CHAPTER 1: INTRODUCTION

Muscle soreness that often follows a bout of hard or unaccustomed exercise, is a problem that most people experience following training or competition. It has been studied quite extensively, and its cause and mechanism are thus well documented. (Eston & Peters 1999; Proske & Morgan 2001; Connolly; Sayers & McHugh 2003; Toumi & Best 2003). However a means for alleviating or preventing it has been elusive. This project is an investigation into the efficacy of the flavonoid group of anti-oxidants for reducing such muscle soreness. This section provides an overview of current research and follows progress in the line of nutritional supplementation for reducing delayed onset muscle soreness (DOMS). In particular, the relatively new identification of the role of flavonoids in metabolism will be described, and their potential role in preventing muscle damage reviewed.

1:1 Delayed onset muscle soreness (DOMS) – Background & Mechanism

DOMS is a form of exercise induced muscle injury. It can occur from any unaccustomed exercise, or unusually hard exercise and is particularly associated with eccentric muscular contractions e.g. in the quadriceps muscles, when running downhill. It causes pain, stiffness, loss of power and loss of function and impairs restoration of muscle glycogen stores in the affected muscle and therefore significantly affects athletic training and performance (Jeukendrup & Gleeson 2004).

During eccentric exercise the contracting muscle is forcibly lengthened, usually as muscles contract to control their rate of lengthening against the force of gravity. During this phase the actin and myosin cross bridges within muscle fibres are under considerable mechanical stress, making them particularly vulnerable to damage. There
is no pain experienced immediately after the exercise, but the symptoms of DOMS begin several hours later, typically peaking between 24 and 48 hours after eccentric activity (Jeukendrup & Gleeson 2004). Electron microscope examinations of muscle tissue show that there are structural signs of muscle damage: disrupted sarcomeres in myofibrils and damage to the excitation-contraction coupling system. However there is not consensus which of these occurs first (Prosko & Morgan 2001). Whichever is the case, there is clearly a mechanical injury when contractile elements of muscle and connective tissue structures are overloaded. This includes fragmentation of the sarcoplasmic reticulum, lesions to the plasma membrane, cytoskeletal damage and swollen mitochondria (Eston & Peters 1999, Toumi & Best 2003). The stronger argument seems to be that the cell membrane damage disrupts Ca++ balance in the fibres, which in turn effects muscle contractions, while interstitial fluid accumulation stimulates sensory nerve endings, contributing to pain sensation and characteristic stiffness.(Armstrong 1984, Prosko & Morgan 2001). Sarcomere disruption does not extend the full length of a myofibril and usually does not extend across a whole muscle fibre, so that adjacent fibres can appear quite normal (Connolly et al. 2003).

The degree of damage sustained is dependent on how trained the muscle is, the extent of lengthening, the force and speed of the exercising muscle, and the degree of existing muscle stiffness. The greater any of these four characteristics, the greater the damage likely to occur. e.g. a recreational runner who takes part in some unaccustomed sprint sessions, possibly involving some hill efforts, will experience DOMS, whilst another runner in the group who partakes in a similar session weekly, may not have any after effects. It has been found that even a single bout of eccentric exercise can reduce DOMS on a subsequent bout within a 6 week period, and regular
eccentric muscle training thus provides a high degree of protection (Byrnes et al. 1985, Kanter 1998, Proske & Morgan 2001). Specifically how the trained muscle sustains less damage has yet to be fully researched.

The intriguing aspect of this type of muscle injury compared to other types, is the fact that the signs and symptoms are delayed by at least 24 hours. An acute muscle tear is felt immediately, and overuse injuries begin by being felt about an hour or two after exercise, and progress to becoming painful during exercise. Clearly the extent and distribution of fibre damage is a factor in explaining these differences, but other factors must be involved. A clearer understanding of what happens in the period between engaging in muscle damaging exercise, and the onset of DOMS, may also lead to better understanding and intervention in other kinds of muscle injury.

The initial mechanical damage that occurs during exercise, is now believed to be followed by further secondary, or oxidative damage, that is initiated by the normal inflammatory response to injury (Connolly et al. 2003). This explains the delay in soreness experienced by the athlete 24 to 48 hours after exercise (Connolly et al. 2003). Inflammation from the primary muscle damage leads to release of prostaglandins and leukotrienes. The leukotrien response involves neutrophil activation and the accumulation of neutrophils within the injured muscle, as early as one to two hours after exercise (Toumi & Best 2003). Cellular debris from the primary injury is removed by the phagocytic action of the neutrophils, to make way for a regenerative response. Increased presence of white blood cells in exercised muscle after eccentric exercise was confirmed by MacIntyre, Reid, Lyster, Szasz, and McKenzie (1996). However neutrophils contain more than 40 hydrolytic...
enzymes and can generate reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide and hypochlorous acid. All are types of free radical, capable of causing lipid peroxidation, and thus damage to cell membranes as well as to proteins and DNA (Jeukendrup & Gleeson 2004). Such damage gives rise to release of myoglobin and the muscle enzyme creatine kinase (Antonio & Stout 2001). Both of the latter are used in research studies to observe and quantify microscopic skeletal damage. Even though inflammation is a positive healing process, its short term effects seem to result in elevated pain, and inhibited short term recovery of muscle function (Connolly et al. 2003).

It is only in the past two decades that biochemists have realised the profound significance of free radical peroxidation in vivo, and its involvement in many diseases. In cells subjected to severe oxidative stress, antioxidant defences may become depleted and it is now accepted that many common diseases are associated with lipid peroxidation and low anti-oxidant status (Scott 1997, Prior 2004). It is thought that ROS can be involved in the initiation and/or development of cell damage, and in the case of DOMS it would be the latter that is expected to happen. Increased levels of ROS have been measured in muscle after intense exercise, which is thought to be the consequence of microscopic muscle damage (Scott 1997). This has been supported by detection of increased ethane and pentane in the breath after exercise (Scott 1997). Hence the secondary, delayed source of muscle damage

The human body has enzymatic and nutritional defence mechanisms to protect itself from free radicals, which help to maintain an anti-oxidant balance in the system, and control cellular damage (Antonio & Stout 2001). It has been noted that training
strengthens the antioxidant defence system by increasing levels of superoxide
dismutase (Evans 2000). However it appears that intense physical exercise may
over-reach the capacity of these defences, leading to skeletal damage. Improvement
of intrinsic antioxidant capacity, may be one of the adaptive responses that occurs
when muscles are trained repeatedly in eccentric patterns, e.g. fell runners who
regularly train to run up and down hill, generally avoid DOMS if running familiar
distances, speeds and inclines.

Figure 1.
Schematic representation of the flow of events following muscle-damaging exercise.

I Mechanical damage II Inflammation & Swelling III Free radical activity
(from Connolly et al. 2003 p 198)

Research has been conducted into various means of reducing DOMS, including use of
non-steroidal anti-inflammatories (NSAIDS), icing, massage, stretching, micro current
electrical stimulation and more recently, anti-oxidant therapy. (Rokitzki et al. 1994;
Weber, Servedio & Woodall 1994); Of these, anti-inflammatory drugs and
antioxidants show the best results (Connolly et al. 2003), although even these vary in their success between studies. Hasson et al. (1993) found that a prophylactic dose of 400mg ibuprofen did not prevent CK release from muscle, but did reduce perceived muscle soreness and may assist with recovery. (The latter study only had 5 subjects per group). However Gulick; Kimura; Sitler; Palone; and Kelly (1996), suggest that NSAIDS may impede recovery of muscle function. Immersion in cold water post exercise has shown some benefits, (Eston & Peters 1999), but is very impractical to apply in most sporting situations. Weber et al. (1994) found no difference in DOMS response between massage, microcurrent stimulation, upper body ergometry and their control group. It appears there has been no remarkable advance in elucidating a practical, inexpensive and effective means of combating DOMS. Thus further attention has been paid to the potential role of anti-oxidant therapy, as a means of combating the effects of ROS. By supplementing the athlete with an anti-oxidant source, the pro-oxidant/anti-oxidant balance in muscle cells may be maintained and hence lipid peroxidation reduced.

1:2 Anti-oxidants – role in free radical scavenging

An anti-oxidant is a compound that protects biological systems against the deleterious effects of excessive ROS. (Jeukendrup & Gleeson 2004). Intrinsic or endogenous antioxidants are synthesised in the body e.g. glutathione, coenzyme Q10, superoxide dismutase & glutathione peroxidase. Extrinsic anti-oxidants must be obtained from the diet in the form of nutrients such as Vitamin A,C, E, carotenoids, flavonoids and some minerals. Physical training is associated with an increase in levels of endogenous anti-oxidant enzymes, which would appear to be an adaptive response to
an increased requirement for these compounds, in order to try and maintain oxidant/anti-oxidant homeostasis (Jeukendrup & Gleeson 2004).

1:3 Phytonutrients: Flavonoids

Flavonoids are biologically active compounds derived from plants that have great potential for human health. They are similar in structure to other anti-oxidant nutrients, and prevent or limit the actions of free radicals, usually by removing their unpaired electron, and thus making them less reactive (Jeukendrup & Gleeson 2004). Flavonoids are a derivative of the polyphenol group, and there are thousands of flavonoid compounds that occur naturally, including the flavonols, flavonones, proanthocyanidins and anthocyanidins. Typically these are compounds which give colour to fruit and vegetables. In vitro studies show that flavonoids are 3 to 10 times more effective than Vitamin E in scavenging free radicals (Jeukendrup & Gleeson 2004). Some flavonoids have been researched to establish their absorption rate and metabolic activity, and these are the ones that are most widely found in anti-oxidant supplements available across the counter. However there is still a dearth of information on these substances, particularly about the absorption process and metabolically active timescale of flavonoids in human metabolism. Examples of flavonoids and their biochemical activities are described below.

Grape seed proanthocyanidins (OPC’s) have been found to have a number of antioxidant activities in the laboratory, including scavenging of hydroxyl and peroxyl radicals and inhibition of oxidation of low density lipoprotein (LDL). The greater the number of catechin and epicatechin units, the greater the inhibitory potential (pdrhealth.com). OPC’s appear to have an affinity for vascular tissue and strongly
inhibit several enzymes involved in degredation of collagen and elastin (unnamed author in Alternative Medicine Review 2003). Comparative tests carried out by Les Derives Resiniques et Terpenique, the manufacturer of Vitaflavan, (grapeseed extract), found that it was 14 times more active than Vitamin C and significantly more active than catechin alone, in its anti-free radical effect (Assouad 2004). OPC’s have been shown to reduce superoxide anions and hydroxyl radicals better than Vit C & E, whilst simulataneously aiding regeneration of Vitamin C and upregulating endogenous antioxidant enzymes (Antonio & Stout 2001). Absorption is variable among individuals. (pdrhealth.com, Assouad 2004).

Green tea is produced from steaming fresh tea leaves at high temperatures, thereby inactivating oxidising enzymes and preserving the polyphenol content. The polyphenols are catechins (epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate), and comprise 30-40% of the extractable solids of dried green tea. (unnamed author in Alternative Medicine Review 2000). Green tea has anti-oxidant potential and has also been noted to inhibit arachidonic acids, and therefore has an additional anti-inflammatory effect.

Hesperidin, rutin and quercetin are chemically very similar in structure. Hesperidin is the predominant flavonoid in lemons and oranges, found mainly in the peel and pulp of the fruit. Rutin is found in buckwheat, black tea and apples, and like hesperidin, is only partially soluble in water. Rutin has been shown to chelate metal ions such as ferrous cations, which reduces their capacity to generate reactive oxygen species, rutin may modulate the respiratory burst of neutrophils, and help maintain levels of cellular glutathione. (pdrhealth.com).
1:4 Current research utilising anti-oxidant supplementation to reduce DOMS

Most studies that have attempted to determine whether treatment with antioxidants can reduce or alter the DOMS response to exercise induced muscle damage, have used either Vitamin C, (Kaminski & Boal 1992; Thompson et al. 2003), Vitamin E (Mc Bride; Kraemer; McBride; & Sebastianelli 1998; Cannon et al. 1990), or a combination of both. (Bloomer; Goldfarb; McKenzie; You & Nguyen 2004; Goldfarb; Bloomer; & McKenzie 2005; Gauche et al. 2006). Of these, some used the supplement before the exercise trial, and some after, while more recently, the supplement has been given both before and after eccentric exercise. Outcomes have been equivocal (Kanter 1998). The problem with comparing outcomes from different trials, is that there is a wide variation in the amounts and timings of the supplements used. e.g. Mc Bride et al. (1998) used 1200 iu Vitamin E for 14 days before the trial, Cannon et al. (1990) 800 iu Vitamin E for 48 days before the trial and Bloomer et al. (2004) 400 iu Vitamin E for 14 days before and 3 days after their trial. There has been criticism that CK and myoglobin enzymes markers and ethane and pentane expired breath, are only indirect measures of oxidative stress and/or muscle damage. However in the absence of a practical alternative, they provide information about metabolic responses, and studies using the same biochemical markers to evaluate DOMS, are thus easier to compare. e.g. Cannon et al. 1990; Rokitzki et al. 1994; Kanter et al 1997; McBride et al. 1998). At the same time, most current research designs (Thompson et al. 2003; Bloomer et al. 2004; Gauche et al. 2006) also look at measures of performance, such as peak torque and range of movement, to assess effects of DOMS on muscle action in a more practical way.
Kaminiski and Boal (1992) supplemented subjects with Vitamin C for 7 days post exercise, which reduced muscle soreness scores. The investigators did not measure any biochemical markers such as creatine kinase (CK) or malondialdehyde (MDA), and there may have been a carry over effect from the cross over design of the trial. This study was improved upon by Thompson et al. (2003), who supplemented with Vitamin C for 3 days post exercise, but used independent groups, and subsequently measured CK and myoglobin concentration, as well as muscle soreness and muscle function. They found no difference between the trial group and the placebo group in any response. This suggests that Vitamin C alone, is not effective in reducing DOMS, or as the authors themselves point out, that Vitamin C consumed wholly after exercise, is unable to deliver antioxidant effect to the muscles with sufficient expediency to improve recovery.

McBride et al. (1998), used 1200iu Vitamin E for 2 weeks prior to an exercise trial. (Vitamin E levels are elevated in plasma after 14 days supplementation, and in skeletal muscle after 7 days supplementation). (Bloomer et al. 2004). Although CK levels were significantly lower than in the control group at 24hrs post exercise, perceived soreness and MDA plasma concentration did not differ between the groups at any time point. Whilst there appears to be an effect from Vitamin E on biochemical reactions at the 24hr point, it seems rather optimistic of the authors to state that: "vitamin E is effective in attenuating muscle membrane disruption." (McBride et al. 1998 p67). A more positive effect of vitamin E supplementation was found by Cannon et al. (1990), where the supplement was found to eliminate the difference in DOMS response between two age groups, effectively reducing DOMS in an over 55
year old group to the same as that of an under 30 year old group. This would suggest that in the older age category, endogenous antioxidants may not be as effective as in younger subjects, and so the vitamin E supplement was able to make up the difference. A possible reason for this might be that normal dietary vitamin absorption is different in an older population. In this case the supplement was given for 6 weeks prior to the trial, which in itself could account for the difference in result. Rokitzi et al. (1994) and Kanter et al. (1997), report declines in post exercise serum enzymes, CK & MDA, indicative of muscle damage or LDL oxidation rate, in subjects who consumed between 300 and 1000 IU vitamin E for between 1 and 8 weeks. In contrast, Warren et al. (1992) did not find that a 5 week program of high dose Vit E reduced muscle damage in any way, but his study was done with rats, where the supplemented rats had 250 times as much Vit E as the control rats.

Recently, Bloomer et al. (2004), investigated a combination of vitamin C, Vitamin E and selenium on DOMS response in women after a downhill run. This study was more comprehensive in its measurement criteria, including muscle soreness (MS), maximal isometric force (MIS), range of movement, (ROM) and creatine kinase, (CK) release. The supplement was taken for 2 weeks prior to and 3 days after the trial. These factors together, making it a better protocol than earlier work. Main findings were that the combination of antioxidants significantly reduced CK activity during the 4 days after eccentric exercise, suppressed MS during the second and third day, but had little impact on MIF or ROM loss. These results are more positive, indicating that increased plasma levels of Vit C, E & selenium can alter the biochemical processes taking place during and after eccentric exercise. However the fundamental search is for a change in the performance criteria (in this case MIF &
ROM), which would show that the trial subjects were recovering their ability to perform more quickly than the placebo group. Gauche et al. (2006), tested a combination of anti-oxidants on a sample of 22 endurance runners, supplementing the runners before and after a 55km mountain race. Their interest was primarily neuromuscular recovery, and they found that Maximum Voluntary Contraction (MVC) returned to baseline 24hrs sooner in the supplemented group. Loss of contractile function immediately after the race was not attenuated, but it appears that recovery was enhanced by this anti-oxidant combination.

This review illustrates a trend towards increasing effectiveness of the anti-oxidant intervention over time. The effects are relatively small and not systematic, but they offer encouragement for further investigation.

1:5 A future without DOMS?

The symptoms of DOMS have a significant effect on athletic performance. The soreness and altered function of muscles interrupts training as best, and at worst reduces competitive performance (Connolly et al. 2003). Many sports involve multi-day competition, where the athlete has to perform at the same event on consecutive days, for extended periods of time, or in some cases in differing disciplines on consecutive days. An example in the case of distance running is the mountain marathon, e.g. the OMM (Original Mountain Marathon). This event involves running over hilly terrain for approximately 8 hours on two consecutive days. The possibility of avoiding or reducing DOMS in such situations would be very advantageous. In addition to the local muscle soreness and reduced muscle strength, Gleeson; Blannin; Zhu; Brooks and Cave (1995) and Gleeson; Blannin; Walsh; Field; and Pritchard (1998), indicate that lactate response and possibly cardiovascular response, to a given
exercise load, is greater two days after having performed eccentric exercise, than concentric exercise. This is indicative of the greater relative exercise stress and such effects "will significantly limit the level & duration of exercise that can be achieved in subsequent training bouts over several days." (Gleeson et al. 1995 p 471).

Antioxidant therapy has great appeal for relieving the effects of DOMS, because of its practical ease of application. Many of the modalities described earlier, e.g. ice baths and electro-therapy, to reduce DOMS, are vastly impractical for the majority of athletes, even if they did work. In contrast, antioxidant supplements are cheap, transportable and could easily be used safely by most sports participants. Given the proven in vitro effects of flavonoids, and the widespread medical interest in their health benefits, it would be exciting to explore whether they have similar or even more powerful effects, than those achieved with other anti-oxidant nutrients.

Another reason for pursuing this line of research, is that it has a link to other kinds of muskulo-skeletal injury. The basic premise that inflammatory processes produce free radicals, is true of eccentric- exercise- induced muscle injury and almost every other kind of muscle injury. If flavonoids- based supplements do reduce secondary inflammatory damage by their antioxidant activity, then there is the possibility that they may play a similar role in other kinds of inflammatory tissue damage apart from DOMS. In which case, this research may go a small way to encouraging new investigations to look at other applications for flavonoid therapy, particularly in the field of clinical sports injury treatment.
This study will investigate whether an athlete taking extra anti-oxidants in the form of a flavonoid supplement, at critical time points, will reduce the effects of DOMS induced by eccentric exercise.

1:6 Research Questions

1. Does supplementation with a flavonoid antioxidant, pre and post eccentric exercise reduce the biochemical marker of muscle damage (CK), 24 & 48 hours later, compared with a placebo?

2. Does supplementation with a flavonoid antioxidant, pre & post eccentric exercise reduce perceived muscle soreness 24 & 48 hours later, compared with a placebo?

3. Does supplementation with a flavonoid anti-oxidant decrease recovery time to baseline strength measures compared with a placebo?

1:7 Hypothesis

Consumption of a high dose flavonoid supplement both before and after participating in eccentric exercise, will reduce production of creatine kinase, reduce perception of muscle soreness and reduce time required to recover muscle strength.