea consumption has also been linked to the prevention of many types of cancer, including lung, colon, esophagus, mouth, stomach, small intestine, kidney, pancreas, and mammary glands (Koo et al. 2006). Several epidemiological studies and clinical trials showed that green tea (and black and Oolong teas to a
lesser extent) may reduce the risk of many chronic diseases (Zaveri. 2006). This beneficial effect has been attributed to the presence of high amounts of polyphenols, which are potent antioxidants. In particular, green tea may lower blood pressure and thus reduce the risk of stroke and coronary heart disease. Some animal’s studies suggested that green tea might protect against the development of coronary heart disease by reducing blood glucose levels and body weight (Tsuneki. 2004). However, all these data are based on middle-aged animals’ populations, not the elderly populations, which nutritional status tends to be more adversely influenced by age-related biological and socioeconomic factors (Meydani. 2001)

Tea components possess antioxidant, anti-mutagenic, and anti-carcinogenic effects and could protect humans against the risk of cancer by environmental agents (Mukhtar & Sano et. al., 1992). The inhibitory effects of green tea leaves against tart-butyl hydroperoxide-induced lipid peroxidation, and a similar antioxidant effect on the kidney was observed after oral administration of the major tea polyphenol EGCG. The antioxidative potency of crude catechin powder and individual catechins was tested in experiments using the active oxygen method. Crude catechins reduced the formation of peroxides far more effectively than dl-a-tocopherol (Hara.Y. 1990. Shim et al. 1995) studied the chemo preventive effect of green tea among cigarette smokers and found that it can block the cigarette-induced increase in sister chromatid exchange frequency (Shim et al. 1995).

The effectiveness of green tea in treating any type of diarrhea and typhoid has been known in Asia since ancient times (McKay.D.L. 2002, Lu et al. 2003, and Wu et.al. 2003). Green tea catechins have an inhibitory effect on Helicobacter pylori infection (Takabayashi et al. 2004, Yee et al. 2002). Effects of green tea against the influenza virus, especially in its earliest stage, as well as against the Herpes simplex virus have also been demonstrated (Toda.et.al 1989, Mukoyama et al. 1991, Yama.et.al 1997). Furthermore, Weber et al. observed that adenovirus infection is inhibited in vitro by green tea catechins (Weber et al. 2003).

In humans, Hirasawa and Takada (2004) studied the antifungal activity of green tea catechins against Candida albicans and the convenience of a combined treatment with catechins and lower doses of antimycotics, which may help to avoid the side effects of antimycotics. Green tea consumption has also been associated
with increased bone mineral density, and it has been identified as an independent factor protecting against the risk of hip fractures; this effect was considered independent of smoking status, hormone replacement therapy, coffee drinking, and the addition of milk to tea (Muraki et al. 2003).

Park et al. (2003) observed the positive effects of green tea extracts and GTPs on the proliferation and activity of bone cells. The proliferation of hepatic stellate cells is closely related to the progression of liver fibrosis in chronic liver diseases, and EGCG has a potential inhibitory effect on the proliferation of these cells (Dorchies et al. 2003, Sakata et al. 2004). Green tea strengthens the immune system action because it protects it against oxidants and radicals. Recent studies suggested that GTPs might protect against Parkinson’s and Alzheimer’s diseases and other neurodegenerative diseases (Weinreb et al. 2004, Pan et al. 2003). Studies have demonstrated GTP neuroprotectant activity in cell cultures and animal models, such as the prevention of neurotoxin-induced cell injury (Pan et al. 2003). Green tea is considered to be useful for insect stings due mainly to its anti-inflammatory effects and its capacity to stop bleeding (Sagesaka et al. 1998, Dvorakova et al. 1999). Some studies have suggested an inverse association between green tea consumption and the risk of kidney stone formation (McKay, 2002, Ishizuka, 2003). In an experimental cataractogenesis system, green tea acted by preserving the antioxidant defense system of the lens (Gupta et al. 2002).

Skrzydlewska et al. (2002) indicated a beneficial effect of green tea in alcohol intoxication. In addition to all of these reported properties, which have helped the recognition of green tea as functional food by some authors (Ferrari, CKB and Torres EAFS. 2003) green tea is also currently used in the preparation of a variety of foods, pharmaceutical preparations, dentifrices, and cosmetics (Arburjai. T and Natsehe. FM: 2003).

Tea has been shown anti-carcinogenic effects against breast cancer in experimental studies (Min Zhang et al. 2005). However, epidemiologic evidence that tea protects against breast cancer has been inconsistent (Min Zhang et al. 2005). A case-control study was conducted in southeastern China between 2004 and 2005 (Zhang et al. 2008). The incidence cases were 1009 female patients aged 20-87 years with histologically confirmed breast cancer, and the 1009 age-matched controls were healthy women randomly recruited from breast disease clinics. Information on
duration, frequency, quantity, preparation, and type of tea consumption as well as diet and lifestyle were collected by face-to-face interviews using a validated and reliable questionnaire. In comparison with non-tea drinkers, green tea drinkers tended to reside in urban settings, to have more education, and to consume more coffee, alcohol, soy, vegetables, and fruits. After adjusting established and potential confounding factors, green tea consumption was associated with a reduced risk of breast cancer. Similar dose response relationships were observed for duration of drinking green tea, number of cups consumed, and new batches prepared per day.

Hsu.SP, et al. (2007) demonstrated the effects of supplementation with decaffeinated green tea extract (catechins) on hemodialysis-induced reactive oxygen species, atherosclerotic disease risk factors, and proinflammatory cytokines. The pharmacokinetics of one oral dose of catechins was compared between healthy subjects and hemodialysis patients. The authors compared the antioxidant effects of three different doses (0, 455, and 910 mg) of oral catechins with that of oral vitamin C (500 mg) during a hemodialysis session. In patients, catechin supplementation reduced hemodialysis-enhanced plasma hypochlorous acid activity more effectively than did placebo or vitamin C. Between the treatments with 455 and 910 mg catechins, no significant difference was found in the reduction of plasma hypochlorous acid activity. Catechins also significantly reduced proinflammatory cytokine expression enhanced by hemodialysis.

2.29. Effects on antioxidant markers and oxidative stress

Green tea is a popular neutraceutical as an antioxidant. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals, and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage (Halliwell et al. 1985). Catechins are hypothesized to help protect against these diseases by contributing, along with antioxidant vitamins (i.e., vitamins C and E) and enzymes (i.e., SOD and catalase), to the total antioxidant defense system (Abdel-Raheim et al. 2009).

The four main catechins in green tea are (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epicatechin (EC) (Lee, et al. 2002). The catechins EGCG, EGC, and EC are thought
to be the most active as radical scavengers (Lee, et al. 2002). Peak plasma concentrations of green tea polyphenols occurs approximately 1.5 to 2.5 hours post consumption. The half-life of these polyphenols varies from 3 to 5 hours. It should be noted that the polyphenols yield several metabolites that keep the antioxidant status elevated several hours post consumption (Lee, et al. 2002). Green tea infusions have been shown to increase the total antioxidant capacity in vivo with human subjects 60-120 minutes post consumption (Sung, et al. 2000). The levels return to baseline at 24 hours post consumption. Unlike vitamin E, green tea catechins are water-soluble and do not build up in the body (Lee, et al. 2002). Supplementation for multiple weeks would thus be unnecessary. Black tea, oolong tea, and green tea are produced from the same plant (Camellia sinensis). The difference between the types of tea is due to the processing of the tea leaves (Frei and Higdon, 2003). Black tea results from rolling and crushing tea leaves and allowing them to ferment. This process allows catechins to be converted into theaflavins and thearubigins. The process of making oolong tea is intermediate between black tea and green tea and results in a tea possessing properties of both black and green tea. To make green tea, the leaves are wilted and steamed to inactivate polyphenol oxidase and prevent catechins from being converted. This results in a minimally processed tea that is high in catechins (Higdon, and Frei 2003).

*In vivo* studies showed that green tea catechins increase total plasma antioxidant activity (Yokozawa et al. 2002, Skrzydlewska et al. 2002). Intake of green tea extracts also increases the activity of superoxide dismutase in serum and the expression of catalase in the aorta; these enzymes are implicated in cellular protection against ROS (Skrzydlewska et al., 2002, Negishi et al. 2004). This action is combined with direct action on oxygen species by a decrease in the nitric oxide plasma concentration (Yokozawa et al. 2002). Malondialdehyde, a marker of OS, also decreases after green tea intake (Yokozawa et al. 1999, 2002). These results suggest that catechins could have a direct (antioxidant) or indirect (increase of activity or expression) effect. Since catechins can act as antioxidants in vitro, they might prevent the oxidation of other antioxidants, such as vitamin E. However, ingestion of green tea catechins does not modify the plasma status of vitamins E and C in vivo (Skrzydlewska et al. 2002, Tijburg et al. 1997, Alessio et al. 2003).
Nevertheless, one study reported that catechins increase vitamin E concentration in low-density lipoprotein (Tijburg et al. 1997) and in this way could protect low density lipoprotein against peroxidation (Yokozawa et al 2002).

**Pilipenko et al. (2008)** assessed the tolerance of tableted green tea and its effect on the antioxidant status indices. Twenty-five patients with different gastrointestinal pathologies were included in the study and divided into treatment and control groups. The tolerance of tableted green tea was good in the treatment group, who showed better dynamics of quality-of-life indices, especially in scales of body pain and social functioning. There were no significant differences in biochemical analysis between the groups, which may indicate the safety of this product. Analysis revealed that the treatment group showed a decreased level of all antioxidant status indices, as reflected in a significant decreasing of the lipid peroxidation index from 4.63 to 4.14.

### 2.30. Adverse effects of green tea

Although green tea has several beneficial effects on health, the effects of green tea and its constituents may be beneficial up to a certain dose yet higher doses may cause some unknown adverse effects. Moreover, the effects of green tea catechins may not be similar in all individuals. EGCG of green tea extract is cytotoxic, and higher consumption of green tea can exert acute cytotoxicity in liver cells, a major metabolic organ in the body (Schmidt et al. 2005). Another study found that higher intake of green tea might cause oxidative DNA damage of hamster pancreas and liver (Takabayashi et al. 2004).

**Yun et al. (2006)** clarified that EGCG acts as a pro-oxidant, rather than an antioxidant, in pancreatic b cells in vivo. Therefore, high intake of green tea. may be detrimental for diabetic animals to control hyperglycemia. At a high dose (5% of diet for 13 wk), green tea extract induced a thyroid enlargement (goiter) in normal rats (Sakamoto et al 2001, Satoh et al 2002). This high-level treatment modified the plasma concentrations of the thyroid hormones. However, drinking even a very high dietary amount of green tea would be unlikely to cause these adverse effects in humans.
Harmful effects of tea overconsumption (black or green) are due to three main factors: (a) its caffeine content, (b) the presence of aluminum, and (c) the effects of tea polyphenols on iron bioavailability. Green tea should not be taken by patients suffering from heart conditions or major cardiovascular problems. Pregnant and breastfeeding women should drink no more than one or two cups per day, because caffeine can cause an increase in heart rhythm. It is also important to control the concomitant consumption of green tea and some drugs, due to caffeine’s diuretic effects (Bruneton et al. 2001). Some studies revealed the capacity of tea plants to accumulate high levels of aluminum. This aspect is important for patients with renal failure because aluminum can be accumulated by the body, resulting in neurological diseases; it is therefore necessary to control the intake of food with high amounts of this metal (Costa et al. 2002). Likewise, green tea catechins may have an affinity for iron, and green tea infusions can cause a significant decrease of the iron bioavailability from the diet (Hamdaoui et al. 2003).