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## The role of oestrogen in exercise-induced muscle damage

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# THE ROLE OF OESTROGEN IN EXERCISE-INDUCED MUSCLE DAMAGE

**PhD THESIS**

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## SUMMARY

Oestrogen is believed to be a potent antioxidant, with potential membrane stabilising and gene regulatory effects. Oestrogen has been shown to be an effective cardioprotectant, with for example a lower incidence of atherosclerosis in pre menopausal females compared to age-matched males and in post menopausal females on hormone replacement therapy compared to age-matched males. What has yet to be determined is the extent to which oestrogen can protect skeletal muscle. It has been shown that certain markers of exercise-induced muscle damage (EIMD) are lower in females in both animal and human studies, but as yet no conclusive evidence from human studies has shown that oestrogen provides a protective mechanism against EIMD or whether susceptibility to EIMD varies across the normal menstrual cycle, where oestrogen fluctuations are high. Furthermore, if oestrogen provides a protective mechanism against EIMD, it is unknown at which phase during the muscle damage and repair cycle this occurs. It is also debatable if the potential inhibitory effects that oestrogen has on the inflammatory response, with regard to repair and regeneration of the skeletal muscle are positive or negative.

The thesis is comprised of a critical review of the nature of EIMD and the potential effects that oestrogen has on the muscle damage and repair cycle. This is followed by three empirical studies which were designed to explore this question. These are outlined below:

**Study 1**

Study one was in two parts. The first part of this study aimed at determining if the phase of the menstrual cycle, could in anyway affect eumennorheic (normally

menstruating) females in their susceptibility to exercise-induced muscle damage. An eccentric exercise procedure (elbow flexor muscle group) was performed on a randomly assigned arm during either the menses or ovulatory phase of the menstrual cycle. The contra-lateral limb underwent the same procedure during the alternate phase (random assignment determined in which phase the participant was first damaged). Simple markers of EIMD were assessed at baseline and every 24 h up to three days post exercise, during both phases. No significant differences were seen in any markers of EIMD across phases of the menstrual cycle.

The second part of this study investigated whether prolonged ingestion of exogenous oestrogen, in the form of the combined oral contraceptive pill attenuated any of the symptoms associated with EIMD. The only symptom to show a significant interaction between groups was perceived soreness, with the pill users reporting significantly ($P<0.01$) less soreness than the eumennorheic females in the days following the exercise protocol. This suggested that oestrogen may modulate the pain associated with EIMD.

**Study 2**

The second study focussed on gender differences in exercise-induced muscle damage, with particular focus on the secondary symptoms and events which occur following EIMD. Male and female participants performed a bout of eccentrically biased exercise. Markers of both EIMD and inflammation were taken prior to the eccentric exercise and across a 7-day follow up period. Gender differences in the response to EIMD were seen in creatine kinase activity and mid-thigh circumference, with males showing a larger response on both variables. In addition to this, males reported significantly less soreness than females following the exercise protocol.

Interestingly, with the exception of neutrophil elastase release, there were no differences in other markers of inflammation between men and women. Total elastase concentration, a marker of neutrophil activation, did not differ between genders. However, elastase release per neutrophil was significantly lower in females, which may be indicative of gender differences in the inflammatory response associated with EIMD.

**Study 3**

With the recognition that oestrogen could potentially reduce or inhibit the inflammatory response the third and final study investigated whether female skeletal muscle was more susceptible to exercise-induced muscle damage after a second bout of eccentric exercise, due to poor regeneration and repair following the initial bout. Males and females performed a bout of eccentrically biased exercise. Markers of EIMD included creatine kinase, soreness, isometric strength and isokinetic strength assessment. The procedure was then repeated two weeks later to determine if gender differences existed in terms of the repeated bout effect associated with EIMD. Only one variable showed a gender x time x bout interaction ($P<0.05$), that was the fatigue index. It was shown that following the initial bout of damage, males and females responded very differently, with female muscle being less fatiguable in the 48 h following damage compared to the males, but with both groups responding very similarly in the repeated bout. This may be due to differences in gender and their response to EIMD, or due to differences in fibre type between genders.

# CHAPTER 1

# INTRODUCTION AND REVIEW OF LITERATURE

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### 1.2 Model of muscle damage

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#### 1.2.2 Initial stimulus of damage

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## 1.8 Research implications

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## Section 1

### 1.1 Exercise-induced muscle damage

It is well documented that strenuous and repeated eccentric contractions are associated with exercise-induced muscle damage and delayed onset muscle soreness (DOMS) (Newham et al., 1983; Armstrong, 1984; Clarkson & Newham, 1994).This occurs in both recreational and elite athletes. With elite athletes, these responses are often related to relatively sudden increases in the volume or intensity of training, or following prolonged rest or injury (Pyne, 1994). For sedentary individuals, a single episode of exercise involving eccentric muscular contractions may produce significant muscle soreness and damage (Pyne, 1994).

The muscle's ability to resist force is approximately 30% greater during a maximal voluntary eccentric contraction than its ability to exert force during a concentric contraction (Wilmore & Costill, 1994). Although current research is inconclusive, several studies have advocated the importance of including the eccentric phase of muscle action, in addition to the concentric phase, to maximise gains in strength and size ( Hortobagyi et al., 1996; Lastayo et al., 1999). It is therefore considered to be an important inclusion in strength training programmes.

During eccentric contractions the length of the muscle increases whilst the muscle itself attempts to contract. Compared to concentric contractions, the mechanical strain per muscle fibre is higher, as fewer fibres are recruited (Enoka, 1996; Bär et al., 1997). This 'loading profile' places a high stress on the tissues involved and is most likely a primary factor of muscle damage (Enoka, 1996). During shortening contractions, work is done by the muscle, but during eccentric contraction, work is

done on the muscle by the external lengthening forces (Clarkson & Newham, 1994). As eccentric muscle actions occur frequently in everyday life and during sporting activities, exercise-induced muscle damage is a common experience to most individuals.

Eccentric contractions can cause severe morphological changes in the muscle fibres (McComas, 1996). According to the sliding filament theory, myosin cross-bridges make repeated connections with actin filaments throughout the duration of the active state of the muscle fibre. However, during eccentric contractions, instead of the actin filament being propelled toward the centre of the myosin filament they are pulled in opposite directions by the external forces acting on the muscle (McComas, 1996).

The injury can involve primary and secondary sarcolemmal disruption, swelling or disruption of the sarcotubular system, disruption of the myofibre contractile components, cytoskeletal damage and extracellular myofibre matrix abnormalities (Friden & Lieber, 1992). High-tension, eccentric contractions are thought to stretch or break the intermediate filaments between the z disks, additionally disrupting the double/intermediate filament z disk ring (Friden & Lieber, 1992).

The symptoms of exercise-induced muscle damage include: soreness (Talag, 1973; Howell et al., 1993; Clarkson & Newham, 1994); increase in volume of limb with injured muscle (Talag, 1973); increase in circumference of limb with injured muscle (Cleak & Eston, 1992a; Howell et al., 1993; Eston & Peters, 1999); decrease in resting limb angle (Talag, 1973; Cleak & Eston, 1992a; Howell et al., 1993; Eston & Peters, 1999); decrease in range of motion of the affected limb (Yackzan et al., 1984;

Cleak & Eston, 1992a; Clarkson & Newham, 1994); decrease in muscular strength (Talag, 1973; Clarkson & Newham, 1994; Eston et al., 1996); decrease in muscular power (Byrne & Eston, 2002 a,b) swelling and structural damage (Clarkson & Newham, 1994); leakage of myofibre proteins into the blood, the most commonly measured being creatine kinase (CK) (Jones et al., 1986; Clarkson et al., 1992; Clarkson & Newham, 1994; Child et al., 1998). However, the relationship between CK and muscle damage is poorly understood and disputed. For example, the temporal pattern of CK appears to vary depending upon the method of inducing the damage, the severity of the damage and the size of the muscle group involved. For example, peak CK concentrations have been shown to vary from 24 hours post exercise following a squatting protocol (Byrne & Eston, 2002b) to 4 days post exercise following an electrically stimulated eccentric elbow flexion protocol (Nosaka et al., 2002) to 7 days post exercise following an isokinetic leg exercise protocol (Byrne et al., 2001).

The most frequently reported symptom of muscle damage is delayed-onset muscle soreness. The soreness associated with this type of activity appears between eight and twenty-four hours following the damaging exercise and peaks between twenty-four and forty-eight hours later, but can remain for up to seven days (Cleak & Eston, 1992b; Howell et al., 1993; Nosaka & Clarkson, 1995).

The sensation of pain in skeletal muscle is transmitted by myelinated group III and unmyelinated group IV afferent fibres (Armstrong, 1984; Bär et al., 1997). The myelinated group III fibres are believed to transmit sharp pain, where as the unmyelinated transmit dull aching pain (Armstrong, 1984; Jones & Round, 1990),

which is more commonly associated with delayed-onset muscle soreness and muscle damage. The mechanism for this discomfort is poorly understood. It is believed that the pain afferents may be sensitised by chemicals released during the muscle damage and repair cycle (Armstrong, 1984; Bär et al., 1997), which include prostaglandin, bradykinin, serotonin, histamine and potassium (Armstrong, 1984; Bär et al., 1997). Alternative explanations include inflammation of the connective tissue, which may sensitise mechanoreceptors, responding when the muscle is stretched or pressed (Jones and Round, 1990). As explained by Jones and Round (1990) there is a sensation of mechanical stiffness which gives rise to the feeling that the muscle has become shorter. They observed that the stiffness and contracture which follows eccentric damage to the elbow flexors is electrically silent. This implies that the fibres themselves are not contracting, and some other structure must be responsible. It is expected that the likely explanation for this shortening is due to shortening of the connective tissue arranged in parallel with the muscle fibres (Jones & Round, 1990).

## 1.2 Model of muscle damage

An integrated model of muscle damage has been proposed by Armstrong (1990) which defines four stages: 1) initial events 2) autogenic processes 3) phagocytic stage and 4) regenerative phase. The processes involved in muscle damage shall be discussed further using this model as a basis. It should be made clear that while muscle damage can be divided up into these separate processes, they overlap enormously, and the exact nature of the muscle damage, the mechanisms responsible and processes involved are not fully understood. Before dividing the processes up according to the above model, reactive oxygen species (ROS) will be discussed separately, particularly as these may play a role in the process of muscle damage.

Furthermore, there are important hypothetical mechanisms for the role of oestrogen in preventing the potential destructive activities of these reactive species.

### 1.2.1 Free radicals and muscle damage

A common feature throughout the muscle damage and repair cycle is the production of free radicals. It is recognised that there are a number of potential sites for the production of free radicals (McArdle & Jackson, 1997), across all stages of the theoretical model.

Free radicals are molecules or molecule fragments containing an unpaired electron in their outer valence shell (Jenkins, 1988). The unpaired electron is usually extremely exchangeable, which is the chemical and physical reason for the reactivity of radical species (Karlsson, 1997). They have a potent oxidising effect which is the basis for its destructive effect against lipids, proteins, nucleic acid and the extracellular matrix (Niess et al., 1999).

McArdle and Jackson (1997) explained that free radicals can be generated through the mitochondrial electron transport system (Boveris & Chance, 1973), membrane bound oxidases (Crane et al., 1985) and infiltrating phagocytic cells (Font et al., 1977). It is known that inflammatory events involve the generation of free radicals via NADPH and myeloperoxidase (Hellsten et al., 1997; Tiidus, 1998). More recent evidence suggests that superoxide radicals are also of importance in neutrophil attraction and neutrophil adherence to the endothelium (Hellsten et al., 1997).

Free radicals can cause damage by lipid peroxidation of unsaturated fatty acids in the muscle membrane (Hellsten et al., 1997). They can also cause oxidative damage to

DNA and proteins (Halliwell & Gutteridge, 1985; McArdle & Jackson, 1997). It is suggested that as well as playing a role in direct tissue damage, the generation of reactive oxygen species (ROS) may also amplify the body's general inflammatory response and promote further cell injury, for example through up-regulation of pro-inflammatory cytokines (Best et al., 1999).

Oxygen radicals generated via the neutrophil respiratory burst are vital in clearing away muscle tissue that has been damaged by exercise and may also be responsible for propagation of further damage (Tiidus, 1998). There is abundant evidence for the involvement of neutrophil-generated ROS in the inflammatory response of tissues to various types of injury (Tiidus, 1998) and growing evidence of their involvement in post-exercise muscle inflammatory response and damage (Pyne, 1994; Eston et al., 1996b). However, the results from one recent study infer that the effect of neutrophil-generated ROS activity may not be a significant factor in the muscle damage and repair cycle, with neutropenic rats showing the same time course of muscle damage to non-neutropenic controls (Lowe et al., 1995).

Since eccentric exercise requires less oxygen consumption than equivalent concentric exercise (Lastayo et al., 1999), yet induces significantly greater damage, it is unlikely that oxygen free radicals are always the primary cause of exercise-induced muscle damage. Nevertheless, exercise-induced muscle damage, is characterised by neutrophil and macrophage infiltration into muscle and a subsequent inflammatory and repair process (Tiidus, 1998), which is promoted by free radical activity.

It is not possible to place the generation of free radicals within a certain phase of the muscle damage and repair cycle, as it appears to be a very important factor throughout and will be discussed further throughout this review.

### 1.2.2 Initial stimulus of damage

The initial events are thought to occur either via mechanical stress or metabolic stress (Armstrong, 1990; Byrd, 1992; Pyne, 1994). In terms of mechanical stress, high tension or a tension imbalance (Friden & Lieber, 1992; Armstrong et al., 1991; Lieber & Friden, 1993) (associated with eccentric contractions) can disrupt the sarcolemma (resulting in calcium entry), the sarcoplasmic reticulum (resulting in impaired calcium sequestration) and myofibrillar structures (Armstrong, 1990). Metabolic events include high temperature, lowered pH, insufficient mitochondrial respiration and oxygen free radical production (Armstrong, 1990). A number of paradigms, including rhythmic exercise and repeated eccentric muscle activity have related reactive oxygen species production to muscle inflammation and injury (Best et al., 1999).

Oxygen-free radicals are produced in tissue which is highly metabolically active. These substances can cause irreversible damage to many cellular constituents (Byrd, 1992). Oxidation of the sulfhydryl groups of the ATPase pump by free radicals, is highly related with a reduction in the rate of $Ca^{2+}$ uptake by the sarcoplasmic reticulum (Byrd, 1992) resulting in a loss of $Ca^{2+}$ homeostasis.

A reduction in local ATP and/or a reduction in the free energy from hydrolysis of ATP due to increased ADP could reduce the rate of ATP splitting and slow $Ca^{2+}$ pumping by the sarcoplasmic reticulum pump (Byrd, 1992).

The increase in hydrogen ions (decrease in pH) which occurs during strenuous exercise, has a profound effect on the ability of the sarcoplasmic reticulum to take up $Ca^{2+}$. This has been attributed to the $H^+$ and $Ca^{2+}$ ions competing for the $Ca^{2+}$ binding site on the ATPase pump (Byrd, 1992).

Temperature increases to above 38°C have also been shown to uncouple the $Ca^{2+}$ stimulated ATPase activity from $Ca^{2+}$ transport by the sarcoplasmic reticulum (Byrd, 1992). High temperatures, similar to those obtained during fatiguing exercise, may alter the fluidity of the lipid membrane surrounding the ATPase pump and somehow impair its ability to sequester $Ca^{2+}$ (Byrd, 1992).

Metabolic and mechanical stress from exercise may occur separately or simultaneously. The contribution to muscle damage depends on the exact nature of the physical activity undertaken (Pyne, 1994), that is, whether it is eccentric in nature and involving a relatively low metabolic demand, or a sustained highly metabolic activity.

### 1.2.3 Autogenic processes

The common factor that emerges in the initial phase of exercise-induced muscle damage is the loss of $Ca^{2+}$ homeostasis. Regardless of the initiating stimulus, it

would appear that there follows a rapid activation of autogenic destructive processes that originate in the muscle fibres (Armstrong, 1990).

### 1.2.3.1 Role of calcium

The mechanisms underlying this phase of the injury and repair process are not known, although the loss of intracellular $Ca^{2+}$ homeostasis could play a primary role (Armstrong, 1990). Empirical evidence supports the theory that $Ca^{2+}$ release from the sarcoplasmic reticulum is an important factor in exercise-induced muscle damage.

Experimental work has demonstrated that an increase in intracellular calcium content causes damage to the myofilaments of skeletal muscle (Duncan, 1978; McArdle & Jackson, 1997). Studies which have inhibited the flux of $Ca^{2+}$ across the sarcoplasmic reticulum, following an exercise protocol in rats, have demonstrated a decrease in damage (Amelink et al., 1990; Byrd, 1992). It has also been postulated that elevated $Ca^{2+}$ appears to cause a release of muscle enzymes through activation of phospholipase $A_2$, which in turn may induce injury to sarcolemma through production of leukotrienes and prostaglandins through free oxygen radical formation and/or through release of detergent-like lysophospholipids (Armstrong, 1990). This in turn, will affect the fluidity of the membrane resulting in a "leaky" membrane, loss of intracellular enzymes and an efflux of lysosomal enzymes (Jenkins, 1988). It is also believed that $Ca^{2+}$ stimulates proteases (calpains) that are thought to act directly on the proteins in cell membranes, and proteases that act specifically on the z lines (Byrd, 1992; Clarkson & Sayers, 1999).

Low $Ca^{2+}$ is necessary for cell function, whereas high $Ca^{2+}$ has long been associated with cell dysfunction and cell death. The sudden increase in $Ca^{2+}$ is regarded as an important step in the cascade of events that result in cellular damage following exercise (Byrd, 1992). Calcium overload results in ultrastructural changes in the muscle cell, including swollen and disrupted mitochondria, dilated t-tubules and sarcoplasmic reticulum, general cellular oedema, and disruption of the myofilaments (Byrd, 1992).

Processes proposed to explain how muscle could be damaged following an elevation of intramuscular calcium content, include: stimulation of calcium-activated proteases; activation of lysosomal proteases; mitochondrial overload; and activation of lipolytic enzymes. The two most important processes appear to be the activation of lipolytic enzymes and calcium-activated proteases, calpain (McArdle & Jackson, 1997). A calpain hypothesis was proposed by Belcastro et al. (1998), who provided evidence for the importance of this protease in the muscle damage and repair cycle.

In addition to the cascade of autogenic processes that follow a loss of $Ca^{2+}$ homeostasis, elevated $Ca^{2+}$ has also been associated with a disruption of the excitation-contraction coupling process (Ingalls et al., 1998). This in turn has been related to the reduction of maximal isometric titanic force associated with eccentric exercise (Ingalls et al., 1998).

In summary, the loss of calcium homeostasis following the mechanical/metabolic insult may activate phospholipases and proteases. The free fatty acids liberated will

in turn have a detergent effect on the cell membrane and may be vulnerable to free radical attack.

The processes that follow the initial events can eventually lead to complete repair of the damaged muscle but rely upon the activities of non-muscle cells (Tidball, 1995). A rise in free cytolsolic calcium may also be related, independently to the activation of the respiratory burst in phagocytic cells (Pyne, 1994). This phenomena suggests links between the mechanisms involved in the early stages of exercise-induced tissue damage and the activation of cells involved in non-specific immune responses (Pyne, 1994).

### 1.2.3.2 Calpain

While loss of $Ca^{2+}$ homeostasis has been suggested as a primary factor in producing muscle damage, Belcastro et al. (1998) proposed a calpain hypothesis of exercise-induced muscle damage. As explained previously, it is believed that $Ca^{2+}$ stimulates proteases, such as calpain, which directly act upon proteins within the muscle. Belcastro et al. (1998) reported that this non-lysosomal protease contributes to the initiation of immediate protein degradation whereas lysosomal proteases from extracellular sources (monocytes and macrophages) play a primary role in protein turnover several days after exercise.

The isoenzymes of calpain are typically localised throughout the muscle cell in the I and Z band regions (Belcastro et al., 1998). It is hypothesised that when calpain is activated, selective proteolysis of various contractile, metabolic and structural elements occurs. It is also believed that calpain or the resultant peptide fragments

may be associated with the neutrophil chemotaxis reported to occur during or immediately following exercise (Belcastro et al., 1998), thus, aiding the inflammatory response and repair.

### 1.2.4 Inflammatory and immune response to muscle damage

Tissue damage and infection both initiate a coordinated sequence of events that are collectively known as the acute phase response (Evans & Cannon, 1991). These events initially facilitate antibacterial and anti-viral responses before promoting the clearance of debris and tissue fragments. This leads into the regenerative phase with growth and repair of tissues and restoration of normal function (Pyne, 1994).

Inflammation is characterised by the movement of fluid, plasma proteins and leukocytes into the tissue in response to injuries, infections or antigens (MacIntyre et al., 1995). Signalling occurs between the injured muscle cells and the mononucleated cells that subsequently appear at the site of injury (Tidball, 1995). At least two cell populations respond to muscle injury; inflammatory cells involved in the removal of cellular debris and myogenic cells involved in replacement of the damaged muscle (Tidball, 1995). Infiltration of these cells into the muscle is orchestrated by specific cytokines (Pyne, 1994).

#### 1.2.4.1 Cytokines

Cytokines are small polypeptides that are considered to be an important link between the immunological and neuroendocrinal systems involved in inflammation, fever, chemotaxis, the acute phase response and tumour regression (Pyne, 1994; MacIntyre et al., 1995). Host defense cytokines are produced by circulating and tissue resident

leukocytes as well as other cells (Bagby et al. 1996). It is believed that a small group of cytokines, including interleukin 1 (IL-1), interferon, IL-2, IL-6 and tumour necrosis factor α (TNFα), are the principle mediators of inflammation (Imura et al., 1991). Interleukin-1 is expected to have broad and important influences in muscle inflammation, as well as possible roles in stimulating protease synthesis (Tidball, 1995). TNFα and Il-1 have overlapping mechanisms within the body and have been shown to increase leukocyte adhesion, priming leukocyte function and causing macrophage activation (MacIntyre et al., 1995). In addition, IL-1 is believed to induce the expression of many other cytokines including IL-2, IL-3, IL-6, TNFα and interferons (Tidball, 1995). Exercise and muscle injury have been shown to increase the concentration of IL-1 in serum and muscle, which is expected to play a substantial role in promoting muscle inflammation (Tidball, 1995). To stimulate the activity of antigen specific host defenses, these cytokines regulate the growth, differentiation and functional activities of T and B lymphocytes (Pyne, 1994).

### 1.2.4.2 Leukocytes

Leukocytes, primarily neutrophils and monocytes/macrophages are thought to perform a wide range of functions during the inflammatory response associated with muscle damage. It is generally believed, although still not thoroughly understood, that these cells perform three functions within the muscle damage and repair cycle (Tidball, 1995; Clarkson & Sayers, 1999). Attack and breakdown of debris (neutrophils and macrophages), removal of cellular debris (macrophages) and regeneration of cells (macrophages).

Leukocytes are attracted to injured muscle cell, via various chemotactic factors, possibly including resident leukocytes (Tidball, 1995), calpain or peptide fragments (Belcastro et al., 1998), and cytokines (Pyne, 1994; Neiss et al., 1999). To enter the inflamed tissue, leukocytes bind to specific adhesion molecules of endothelial cells that line blood vessel walls (St Pierre Schneider et al., 1999).

The neutrophil is one of the first cells to arrive at sites of injury and infection, where it releases a number of chemoattractants to amplify the response by recruiting additional neutrophils and mononuclear cells. Neutrophils generate superoxide and other reactive oxygen species via a respiratory burst, which is catalysed by the enzyme NADPH oxidase, located in the plasma membrane (Pyne, 1994).

It has been suggested that the neutrophil is programmed for overkill not caution (McCord, 1995). It has little intrinsic ability to distinguish between foreign and host antigens, thus destroying healthy as well as damaged cell and debris (Pyne, 1994). Macrophages, like neutrophils are capable of producing oxygen free radicals (MacIntyre et al., 1995). Macrophages also give rise to cytokines, which in turn may exacerbate damage by potentiating cytotoxic mechanisms of other inflammatory cells to enhance free radical production and enzyme releases (Evans & Cannon, 1991).

Following degradation processes, some macrophages may play a role in muscle repair (Tidball, 1995). Two populations have been observed within animal muscle (Tidball, 1995: Clarkson & Sayers, 1999), ED1+ cells act as phagocytes and ED2+ cells regulate the consequent repair process (Clarkson & Sayers, 1999).

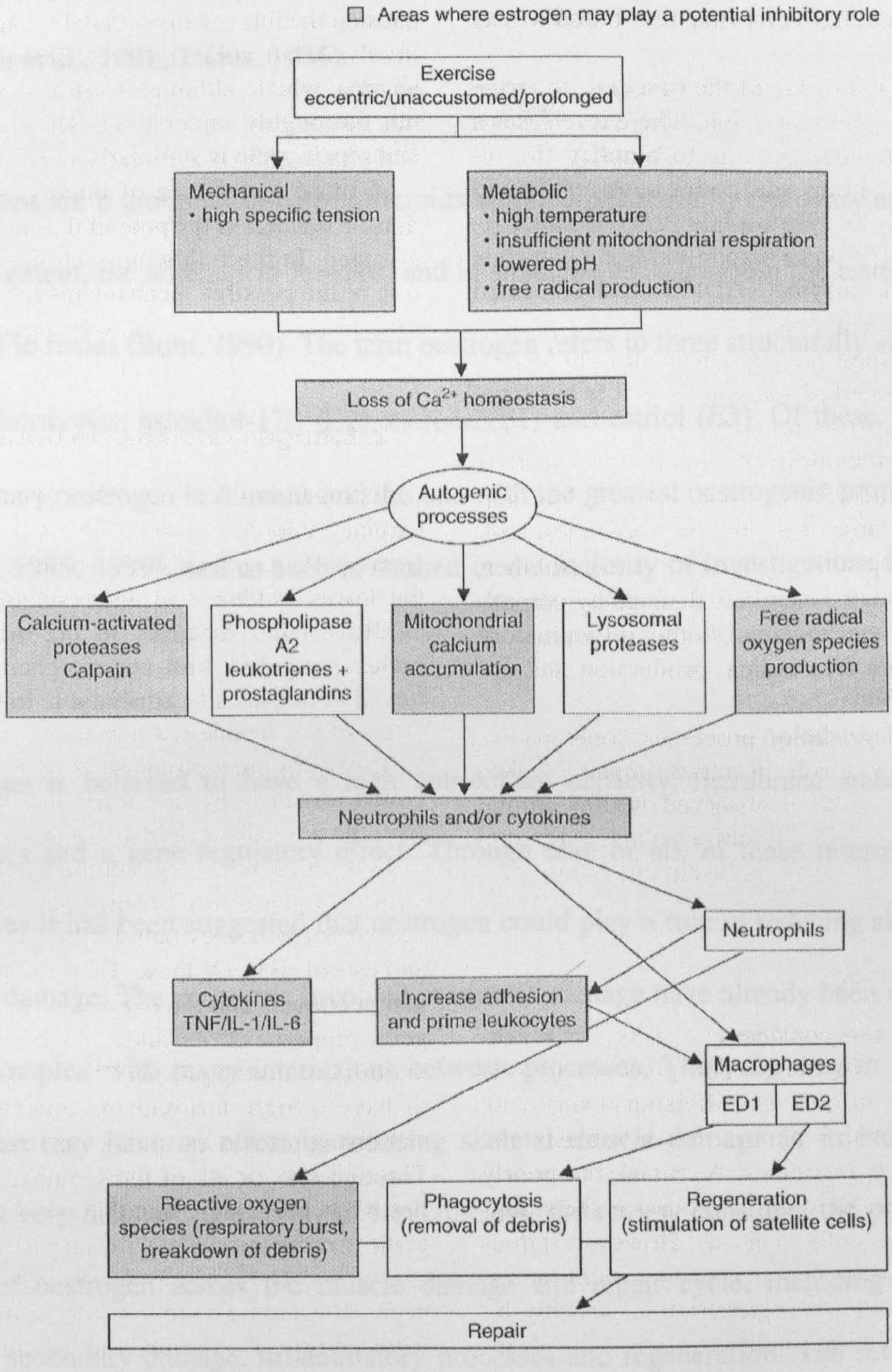
### 1.2.5 Regeneration

Muscles possess considerable powers of regeneration. During the phagocytic phase of muscle damage there is an associated division of surviving satellite cells, which mature into myoblasts and fuse to form new myotubes. A crucial, but poorly understood stage during this process is the stimulation of satellite cells to divide. However, it does appear that invasion by macrophages seems an essential prerequisite for regeneration, possibly by somehow stimulating satellite cell division (Jones & Round, 1990). Indeed, it is strongly suggested that macrophage infiltration is an important part of the regeneration phase particularly in terms of satellite cell proliferation (Cantini & Carraro, 1995; Merly et al., 1999; Lescaudron et al., 1999).

A brief review of the muscle damage and repair cycle has been presented. This has, by no way, exhausted the information available on the processes involved, but does give a general introduction to an area, which although well investigated, is still not thoroughly understood. The muscle damage and repair cycle is summarised in Figure1.1, with the inclusion of areas where oestrogen may play a role.

A more recent area of interest with regard to muscle damage is the potential protective effect of oestrogen. There follows an explanation of the possible mechanisms for the protective role of oestrogen in the muscle damage and repair cycle.

**Figure 1.1:** Illustration of a simple model of the muscle damage and repair cycle.




## 1.3 Oestrogen and muscle damage

Oestrogen has an apparent protective effect on cardiac, smooth and possibly skeletal muscle in terms of damage and inflammation. For example, the lower incidence of atherosclerosis and other cardiovascular diseases in pre-menopausal females when compared to age-matched males is believed to be partially attributable to the

protective effect of the female sex hormone oestrogen (Stumpf et al. 1977; Bush et al., 1987; Gruchow et al., 1988; Barret-Conner & Bush, 1991; Chisholm, 1991; Stampfer et al., 1991; Tiidus, 1995).

Oestrogens are a group of 18-carbon steroids secreted primarily by the ovary and, to a lesser extent, the adrenals in females, and in smaller quantities from the testes and adrenals in males (Bunt, 1990). The term oestrogen refers to three structurally similar steroid hormones, estradiol-17β (E2), estrone (E1) and estriol (E3). Of these, E2 is the primary oestrogen in humans and the one with the greatest oestrogenic properties (Tiidus, 1995; 1999), and as such is studied in the majority of investigations (Bunt, 1990).

Oestrogen is believed to have a high antioxidant capacity, membrane stabilising properties and a gene regulatory effect. Through one, or all, of these interrelating properties it has been suggested that oestrogen could play a role in reducing skeletal muscle damage. The processes involved in muscle damage have already been shown to be complex with many interactions between processes. Thus, the way in which oestrogen may have an effect in reducing skeletal muscle damage (if in indeed it does) is very difficult to determine. Nevertheless, the review considers the possible effect of oestrogen across the muscle damage and repair cycle, including initial events, secondary damage, inflammatory processes and regeneration. The review is presented within subsections but the interaction between processes and thus subsections should not be forgotten.

### 1.3.1 Oestrogen as an antioxidant

An antioxidant is a molecule with a relatively strong reductant property to quench/scavenge/neutralise the unpaired electron from free radical species (Karlsson, 1997). A common denominator for these compounds is that their molecular structure is based on a carbon-ring structure, which originates from phenol species (Karlsson, 1997). Phenol species have one or more hydroxyl groups, which have a unique property to reduce electrons (Karlsson, 1997).

Lipid peroxidation is a free radical mediated chain reaction, which can be initiated by the hydroxyl radical attacking polyunsaturated fatty acids in membranes which results in oxidative damage (Wiseman & O'Reilly, 1997) and ultimately affects membrane stability (Tiidus, 1995). It has been demonstrated *in vitro* and *in vivo* in both rat and human investigations, that oestrogen (at physiological and supraphysiological concentrations) possesses a potent antioxidant characteristic (Yagi & Komura, 1986; Sugiola et al., 1987; Huber et al., 1990; Mooradian, 1993; Subbiah et al., 1993; Tiidus, 1995; Ayres et al., 1996; Ayres et al., 1998; Ruehlmann & Mann, 1997; Bär & Amelink, 1997; Wiseman & O'Reilly, 1997), although the mechanisms by which oestrogen acts as an antioxidant have not been fully determined. Oestrogens do possess a hydroxyl group on their A (phenolic) ring, in the same configuration and position as vitamin E (known to possesses a strong antioxidant capacity) (Ayres et al., 1998; Tiidus, 1999; Persky et al., 2000) and similar to thyroxine (Sugioka et al., 1987), which also possesses potent antioxidant activity. Oestrogen may donate hydrogen atoms from their phenolic hydroxyl group, thus terminating peroxidation chain reactions, in a way similar to Vitamin E (Sugioka et al., 1987; Tiidus, 1995; Ayres et al., 1998; Tiidus, 1999; Persky et al., 2000).

An increase in oxygen radical production results in a decrease in vitamin E concentrations as a result of the above reaction (Tiidus, 1999). However, this has only been shown to occur in studies with male and sexually immature female rats (Bowles et al., 1991; Sen et al., 1997), in which oestrogen levels are obviously low. Sexually mature female rats (high oestrogen) are not affected in the same manner, i.e. vitamin E levels are maintained (Tiidus & Houston, 1993). These results suggest that oestrogen may offer an additional line of defence against oxygen free radicals and may render skeletal muscle less susceptible to exercise-induced oxidative damage (Tiidus, 1999).

### 1.3.2 Oestrogen and membrane stabilisation

Due to its figuration and antioxidant capacity, oestrogen is believed to have membrane stabilising characteristics. It has been suggested that oestrogen may protect membranes from peroxidative damage by decreasing membrane fluidity and increasing membrane stability in ways similar to cholesterol (Wiseman et al., 1993; Wiseman & Quinn, 1994). Oestrogen is a fat-soluble hormone and this type of stabilisation involves an interaction between membrane phospholipids and oestrogen in ways similar to the stabilising mechanisms of vitamin E and cholesterol (Wiseman et al., 1993; Tiidus, 1995; Persky et al., 2000). As steroid hormones are lipophilic (Whiting et al., 2000), they intercalate into the bilayer of the cell plasma membrane, potentially altering the fluidity and function of the membrane.

The ability to decrease membrane fluidity has been demonstrated for E2 and related compounds (Wiseman & O'Reilly, 1997). Wiseman and Quinn (1994) demonstrated

a positive association between decreased membrane fluidity and antioxidant ability. They stated that this ability, by oestrogen in particular, to decrease membrane fluidity is a mechanism of their antioxidant action, which results in stabilisation of the membrane against peroxidation.

### 1.3.3 Oestrogen and gene regulation

Pro-inflammatory cytokines, for example IL-6 and TNFα have been shown to increase during the muscle damage and repair cycle (Esperson et al., 1990; Fielding et al., 1993; Bruunsgaard et al., 1997; Hellsten et al., 1997; Pederson et al., 1997; Suzuki et al. 1999; Smith et al., 2000). Nuclear factor kappa B is known to govern gene expression involving various cytokines and cell adhesion molecules (Montgomery et al., 1991; Degitz et al., 1991; Iadermarco et al., 1992; Yoshikawa & Yoshida, 2000) and it has been shown that vitamin E inhibits the activation of this factor (Yoshikawa & Yoshida, 2000). Yoshikawa and Yoshida (2000) demonstrated that vitamin E can prevent leukocyte-endothelial cell adhesion by inhibiting signal transduction. They conclude that vitamin E may have a protective effect against the progression of inflammation. Research suggests that it could be the antioxidant properties of vitamin E which leads to this gene regulatory effect (Yoshikawa & Yoshida, 2000; Sen, 2001; Sen & Roy, 2001). Caulin-Glaser et al. (1996) explained that oestrogen has been shown to have important gene regulatory effects (Beato, 1989; Gaub et al., 1990; Shymala & Guiot, 1992), which again could be explained through its strong antioxidant capacity. Therefore, oestrogen, could affect the expression of adhesion molecules and possibly allay further damage (reduce infiltration of cells such as neutrophils) but in doing so, inhibit inflammatory and repair processes.

It would appear that oestrogen may have a capacity to reduce muscle damage, based on the above three interacting processes. This possible protective role of oestrogen and the subsequent effect upon the muscle damage and repair cycle is presented in Figure 1.2. There follows a review of the research in this area and discussion of how the interacting processes outlined above may account for recent observations in the literature.

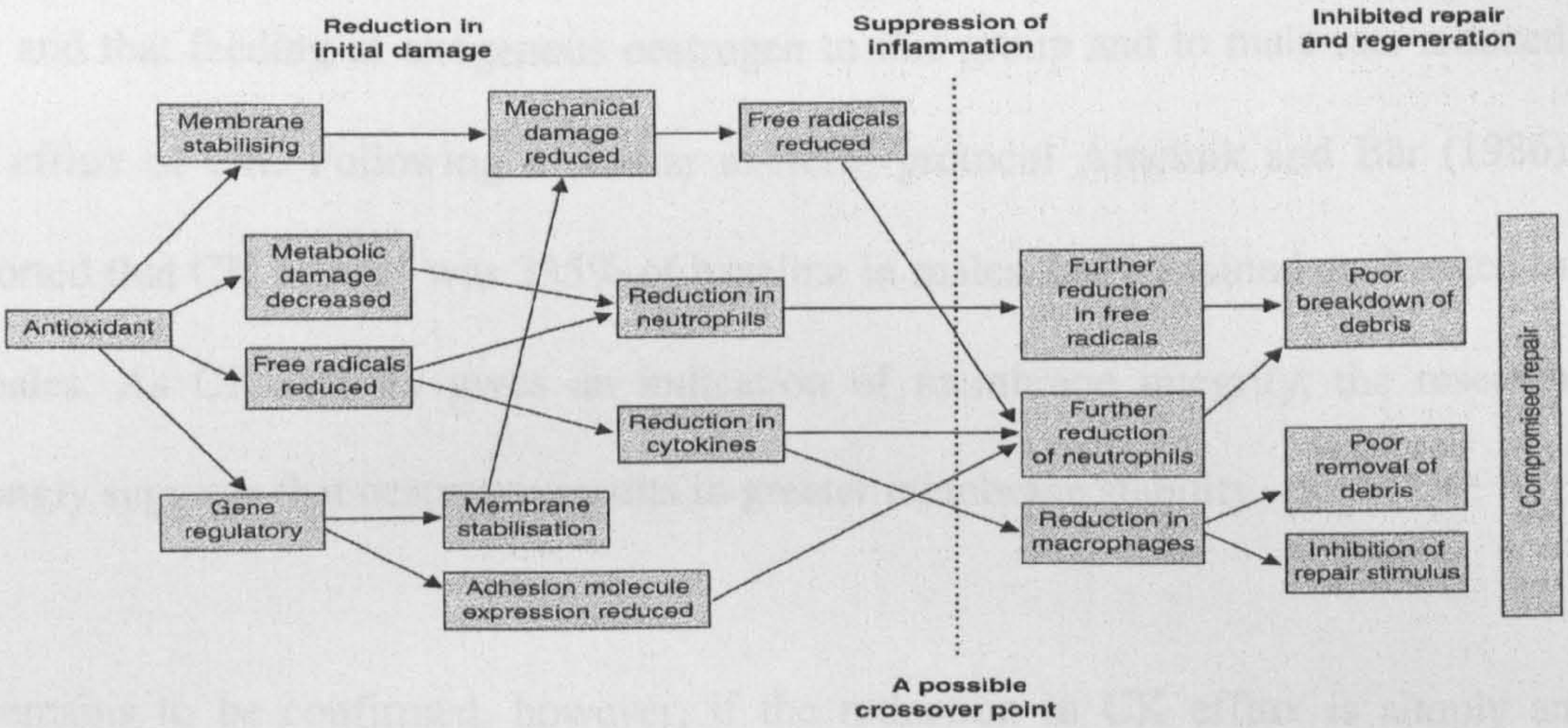



**Figure 1.2:** Summary of how the interacting properties of oestrogen potentially play a role in the muscle damage and repair cycle.
To date it is not known whether oestrogen-mediated inhibition of inflammatory processes would compromise repair. The potential effect of oestrogen during the early stages of the muscle damage and repair cycle may reduce injury sufficiently that an inflammatory cascade would not be initiated. Alternatively, the inflammatory response may be required to allow the muscle to regenerate properly. To the right of the possible crossover point indicated in the figure, inhibition by oestrogen may potentially have a negative effect.

**Section 2**

## 1.4 Effect of oestrogen on creatine kinase activity

Creatine kinase (CK) is a commonly measured marker of muscle damage, although it's inter-subject variability has been criticised (Warren et al., 1999). Despite this,

there has been shown to be a large difference in CK activity between males and females, which has generally been attributed to the effect of oestrogen.

Women have been shown to have lower CK activity at rest (Meltzer, 1971) and show less CK efflux after bicycle exercise (Shumate et al., 1978) or long distance running (Berg & Keul, 1981; Rogers et al., 1985). In addition, Bär et al. (1988) investigated the effects of 2 hours running in rats on CK activity. They observed that ovariectomised females (source of oestrogen removed) showed similar levels to male rats and that feeding of exogenous oestrogen to this group and to male rats reduced the efflux of CK. Following a similar exercise protocol Amelink and Bär (1986) reported that CK activity was 335% of baseline in males, but remained unchanged in females. As CK activity gives an indication of membrane integrity, the research strongly suggests that oestrogen results in greater membrane stability.

It remains to be confirmed, however, if the reduction in CK efflux is simply an indication of increased membrane stability or whether the muscles are in fact receiving less damage. Research in this area has been minimal and inconclusive. Although there is agreement regarding the CK response, the actual protection in terms of the etiology of muscle damage is undetermined. These findings have led to more recent and in-depth investigations into muscle damage and oestrogen.

## 1.5 Histopathological studies

Van der Meulen et al. (1991) and Dumke (1996) measured histological damage and enzyme release following a treadmill protocol in rats. Van der Meulen et al. (1991) found that gender differences in enzyme release after exercise did not reflect

differences in the amount of histological damage (assessed by multiple central nuclei, hyaline aspect, multiple vacuoles, and infiltration by inflammatory cells). Dumke (1996) observed that there were significant differences between a placebo treated and an E2 treated group of ovariectomised females in CK activity (reduced CK activity in E2 treated group), but no significant differences in histological damage. This suggests that oestrogen offers no real protection against damage, but simply helps maintain a more stable membrane. These procedures assume a similar time course for males and females and only measure histological damage once, not following damage fully. It would therefore be difficult to determine if muscle damage was indeed the same in the two groups observed.

A direct contradiction of the findings by Van der Meulen et al. (1991) and Dumke (1996) are those by Reijneveld et al. (1994). A marked attenuation of the CK response, as found in many of the studies in this area (Shumate et al., 1978; Amelink & Bär, 1986; Bär et al., 1988), was concurrent with a decrease in the amount of histological damage. Clearly this area requires further investigation.

Warren et al. (1999) reported that only functional measures could give a true indication of the extent of muscle damage. They indicated that in both human and animal studies, the histopathology of muscle fibres following eccentric exercise correlated poorly with functional measurements, both in terms of magnitude and the time course of the impairment. Furthermore, the histological method of assessing muscle damage has been criticised because specimens obtained from a muscle biopsy represent only a small fraction of the involved muscle. They therefore strongly

recommended that measures that indicated changes in function should be incorporated to assess and follow muscle damage.

## 1.6 The effect of oestrogen on indirect and functional measures of muscle damage

Buckley-Bleiler et al. (1989) investigated the response of women at different ages to exercise-induced muscle damage. Comparisons were made between pre-pubescent, eumennorheic and post-menopausal females. They found no significant differences between these groups in measures of CK, muscle soreness and maximal isometric strength. The results should be interpreted with caution. The method of inducing muscle damage was 40 maximal isometric contractions, which may explain why the reductions in strength and in CK were small. As explained previously, activities that contain eccentric contractions produce more muscle damage and soreness, than other types of contraction. Therefore, this type of exercise protocol was possibly not sufficient to induce significant levels of damage to determine if differences existed in the age groups.

Thompson et al. (1997) investigated the effects of regularly ingesting oestrogen, in the form of oral contraceptives (at least 30 mg ethinyl estradiol for more than 6 months) on post-exercise muscle damage of lower extremities especially the quadriceps, following a bench stepping regimen. The only criterion measure that showed a group by time interaction was soreness, with oral contraceptive users reporting less soreness than the non-users. The authors concluded that oestrogen was ineffective at attenuating muscle damage. However, as the only measures of damage that showed significant differences from baseline were soreness and range of motion,

it is therefore possible that the damage-inducing protocol was not sufficient to elicit significant differences in other factors between the two groups. In addition, the sample size in the two groups was small (control=6, OC group= 7), which again makes any strong conclusions difficult. Nevertheless an interesting observation from this study was the differences between the groups in perceived soreness.

Rinard et al. (2000) investigated the effects of eccentric exercise of the elbow flexors on a large sample of adult males (n=82) and females (n=83). There were no differences between males and females on relative changes in isometric strength and soreness, although women showed the greatest loss in range of motion, as measured by relaxed arm angle. With such a large sample size it would appear that oestrogen has no protective effect on muscle damage. However it should be noted that subjects were selected based on their soreness response, thus making generalisations from the study very difficult.

### 1.7 Oestrogen, the time course of muscle damage and the immune response

In general, the studies reviewed so far have been concerned with the initial effects of muscle damaging exercise. They do not consider the damage over its full time course. In addition, there has been no attempt to consider possible differences in immune response to the insult of damaging exercise between males and females. As explained previously, after the initial mechanical/metabolic damage to the muscle, there follows a multitude of events resulting in secondary damage and eventual repair. Infiltration of the muscles by neutrophils and macrophages may not occur until 24 to 96 hours after the exercise and may persist for over a week (Tiidus & Bombardier, 1999). This is an area that has recently received greater attention, and

has enhanced our understanding of the gender differences in muscle damage and repair.

### 1.7.1 Oestrogen and the general inflammatory response

Komulainen et al. (1999) investigated the time course of changes of structures within the muscle following downhill running. The results demonstrated that the general histopathological changes in both sexes were essentially similar, but that these occurred more slowly (myofibre swelling) and to a lesser extent (necrosis and macrophagy invasion) in females than in males.

Tiidus and Bombardier (1999) hypothesised that as oestrogen may be responsible for differences in gender-related susceptibility of muscle to exercise-induced muscle damage, and may act as an antioxidant, it would be of interest to determine the effects of oestrogen administration on phagocytic cell infiltration of the muscle following exercise. They hypothesised that diminished neutrophil and macrophage infiltration may ultimately reduce the time-course and severity of the inflammatory response of the muscle after exercise and possibly enhance the healing process. Conversely, it could have a negative effect by hindering repair. Tiidus and Bombardier (1999) measured post-exercise tissue myeloperoxidase activity in male and female rats which were treated (40μg/kgBW) and untreated with oestrogen. Their results suggested that oestrogen might significantly affect post-exercise leukocyte infiltration into skeletal muscle. The mechanism by which this may have occurred is difficult to determine, as the control of infiltration by neutrophils and macrophages is complex. Factors affecting these processes include calcium homeostasis and calpain production, cytokines, oxygen free radical activity, and

prostaglandin E2 (Cannon et al., 1991; Belcastro et al., 1998; Tiidus & Bombardier, 1999), to name but a few, with interactions amongst these given factors. This and the possible effect that oestrogen has on some of these factors will be discussed later.

Stupka et al. (2000) reported that the possible difference in the extent of muscle damage between males and females, is due to differences in the inflammatory response and not due to differences in sarcomere damage. They demonstrated that following an eccentric protocol women showed less muscle inflammation compared to men despite the same amount of z-line streaming. This has implications when considering regeneration and in fact the female muscle may be compromised in terms of regeneration, despite suffering a similar amount of initial damage.

St Pierre Schneider et al. (1999) investigated the time course and concentration of leukocyte invasion in injured soleus muscles of male and female mice, to determine if any sex differences existed. Leukocyte invasion began one day after injury in both sexes and diminished on day five in males, but remained evident after day seven in females. During the period of maximal leukocyte invasion at 1 day post-injury, muscle sections from males contained more fibres invaded by acid phosphatase-positive cells than muscle sections from females. They suggested that oestrogen prevented elevated macrophage concentrations in blood vessels by limiting the availability of endothelial cell adhesion molecules. This suggests that oestrogen could reduce macrophage or other leukocyte emigration into inflamed tissue, because fewer endothelial cell adhesion molecules result in an inability of leukocytes to move out of blood vessels and into the area of inflammation. They therefore postulated that removal of damaged myofibres is slower in the female mice.

A direct contradiction to these studies are the findings from MacIntyre et al. (2000), who observed a greater presence of neutrophils in muscles of women four hours after exercise compared to men. They concluded that this was probably due to increased adherence and migration of neutrophils into the muscle. As exercise workload was normalised to body mass and was not significantly different between the two groups, the results suggest that oestrogen may be the most likely factor affecting the infiltration of the inflammatory cells into the muscle.

The observation of differences in leukocyte concentration or time course of infiltration between males and females does not provide substantive evidence that the damage-repair process is mediated by oestrogen concentration. As previously explained, many complex events occur which result in migration and infiltration of these cells and will be discussed further.

### 1.7.2 Oestrogen and specific events associated with inflammation

#### 1.7.2.1 Calcium

As described earlier, loss of $Ca^{2+}$ homeostasis is believed to be a major factor resulting in the cascade of autogenic and inflammatory processes in muscle degradation. Prakash et al. (1999) investigated the effect that oestrogen had on smooth muscle cells. They observed that oestrogen had an inhibitory effect on $Ca^{2+}$ influx, and also enhanced $Ca^{2+}$ efflux via a receptor mediated mechanism. It is expected that this would reduce the $Ca^{2+}$ overload, and therefore suppress the cascade of events which can result in more severe muscle damage. If the same is true

for skeletal muscle, this could be an important process by which oestrogen may affect the inflammatory response to exercise-induced muscle damage.

Jovanovic et al. (2000) demonstrated that physiological levels of E2 provided resistance to female, but not male cardiomyocytes against intracellular $Ca^{2+}$ loading. As in skeletal muscle cells, $Ca^{2+}$ loading in cardiac muscle leads to cell injury. It is possible therefore that the protection conferred by oestrogen in cardiac muscle may also be found within skeletal muscle, preventing, or certainly dampening the secondary muscle damage processes. This however requires empirical verification with skeletal muscle cells.

A recent investigation by Tiidus et al. (2001) seems to suggest that oestrogen does have the potential to reduce $Ca^{2+}$ overload within skeletal muscle following an exercise protocol. In the calpain hypothesis proposed by Belcastro (1998) a very important role is suggested for this $Ca^{2+}$ sensitive protease, including chemotaxis of leukocytes (see section 1). The investigation by Tiidus et al. (2001) demonstrated that following an exercise protocol, ovariectomised rats treated with oestrogen, not only showed significant attenuation of 1 hr post exercise neutrophil number and MPO activity, but also reduced calpain-like activity, compared to placebo treated ovariectomised rats. They suggested that oestrogen supplementation increased post-exercise muscle sarcolemma stability, possibly preventing $Ca^{2+}$ influx into skeletal muscle and therefore preventing the activation of calpain. This would consequently lead to diminished calpain-induced proteolysis and neutrophil chemotaxis.

Although the above study was limited to 1hr post exercise observations only, their findings certainly suggest an important role for oestrogen within this specific ($Ca^{2+}$and calpain) process of the muscle damage and repair cycle.

### 1.7.2.2 Adhesion molecules

To enter the inflamed tissue, leukocytes bind to specific adhesion molecules of endothelial cells that line blood vessel walls (St Pierre Schneider et al., 1999). As described earlier, St Pierre Schneider et al. (1999) attributed the reduced leukocyte infiltration in injured soleus muscle to the inhibitory effect of oestrogen on adhesion molecules.

Previous research has shown E2 can inhibit cytokine-mediated endothelial cell adhesion molecule activation, thereby reducing leukocyte chemotaxis and adhesion (Caulin-Glaser et al., 1996). However, Cid et al. (1994) reported that estradiol treatment of cultured human umbilical vein endothelial cells stimulated up to a twofold increase in TNFα-induced adhesion of leukocytes.

This is a very complex area. In general, it has been found that women of childbearing age are more susceptible to autoimmune diseases (involving an up-regulation of immune response) (Chao et al., 1995; Rider et al., 1998; Ahmed et al., 1999; Tornwall et al., 1999) and this has been suggested to be due to oestrogen. This could in some way explain the findings of Cid et al. (1994) who explained that their results demonstrate that oestradiol has important regulatory functions in promoting leukocyte-endothelial cell interactions. It is unclear why Caulin-Glaser et al. (1996) and St Pierre Schneider et al. (1999) observed a different relationship. To elucidate

further on the potential effects of oestrogen on the specific aspect of muscle damage and the inflammatory response to muscle damage, further research on skeletal muscle is warranted.

Cytokines are important factors in inducing the expression of endothelial cell molecules (Marui et al., 1993). The possible effect that oestrogen has upon cytokines is discussed below. Another important consideration in relation to the influence of oestrogen, is the role that oxidative stress may play in regulating adhesion molecule gene expression. Marui et al. (1993) tested the hypothesis that cytokines selectively induced adhesion molecule gene regulation through an antioxidant sensitive pathway. The findings of their study suggested that regulation of some adhesion molecules are reduced in the presence of a potent antioxidant. As oestrogen has been demonstrated to have strong antioxidant capacity, it is feasible that it could also reduce expression of these molecules through a similar pathway.

### 1.7.2.3 Cytokines

Cytokines are very important in orchestrating inflammatory processes. Thus, any factor that can regulate cytokines will also play an important role in the inflammatory response. As previously explained, there is an increased incidence of autoimmune diseases in pre-menopausal females. Zuckerman et al. (1996) explained that the effect of oestrogen on autoimmunity and inflammation may involve changes in secretion of inflammatory mediators, e.g. cytokines. They showed that treatment of mice with pharmacological doses (0.2-2μg/kg) of estriol resulted in a significant increase in serum TNFα levels and rapid elevation in serum IL-6 levels following challenge.

Both Schwarz et al. (2000) and Angstwurm et al. (1997) investigated the influence of menstrual cycle status upon cytokine levels. Angstwurm et al. (1997) found that during the follicular phase, the increase of E2 was accompanied by an increase in IL-6 ($p=0.07$, $r=0.35$). Following ovulation, when progesterone rose there was a 1.5-4.4 fold drop in plasma IL-6. Schwarz et al. (2000) demonstrated that in male participants no differences existed in cytokine response between initial samples and those taken one to three weeks later. In pre-menopausal females, release of TNFα and IL-6 was significantly decreased in the luteal phase compared to the male group and this difference was more pronounced in females taking oral contraceptive pills. In addition they found a diminished response during the luteal phase compared to the follicular phase. Thus, it would appear that oestrogen has an inhibitory effect on cytokine activation. However, Schwarz et al. (2000) also demonstrate a positive correlation between the concentration of estradiol in plasma and the release of TNFα and IL-6 following a challenge during the luteal phase.

These two papers demonstrate the complicated relationship between oestrogen and cytokines. During the follicular phase (when oestrogen is at its lowest) cytokine release was enhanced compared to the luteal phase. However, during the luteal phase, cytokine release has a positive relationship with oestrogen concentration.

Pottratz et al. (1994) reported an inhibitory effect of E2, within *in vitro* and *in vivo* studies. They observed that ovariectomised mice demonstrated an up-regulation of granulocytes and macrophages and such changes could be prevented by a neutralising antibody against IL-6, as well as oestrogen replacement. They suggested that the inhibition of IL-6 production by E2 was via a receptor-mediated action.

Chao et al. (1995) observed that oestrogen can in some cases depress and in others stimulate cell-mediated immune function. They concluded that within the physiological range of E2, TNFα release is finely regulated and dramatically affected by relatively small changes in hormone concentrations.

## 1.8 Research implications

The complexities of this area are clearly demonstrated in this review. Oestrogen is known to have a potent antioxidant capacity and a membrane stabilising ability. As free radicals are implicated in the development (metabolic stress), propagation of damage (respiratory burst) and even to some extent repair, it would seem a fair assumption that an antioxidant would play a role within this damage, inflammation and repair cycle. In addition to this direct effect upon the skeletal muscle cells, oxygen free radicals may also play a role in mediating other factors associated with the inflammatory response, for example pro-inflammatory cytokines (Tiidus, 1998). This, in turn, can prime leukocytes and thereby enhance superoxide production. Thus, a reduction in initial free radical production, possibly through oestrogen, could feasibly reduce inflammation. In addition to this, oestrogen may also have a direct effect upon specific events within the inflammatory process, for example $Ca^{2+}$ overload, cytokines, and adhesion molecules. These factors of course are themselves not mutually exclusive. A reduction in cytokines will have a direct effect upon adhesion molecule expression. It seems that there are several possible levels at which oestrogen could play a role within the muscle damage and repair cycle, as presented in Figure 1.2.

The literature on the time course and immune response of males and females to exercise-induced muscle damage is sparse, ambiguous and equivocal, and clearly requires further investigation. In addition, further research would help to determine whether the possible inhibition of the immune response either a) aids repair by reducing further secondary damage or b) is negative in terms of the repair and the return of normal function to the muscle. Tidball (1995) stated that inflammatory cells in injured muscle have been implicated in several aspects of successful repair. As explained previously ED2+ cells have been shown to stimulate repair processes. Thus, it could be suggested that inhibition of inflammatory processes has the potential to compromise regeneration. Research into non-steroidal anti-inflammatory drugs and muscle regeneration have been inconclusive, with reports of both compromised (Mishra et al., 1997) and uninhibited repair (Thorsson et al., 1998). Understanding the role of oestrogen in terms of repair would be useful to both recreational and elite sports participants.

If oestrogen is found to play a role within the muscle damage cycle, then implications and applications are considerable and require further investigation. For example, it may not be appropriate to ignore gender when conducting muscle damage research. Fluctuations in oestrogen found across the menstrual cycle may need to be considered within a training environment and schedule.

Another area of research is the possible negative effect of oestrogen on for example tissue regeneration. Are females less protected from the repeated bout effect, due to inhibition of repair following the initial bout of damage?

There are also health considerations for post-menopausal females. Older males have the advantage of testosterone, known for its anabolic properties, to help maintain, in some way, their muscle mass. Post-menopausal females could therefore benefit from oestrogen replacement in terms of protecting their skeletal muscle from degeneration. Phillips et al. (1993) have shown that there is no significant difference in specific force of muscles between young males and pre-menopausal women. However, around the time of the menopause there was a dramatic decline in specific force in women which was prevented by the use of hormone replacement therapy. In men weakness started later and the decline was more gradual. This decrease in strength associated with reduced levels in oestrogen is believed to account for the increases in falling incidents in post-menopausal women (Phillips e al., 1993). Naessen et al. (1997) also found that oestrogen replacement therapy has a positive effect in terms of postural balance in post-menopausal females. However direct contradictions to these findings have also been reported (Seeley et al., 1995; Taafe et al., 1995; Armstrong et al., 1996; Brooke-Wavell et al., 2001).

Research in terms of the protective effect of oestrogen against skeletal muscle damage is sparse, especially in terms of secondary damage and inflammatory processes. Assumptions and theories on the possible protective role of oestrogen on skeletal muscle can be made based upon the known properties of oestrogen and research carried out on cardiac and smooth muscle. However, specific skeletal muscle research is required for any true assumptions and conclusions to be made.

The current thesis aims to advance the knowledge of this complex area and try to determine the extent to which oestrogen plays a role within the muscle damage and repair cycle following eccentrically biased damage inducing exercise in humans.

The following specific questions were addressed:

1) Does the phase of the menstrual cycle in eumennorheic females affect their susceptibility to exercise-induced muscle damage? (Chapter 2)
2) Does prolonged supplementation with exogenous oestrogen, in the form of the combined oral contraceptive pill attenuate any symptoms of exercise-induced muscle damage? (Chapter 2)
3) Are there gender differences associated with symptoms of exercise-induced muscle damage? (Chapter 3)
4) Do markers of inflammation following exercise-induced muscle damage differ between males and females? (Chapter 3)
5) Are females more prone to damage following a second bout of eccentrically biased exercise? (Chapter 4)

# CHAPTER 2

# THE EFFECT OF THE MENSTRUAL CYCLE PHASE AND ORAL CONTRACEPTIVE USE ON SYMPTOMS OF EXERCIS-INDUCED MUSCLE DAMAGE


## 2.1 Abstract

## 2.2 Introduction

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### 2.2.2 Oestrogen and the menstrual cycle

### 2.2.3 Current research

### 2.2.4 Research hypothesis

## 2.3 Methods

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### 2.3.3 Measurement of symptoms of exercise-induced muscle damage

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#### 2.3.3.3 Relaxed arm angle

#### 2.3.3.4 Muscle soreness

#### 2.3.3.5 Upper arm circumference

### 2.3.4 Statistical analysis

## 2.4 Results

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### 2.4.3 Relaxed arm angle

### 2.4.4 Muscle soreness

### 2.4.5 Upper arm circumference

## 2.5 Discussion

### 2.5.1 Evidence of muscle damage

### 2.5.2 Menstrual cycle phase and exercise-induced muscle damage

### 2.5.3 Oral contraceptive use and exercise-induced muscle damage

### 2.5.4 Pain perception and oestrogen

### 2.5.5 Summary and conclusion

This study formed the basis of a verbal presentation to the British Association of Sport and Exercise Sciences conference (2001) and subsequent abstract in Journal of Sports Sciences.

Kendall, B., & Eston, R. (2002). The effect of menstrual cycle status and oral contraceptive use on exercise-induced muscle damage. *Journal of Sports Sciences*, **20**, 54

## 2.1 Abstract

Oestrogen has been suggested as a skeletal muscle protectant. The purpose of this study was to investigate differences in the extent of muscle damage between the follicular and luteal phase of healthy eumennorheic females, following an eccentric exercise protocol of the elbow flexors, and to determine if the regular ingestion of oestrogen, in the form of oral contraceptives, could reduce symptoms of exercise-induced muscle damage following the same protocol.

Nine oral contraceptive users [pill] (age = 21.4 ± 3.0 years, height = 165.4 ± 7.3 cm, mass = 65.5 ± 9.8 kg) and seven non-oral contraceptive users [nonpill] (age = 21.7 ± 4.2 years, height = 166.1 ± 5.3 cm, mass = 64.7 ± 6.3 kg) performed two bouts of damage-inducing exercise of the elbow flexors on opposing arms. The bouts were performed during different phases of their artificial (pill) and actual (nonpill) menstrual cycle. Soreness, CK activity, isometric strength of the elbow flexors, relaxed arm angle, and upper arm circumference were taken prior to exercise, and every 24h for three days following exercise. A mixed model repeated measures ANOVA [group (2) x phase (2) x time (4)] revealed changes in all variables across time, confirming that muscle damage was successfully induced. In addition, soreness was higher on day one in the nonpill group, with differences being maintained across the 3 day monitoring period ($F_{3,42}$= 4.6, $P$<0.01). The data suggest that fluctuations in oestrogen during the normal menstrual cycle do not affect susceptibility to muscle damage. In addition, the results suggest that supplementation of oestrogen in the form of oral contraceptives does not reduce the extent of muscle damage, but does appear to affect the perception of pain associated with this damage.

## 2.2 Introduction

Exercise-induced muscle damage (EIMD) is a well researched but still quite poorly understood occurrence. It is well documented that EIMD is a common experience following eccentric muscle actions (Newham et al., 1983; Clarkson et al., 1986; Jones et al., 1986) or prolonged metabolically demanding activities (Sjöström et al., 1987; Suzuki et al., 1999). Symptoms include stiffness (Jones et al., 1987); loss in strength (Talag, 1973; Newham et al., 1983); delayed onset muscle soreness (Talag, 1973; Newham et al., 1983) especially on activation of the damaged muscle, and leakage of proteins into the blood (Jones et al., 1986), of which the most frequently reported is creatine kinase (CK). Symptoms can last for up to eleven days following the initial insult to the muscle (Cleak and Eston, 1992).

### 2.2.1 Oestrogen and exercise-induced muscle damage

Females exhibit less CK activity both at rest and following exercise (Meltzer, 1971; Shumate et al., 1979; Amelink and Bär, 1986; Bär et al., 1988). Additionally, ovariectomised (removal of oestrogen source) rats and male rats have enhanced CK activity, following a prolonged exercise protocol, compared to sexually mature female rats. This response is reduced with oestrogen treatment (Amelink and Bär, 1986; Bär et al., 1988), which led these authors to suggest that oestrogen may provide a protective effect against skeletal muscle damage. This is further supported by evidence that oestrogen acts as a cardioprotectant (Subbiah et al., 1993; Wiseman, 1997). There is a lower incidence of coronary heart disease in pre-menopausal females compared to age-matched males (Gossland et al., 1987), which ends following the menopause (Ruehlmann and Mann, 1997), unless hormone replacement therapy is used (Bush et al., 1987; Gruchow et al., 1988). The potential

of oestrogen to protect skeletal muscle from EIMD has therefore become a recent focus of research.

Oestrogen is an antioxidant (Subbiah et al., 1993; Tiidus, 1995; Ayres et al., 1996; Ruehlmann and Mann, 1997) with membrane stabilising properties (Wiseman et al., 1993; Tiidus, 1995) and a gene-regulatory effect (Beato, 1989). It is through these interacting properties that oestrogen may intercede at different phases throughout the muscle damage and repair cycle.

Free radicals have been implicated throughout the muscle damage and repair cycle (Child et al., 1998). Thus, an antioxidant may be able to reduce the damage that is associated with these highly reactive molecules, for example by protecting against lipid peroxidation. Stabilising of cell membranes could protect against an onslaught from free radicals amongst other factors (Tiidus, 1999). The gene-regulatory effect of oestrogen may also play a role within the inflammatory response associated with skeletal muscle damage. Cytokines and adhesion molecules, which orchestrate the inflammatory response and infiltration of leukocytes into the injured area, require gene transcription for activation (Montgomery et al., 1991). For a more detailed discussion of these mechanisms see chapter 1.

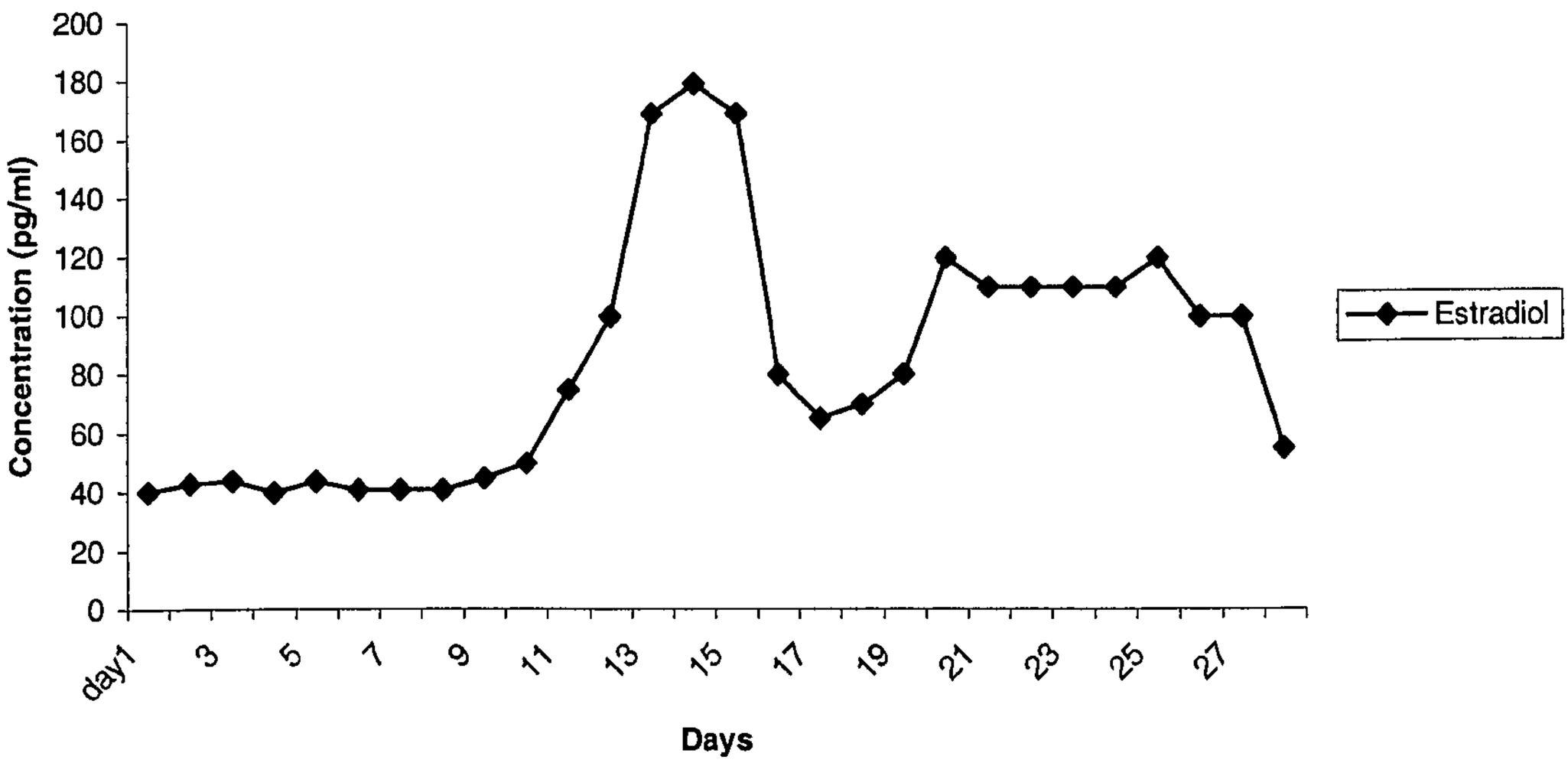
### 2.2.2 Oestrogen and the menstrual cycle

In a healthy eumennorheic female the menstrual cycle lasts on average 28 days. Across this cycle, oestrogen shows very large fluctuations. As demonstrated in Figure 2.1. during the early phases of menses/early follicular oestradiol levels are approximately 40 pg/ml, however at ovulation (approximately day 14) these levels

will increase by approximately 5 fold to around 200 pg/ml. In combined (synthetic oestrogen and progesterone) oral contraceptive users, endogenous oestradiol is reduced to around 15 pg/ml (Savage & Clarkson, 2002), while exogenous and total oestrogen is increased and maintained at high levels.

An area to receive limited attention is the potential for intra-female differences in susceptibility to exercise-induced muscle damage. For example, are females who undergo a bout of damage-inducing exercise more susceptible to the damage if it is performed during the early stages of their menstrual cycle when oestrogen is at its lowest, than if it is performed mid-cycle when oestrogen is at its highest. In addition, can prolonged ingestion of exogenous oestrogen in the form of the combined oral contraceptive pill, attenuate any of the symptoms associated with EIMD?

**Figure 2.1** Oestradiol concentration across the menstrual cycle



### 2.2.3 Current research

Surprisingly, the research in this area is sparse. No studies have looked at differences in susceptibility to EIMD across the menstrual cycle and only three studies have investigated the use of the oral contraceptive pill in attenuating EIMD.

Thompson et al. (1997) investigated simple markers of muscle damage following a bench stepping exercise in pill and non-pill users. It was found that no differences existed in the two groups for strength measures, leg circumference or CK concentrations. However, the results suggest that the level of damage may have been too small for such differences to be determined. Interestingly, it was found that a difference existed between the soreness reported by both groups, with the non-pill users reporting higher levels than the pill users.

Carter et al. (2001) found that oral contraceptive use could attenuate the CK response following a bout of eccentric exercise, but no differences were seen for soreness. While CK is a common marker of EIMD its reliability has been brought into question (Warren et al., 1999) and should therefore be observed with caution.

Finally, Savage and Clarkson (2002) investigated changes in markers of EIMD following an eccentric arm exercise protocol. It was shown that the only difference to exist between pill users and non-pill users was the prolonged reduction in isometric strength found in the pill users.

The purpose of this study was twofold: i) investigate the effect of menstrual cycle status in eumennorheic females, on exercise-induced muscle damage and, ii) to

investigate the effects of oral contraceptive use on symptoms of exercise-induced muscle damage.

### 2.2.4 Research hypothesis

The following hypotheses were investigated in the present study:

a) Eumennorheic females will be more susceptible to a bout of damage-inducing exercise during the menses/follicular phase of their menstrual cycle (when oestrogen concentration is low) compared to the ovulatory/luteal phase;

b) Prolonged ingestion of exogenous oestrogen, in the form of oral contraceptive use will attenuate the symptoms of exercise-induced muscle damage.

## 2.3 Method

### 2.3.1 Participants and design

Sixteen female volunteers from the University of Wales, Bangor (age 21.6 ± 3.5y, ht 165.7 ± 6.3cm, mass 65.2 ± 8.2kg) took part in this investigation. No significant differences existed between groups for age, height or mass. Nine participants had been taking oral contraceptives for the last six months (30-35mg.$d^{-1}$ synthetic oestrogen). The seven non-pill participants reported having regular menstrual cycles for at least the last six months. This study was approved by the ethics committee of the School of Sport, Health and Exercise Sciences, University of Wales, Bangor. All participants completed a health questionnaire and signed an informed consent form.

Participants were assigned to a group based on oral contraceptive pill use. The non-pill group were given a thermometer and a cycle chart, and requested to record oral temperature every morning before rising, for two months prior to the commencement of testing. This enabled phases of the cycle to be determined. In addition, both groups were required to keep record of when they menstruated for at least two cycles before testing. Prior to testing participants were fully familiarised with the techniques involved in the investigation, in particular with the assessment of isometric strength.

The exercise protocol was carried out on the elbow flexors. Random assignment determined which arm (non-dominant or dominant) was exposed to the first bout of damage-inducing exercise, and in which phase. Pill users, who had regular 28 day cycles, were damaged during menses, and between day 13 and 14. These corresponded to low and high oestrogen phases respectively, in a normal menstruating female with a 28 day cycle. The non-pill users were damaged in their low (menses/early follicular) and high (ovulatory-luteal) oestrogen phases based on cycle length and temperature recordings. Individual menstrual cycles vary in length, but the length of the luteal (ovulation to menstruation) phase always remains 14 days (Van Wynsberghe et al., 1995). Based on the above information, the damaging exercise protocol to the non-pill users was conducted during early menses and when ovulating. Thus, in summary, two bouts of damage-inducing exercise were carried out on each participant in two separate phases, on different arms. Phase 1 comprised the damage-inducing exercise and measurements that commenced during menses. Phase 2 comprised the damage-inducing exercise and measurements that occurred around the middle of the cycle (ovulation for non-pill users, day 13-14 for pill users).

Baseline measures of plasma creatine kinase activity, isometric strength of the elbow flexors, relaxed arm angle, soreness and upper arm circumference were taken prior to exercise. All subjects then underwent a bout of eccentric exercise on the assigned arm.

### 2.3.2 Eccentric exercise protocol

The eccentric exercise protocol was performed on the KinCom isokinetic dynamometer (500H, Cattecx, Chattanooga, TN, USA). The exercise involved movement throughout the range of movement of the elbow flexors for each participant. Participants lay in a supine position with strapping around their body in line with their biceps. This reduced the amount of extraneous movement which could be performed by the body, and maintained the arm in a constant position.

The exercise consisted of 5 sets of 10 maximal contractions at $30°s^{-1}$ with a 60 second rest between each set. Participants were told to resist the KinCom maximally as the force extended the elbow. If the participant exerted no force, the movement of the KinCom stopped. In addition to this, verbal and visual feedback was given throughout to try and ensure that effort was maximal.

### 2.3.3 Measurement of symptoms of exercise-induced muscle damage

Measurements during both phases were taken at baseline, then every 24 hours for 3 days following the exercise.

#### 2.3.3.1 Creatine kinase

Plasma creatine kinase (CK) activity was determined from a fingertip blood sample. A warm fingertip was cleaned with a sterile alcohol swab and allowed to dry. Capillary puncture was made with a softclix lancet and a sample of whole fresh blood (30µl) was pipetted from a capillary tube onto the test strip and analysed for CK activity via a colorometric assay procedure (Reflotron, Boehringer Mannheim, Lewes, UK). This system uses a plasma separation principle, which is incorporated in the reagent carrier on the test strip.

### 2.3.3.2 Isometric strength

This was measured on the KinCom isokinetic dynamometer (500H, Cattecx, Chattanooga, TN, USA). The participant performed a brief warm up and practised performing sub-maximal voluntary contractions. They then performed three maximal isometric contractions of the elbow flexors, with a minute rest between contractions. This was performed in the same supine position as the damage-inducing exercise. Participants were strapped in position to minimise movement. The angle at which strength was measured was 90° elbow flexion. The maximum score was recorded.

### 2.3.3.3 Relaxed arm angle

Participants stood in the anatomical position with their hands at their sides. The arm was fully flexed and actively extended into a relaxed position. This angle was recorded using a transparent goniometer. Three points of reference were made to ensure repeatability of measures across the measurement period.

### 2.3.3.4 Muscle soreness

A 0-10 visual analogue scale (VAS), with 0 being no pain and 10 being worst pain ever, was used to measure soreness. The scale was two sided, participants were unable to see the numerical scale, but moved the slide in accordance with an arbitary scale, with *'no pain'* at one end and '*worst pain ever*' at the other. The arm was fully flexed and actively extended, the participants then slid the indicator across the scale in accordance with their perception of pain across that range of movement. The numerical scale allowed the investigator to quantify their reported soreness. This method has been used successfully in previous studies (Cleak and Eston, 1992a).

#### 2.3.3.5 Upper arm circumference

An anatomical tape measure was used to measure the circumference at the mid-belly point on the biceps. This was marked on the anterior and posterior of the arm to ensure repeatability of measure.

### 2.3.4 Statistical analysis

Mixed model repeated measures [group (2) x phase (2) x time (4)] analysis of variance (ANOVA) were applied to the data. Separate ANOVAs were calculated for relaxed arm angle, isometric strength, upper arm circumference soreness and creatine kinase. The assumption of sphericity was tested using Mauchly's test. Any violations of this assumption were corrected using the Greenhouse-Geisser adjustment to raise the critical value of F, by adjusting the degrees of freedom, as indicated by $_{(GG)}$. Statistical significance was set at the 0.05 alpha level. Time main effects were further investigated by pairwise comparisons, with the Bonferroni adjustment technique to eliminate the problem of an inflated Type 1 error risk. An adjusted Tukey's HSD post hoc test was used to investigate any significant interactions (Stevens, 1996). The

mean and standard deviations for each variable across each phase and between each group are presented in Appendix 1

## 2.4 Results

### 2.4.1 Creatine kinase

This measure was log transformed, due to the large variability that is present within this variable. Transformed data showed a main effect for time($F_{(GG)1.8, 25.3} = 10.89$, $P<0.01$), the difference between baseline measures and day 2 were approaching significant elevation ($P = 0.057$) with a significant increase in CK concentration within the blood on day 3 following exercise. The main effects for group and phase were not shown to be significant, neither were the group x time, phase x time, group x phase and group x phase x time interaction. Figure 2.2 illustrates these findings

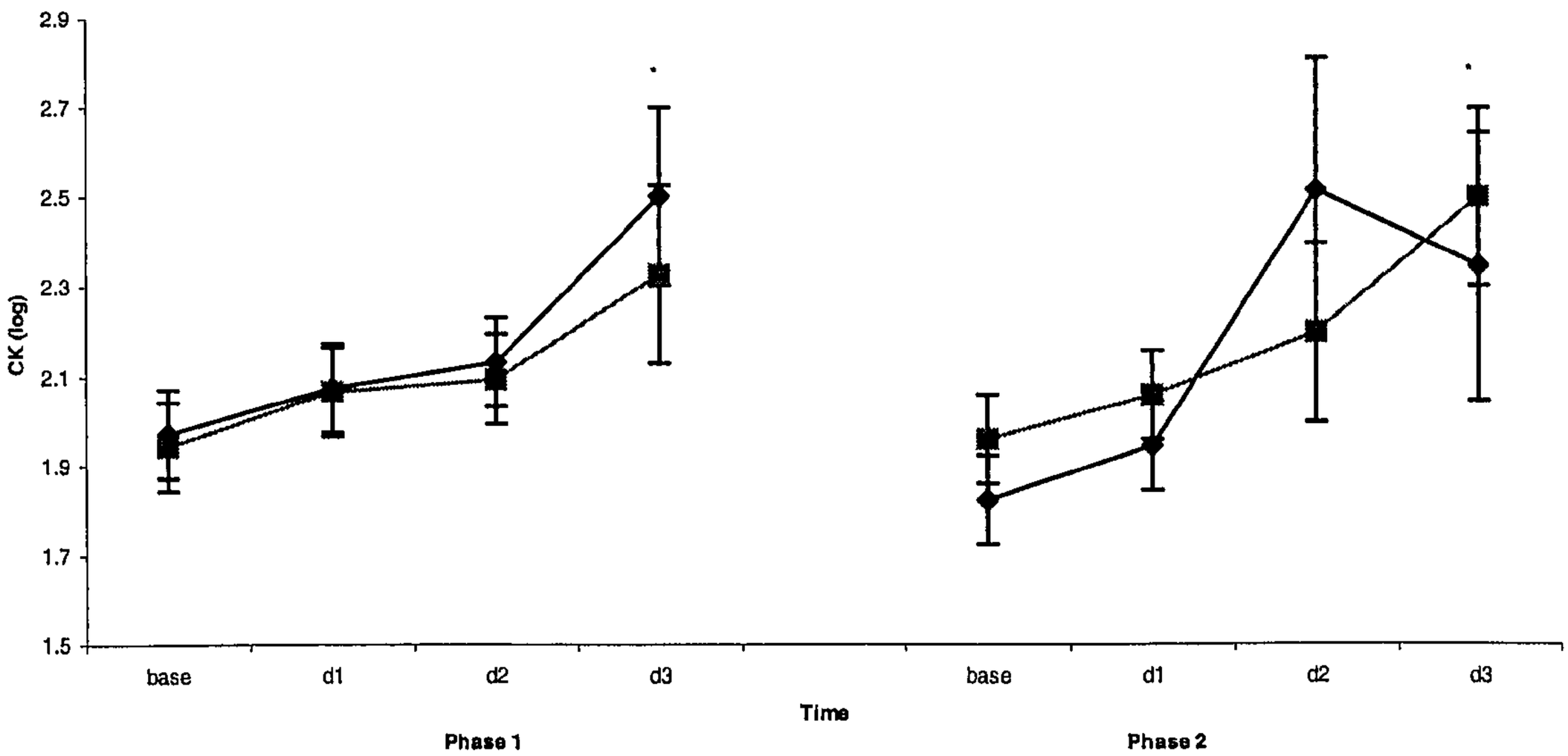



**Figure2.2**: Changes in log CK after eccentric exercise of the elbow flexors in oral contraceptive (pill▪) and non-oral contraceptive users (nonpill ♦) (mean ± S $\overline{x}$ ). * significantly ($P<0.05$) different from baseline

## Isometric strength

There was a significant main effect of time on strength ($F_{3,\ 42}$ = 58.1, $P$<0.01). Significant decrements in strength from baseline were seen on day 1 and were maintained throughout the testing period. The main effects for group and phase were not statistically significant, neither were the group x time, phase x time, group x phase and group x phase x time interaction. Figure 2.3 illustrates these findings

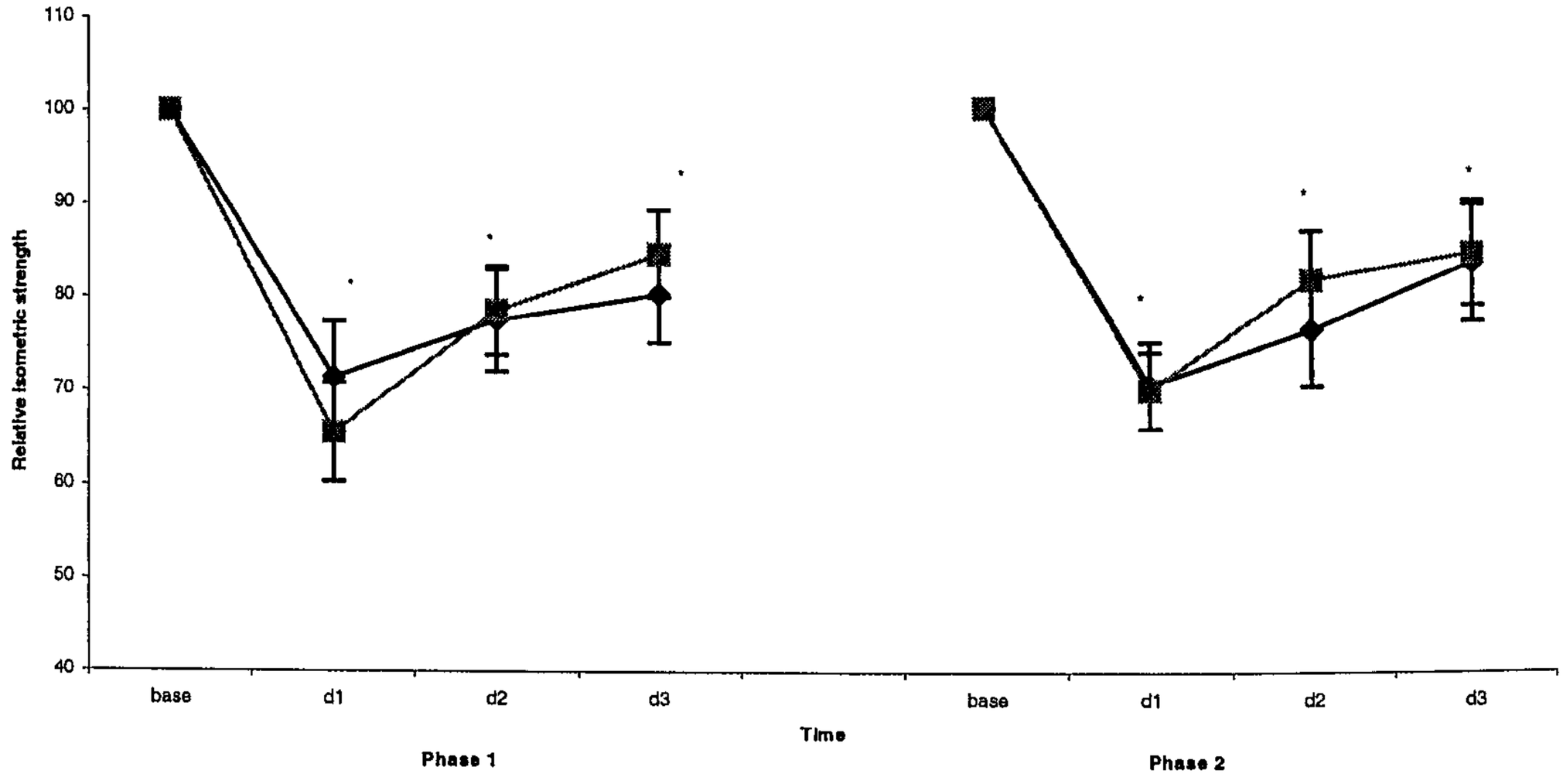



**Figure 2.3**: Relative strength loss after eccentric exercise of the elbow flexors in oral contraceptive (pill ▪) and non-oral contraceptive users (nonpill ♦ )(mean ± S $\overline{x}$ ). * significantly ($P$<0.05) different from baseline

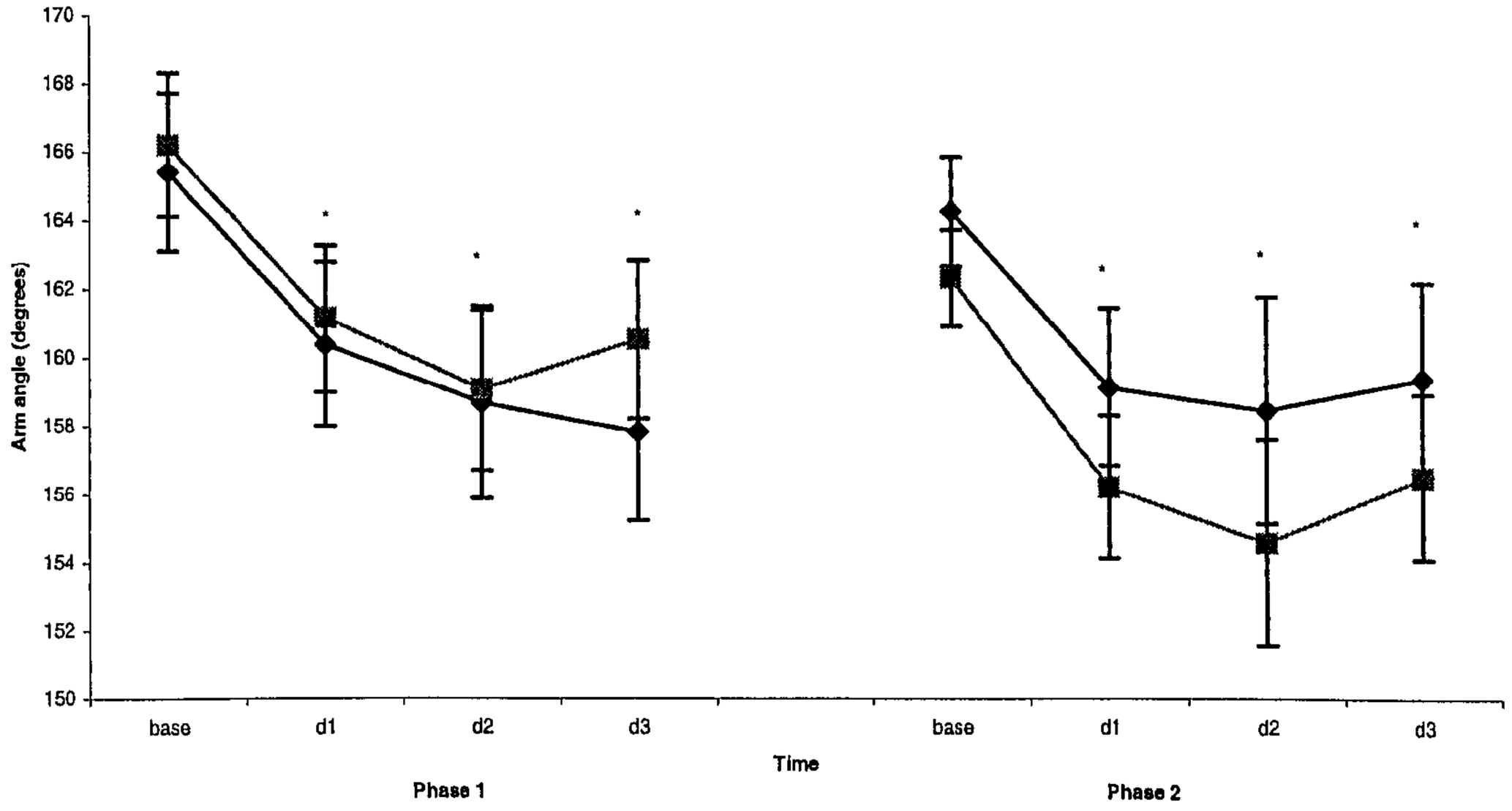



**Figure 2.4**: Changes in relaxed arm angle after eccentric exercise of the elbow flexors in oral contraceptive (pill ▪ ) and non-oral contraceptive users (nonpill ♦ )(mean ± S $\overline{x}$ ). * significantly ($P$<0.05) different from baseline

### 2.4.3 Relaxed arm angle

There was a significant main effect for time on relaxed arm angle ($F_{3, 42}$ = 15.3, $P$<0.01). The resting angle was lower on day 1 which was maintained throughout the testing period. There were no differences between group or phase. There were also no significant group x time, phase x time, group x phase and group x phase x time interaction. Figure 2.4 illustrates these findings.

### 2.4.4 Muscle soreness

There was a significant main effect of time on muscle soreness ($F_{3, 42}$= 42.8, $P$<0.01). Elevated levels of soreness were seen on day 1 and were maintained throughout the testing period. There are also significant differences between groups ($F_{1, 14}$=15.9, $P$<0.01), with higher reported soreness in the non-pill users. The significant group x time interaction ($F_{3, 42}$ = 4.6, $P$<0.01) explain this group main effect. Post hoc analysis revealed that soreness was higher on day 1 in the non-pill group and these differences were maintained throughout the testing period. This is illustrated in figure 2.5.

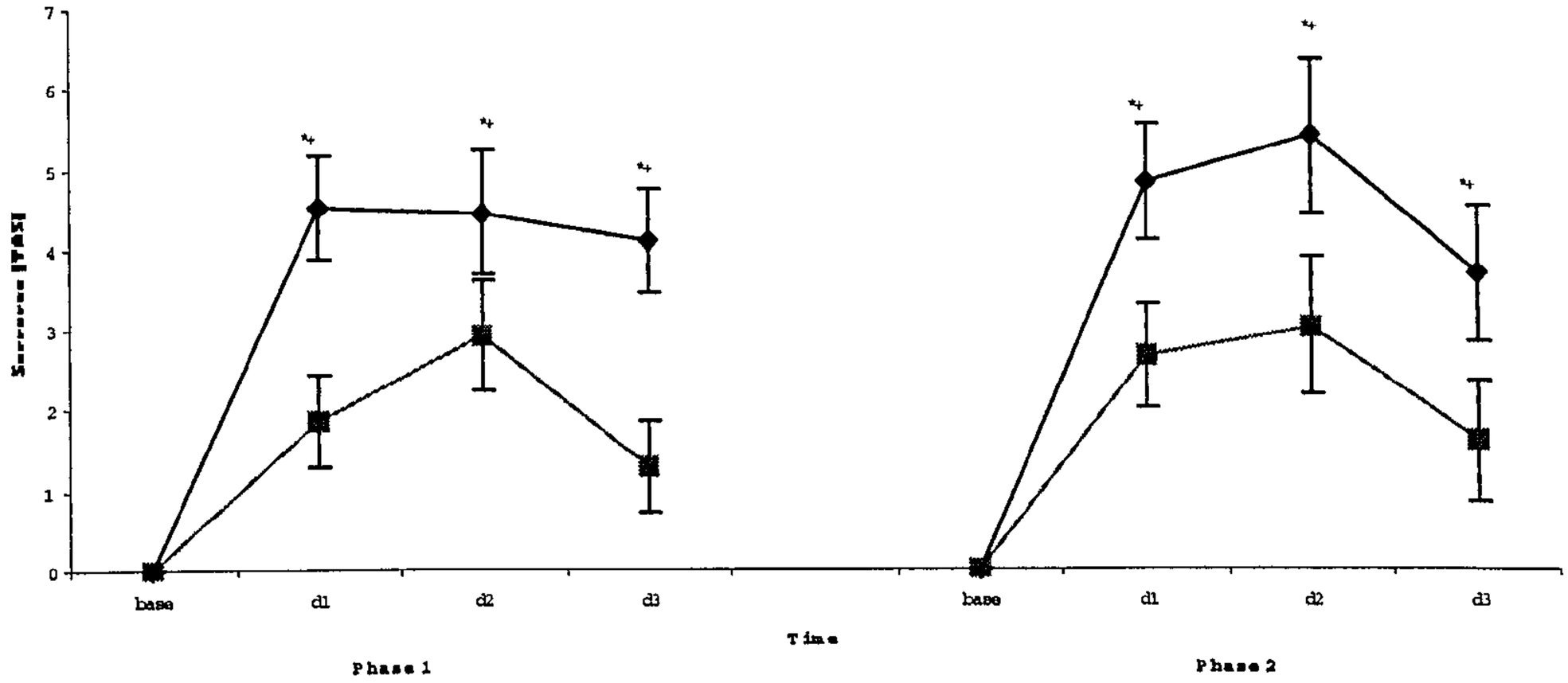



**Figure 2.5**: Incidence of muscle soreness after eccentric exercise of the elbow flexors in oral contraceptive (pill ▪ ) and non-oral contraceptive users (nonpill ♦ )(mean ± S $\bar{x}$ ). * significantly ($P$<0.05) different from baseline, + significant ($P$<0.05) difference between groups.

### 2.4.5 Upper arm circumference

There was a significant main effect of time on upper arm circumference. The main effect for time was shown to be significant ($F_{(GG)}1.6,\ 22.9= 6.7,\ P<0.01$). Circumferences were higher on day 2 and day 3 following the exercise procedure. The main effects for group and phase were not statistically significant, neither were the group x time, phase x time, group x phase and group x phase x time interaction.

Figure 2.6 illustrates these findings.

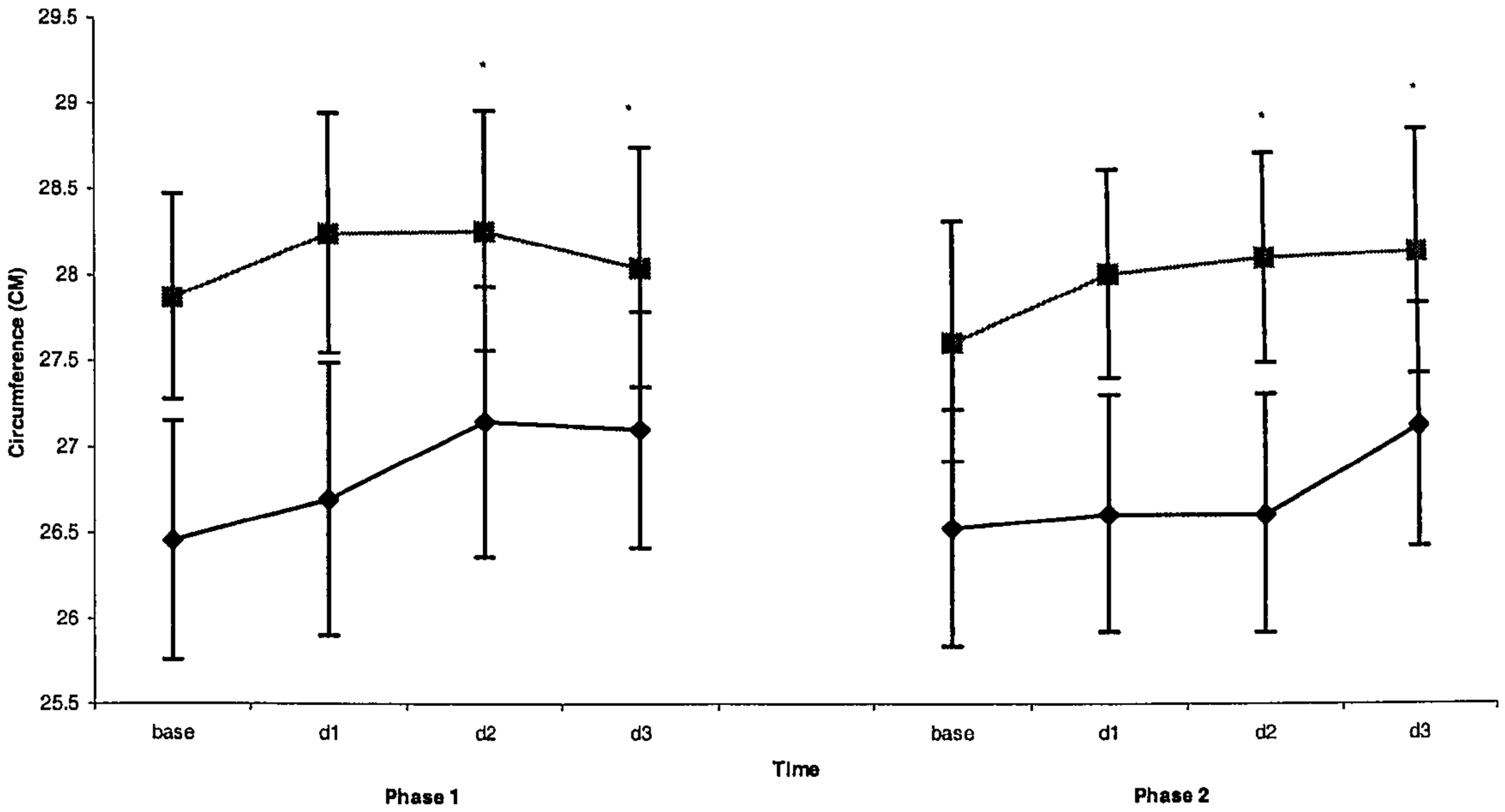



**Figure 2.6**: Changes in upper arm circumference after eccentric exercise of the elbow flexors in oral contraceptive (pill ▪ ) and non-oral contraceptive users (nonpill ♦ )(mean ± $S\bar{x}$ ). * significantly ($P<0.05$) different from baseline

## 2.5 Discussion

The purpose of this investigation was twofold. Firstly, to determine if oestrogen fluctuations across a normal menstrual cycle resulted in differences in exercise-induced muscle damage (EIMD) and secondly, to determine if regularly ingesting oestrogen, in the form of oral contraceptives, could in any way attenuate the symptoms of EIMD.

### 2.5.1 Evidence of muscle damage

All criterion measures of EIMD changed significantly from baseline in both groups, during both phases, confirming that the exercise protocol was sufficient to induce damage in the elbow flexor muscles.

### 2.5.2 Menstrual cycle phase and exercise-induced muscle damage

The current study is the first to investigate differences in susceptibility to EIMD across a 'normal' menstrual cycle. It was thought that because oestrogen did show such large fluctuations across the menstrual cycle and because of the proposed mechanism of oestrogen to attenuate symptoms of EIMD that females may be more prone to EIMD during the early low oestrogen phase of their menstrual cycle. No evidence is presented from the current study to suggest that such an effect occurs. The symptoms of EIMD did not differ significantly across the menstrual cycle. This may be due to prolonged exposure to oestrogen in females.

### 2.5.3 Oral contraceptive use and exercise-induced muscle damage

The only variable to show a difference between the pill and non-pill users, following the damaging protocol, was soreness. At 24 hours post-exercise, the oral contraceptive users reported less soreness than the eumennorheic group. The difference between the groups was maintained throughout the rest of the testing period. This is similar to the findings of Thompson et al. (1997) who, following a bench stepping regimen, observed that oral contraceptive users reported less soreness than non users.

### 2.5.4 Pain perception and oestrogen

It is difficult to explain why the difference between the groups was observed, because the generation of pain following this type of muscle damage is poorly understood. What is known is that the sensation of pain in skeletal muscle is transmitted by myelinated group III and unmyelinated group IV afferent nerves (Armstrong, 1984). It is believed that the dull ache associated with this type of muscle damage is carried along the small unmyelinated type IV fibres, which are sensitive to a variety of chemical, mechanical and thermal stimuli

Inflammation results in the production of chemicals, for example, prostaglandin, bradykinin, serotonin and histamine, which are known to stimulate these pain afferents. Inflammation is also associated with an increase in local temperature and an increase in pressure due to oedema. Thus, any one or all three could lead to the sensation of pain associated with muscle damage (Armstrong, 1984).

Oestrogen may moderate the inflammatory response, with research demonstrating both an up-regulation and down-regulation of the immune response. A more detailed account on the potential effect of oestrogen on general and specific inflammatory events associated with exercise-induced muscle damage is discussed in chapter 1. The potential of oestrogen to inhibit the inflammatory response may help to explain differences in reported pain between the two groups. Less inflammation would reduce mechanical pressure, reduce thermal stimulation of pain afferents, and reduce production of stimulating chemicals, for example prostaglandins.

However, this would also suggest that the symptoms of damage would have been reduced in the pill group, and in this study no significant differences were found in any of the other variables. It could be that the markers used were not sensitive enough to show differences, or that the small sample size didn't allow small differences to be seen. Alternatively, it could be due to the perception of pain. If the generation of pain was the same between these two groups, i.e. chemical, thermal and mechanical stimuli are all the same, it could be that there was a difference in the perception of pain.

The potential ability for sex steroids to modulate pain has been investigated but findings have been inconclusive. In addition, the suggested mechanisms by which sex hormones are able to play a role are equivocal.

A review article by Bethea et al. (1998) indicates that the ovarian steroid hormones, oestrogens and progestins, affect the function of the serotonin neural system. While this has the capability to impact upon mood, cognition and other autonomic functions, serotonin is also associated with pain.

Riley et al.(1999) reviewed 16 studies that investigated pain perception across the menstrual cycle in healthy females. They observed that for pressor stimulation, cold pressor pain, thermal heat stimulation and ischemic muscle pain, a clear pattern emerged. It was shown that during the follicular phase higher pain thresholds were demonstrated. The authors acknowledged the enormous problems within the literature, regarding determination of the menstrual cycle phase, length of phase and even the terminology associated with the phase, making generalisations across

studies difficult. Nevertheless, the results of this review suggest that when oestrogen and progesterone are at their lowest, pain thresholds are at their highest. Potentially, suggesting oestrogen and/or progesterone increase sensitivity to pain.

In contrast, Martinez-Gomez et al. (1994) investigated tail flick latency across the cycle in rats. Shorter latencies were recorded in phases with lower oestrogen. Ovariectomy (removal of oestrogen source) abolished these fluctuations. Administration with oestrogen, but not progesterone increased response time, which suggests that oestrogen increases pain thresholds. Similarly, Rao et al. (1987) carried out a very simple investigation on pain perception across a broad range of participant groups. They showed that the pain threshold was low in oopherectomised women (who were not on HRT) and in boys and girls, intermediate in males, but high in oral contraceptive users and normally menstruating women. Fluctuations in pain thresholds occurred in menstruating women, with higher thresholds at mid cycle, when oestrogen is highest. Thus, although findings are inconclusive, there remains the possibility that oestrogen and progesterone can potentially play a role in the perception of pain.

The human body contains its own mechanism for dealing with pain. Endogenous opioids are neuropeptides that have an analgesic property. These include enkaphalins, endorphins and dynorphins, which have the capability to modify pain transmission and inhibit prostaglandin synthesis (Thomas, 1997). These opioids are released in response to stimuli such as pain or stress, and produce their effect by binding to opioid receptors located in the brain and spinal cord. These receptors include mu ($\mu$), kappa ($\kappa$), and delta ($\delta$) receptors. It has been suggested that both oestrogen and

progesterone could potentially play a role within this natural pain relief system. Antinociception of pregnancy and parturition has been observed in rats (Gintzler, 1980; Kristal et al., 1990; Iwasaki et al., 1992) and in women (Cogan & Spinnato, 1986; Whipple et al., 1990). It has been suggested that the elevated levels of the hormones oestrogen and progesterone can account for these effects. Pseudopregnancy has been investigated, by manipulation of these hormones, to separate the effects of these hormones from other events associated with pregnancy. The findings of these investigations show a higher pain threshold in pseudopregnant rats (Gintzler & Bohan, 1990; Dawson-Basoa & Gintzler, 1996; 1998; Liu & Gintzler, 2000).

Some authors take the standpoint that the high levels of these hormones during pregnancy and pseudopregnancy result in the activation of opiate receptors, mediating the analgesia. Research using receptor antagonists has reinforced this suggestion (Gintzler & Bohan, 1990; Dawson-Basoa & Gintzler, 1998). Conversely, other research has rejected such a conclusion (Gordon & Solimon, 1996).

Oestrogen receptors have also been suggested to play a role in this natural analgesia. Oestrogen receptors are present on small-diameter dorsal root ganglion (DRG) neurons (Dina et al., 2001). Oestrogen has been shown to regulate the expression of mRNA encoding receptors, which modulate nociception (Dina et al., 2001). Additionally, it has been shown that enkephalin synthesising neurons display intracellular oestrogen receptors (Amandusson et al., 1999), which have been demonstrated to increase the gene expression of an enkephalin precursor (Amandusson et al., 1999; Amandusson & Blomqvist, 2001).

Thus, although equivocal, the research demonstrates the potential for oestrogen to moderate the perception of pain. While there is no direct evidence that the combined oral contraceptive pill can modulate pain perception, it seems feasible that the sustained levels of oestrogen and progesterone across the cycle could explain why differences in perceived pain between the two groups occurred. Studies investigating exercise-induced muscle damage and oestrogen levels therefore need to consider the possibility that oestrogen levels may also moderate the perception of pain.

### 2.5.5 Summary and conclusion

The practical implications based upon the findings of this investigation are potentially very complicated and require further investigation. While, it is acknowledged that the muscle damage induced in this study was not severe and the participants were not elite athletes, the findings may have implications for athletic performance.

Soreness as a gauge of muscle damage is important. Prolonged rest from training or reduced intensity in training, due to soreness, may be detrimental to performance. Attenuation of soreness, through, for example oral contraceptive use, may play a positive role, ensuring that training can be maintained at a relatively high intensity, during non-severe muscle damage. Conversely however, masked pain may have long term considerations. A common symptom of overtraining is reported muscular soreness, which should be treated as a very serious complaint in terms of modifying training intensity. Therefore, it is very important that reported muscular soreness is a fair gauge of actual muscle damage. When damage is minimal, training can be maintained at a relatively high level. However, if damage is more severe training

needs to be adapted accordingly. If the findings of this investigation were replicated with a more severe muscle damaging protocol, the potential consequences on performance would need further investigation.

In conclusion, based on the data from this investigation, it would appear that oestrogen fluctuations across the menstrual cycle do not affect the susceptibility of the female muscle to EIMD. Additionally it would appear that prolonged oestrogen supplementation in the form of oral contraceptive pills is unable to attenuate the majority of criterion measures of EIMD, with the exception of pain. The potential role of oral contraceptives to influence pain perception requires further investigation, possibly with opioid receptor antagonists to decipher where, if at all, they interact with the natural pain relief system.

As there appears to be no difference in susceptibility to EIMD across the menstrual cycle and oral contraceptive use does not appear to reduce the extent of the symptoms of EIMD, the focus of the next study was to explore the potential protective role of oestrogen and gender differences in the time-course of symptoms and inflammatory markers associated with EIMD.

# CHAPTER 3

# GENDER DIFFERENCES IN MARKERS OF THE INFLAMMATORY RESPONSE ASSOCIATED WITH EXERCISE-INDUCED MUSCLE DAMAGE


## 3.1 Abstract

## 3.2 Introduction

### 3.2.1 Inflammatory response associated with exercise-induced muscle damage

### 3.2.2 Free radicals and reactive oxygen species

### 3.2.3 Oestrogen, exercise-induced muscle damage and inflammation

### 3.2.4 Research hypotheses

## 3.3 Methods

### 3.3.1 Participants and design

### 3.3.2 Measurement of symptoms of exercise-induced muscle damage

#### 3.3.2.1 Soreness

#### 3.3.2.2 Creatine kinase

#### 3.3.2.3 Isometric strength

#### 3.3.2.3 Jump height

#### 3.3.2.4 Mid-thigh circumference

### 3.3.3 Blood sampling and handling

#### 3.3.3.1 White blood cell counts

#### 3.3.3.2 Plasma elastase and lipopolysaccharide-stimulated neutrophil degranulation

#### 3.3.3.3 C-Reactive protein

#### 3.3.3.4 Oxidative burst activity

### 3.3.4 Exercise protocol

### 3.3.5 Statistical analysis

## 3.4 Results

### 3.4.1 Soreness

### 3.4.2 Creatine kinase

### 3.4.3 Isometric strength

#### 3.4.3.1 Isometric strength at 10 degrees knee flexion

#### 3.4.3.2 Isometric strength at 20 degrees knee flexion

#### 3.4.3.3 Isometric strength at 80 degrees knee flexion

### 3.4.4 Jump height

### 3.4.5 Mid-thigh circumference

### 3.4.6 White blood cell count

### 3.4.7 Elastase concentration

### 3.4.8 C-reactive protein

### 3.4.9 Oxidative burst activity

## 3.5 Discussion

### 3.5.1 Indirect markers of exercise-induced muscle damage

#### 3.5.1.1 Creatine kinase

#### 3.5.1.2 Soreness

#### 3.5.1.3 Mid-thigh circumference

### 3.5.2 Inflammatory markers

#### 3.5.2.1 Neutrophil count

#### 3.5.2.2 Elastase

### 3.5.3 Summary and conclusion

Some data from this study formed the basis of a poster presentation to the British Association of Sport and Exercise Sciences conference (2001) (12th International Commonwealth Sport Conference) and subsequent abstract in Journal of Sports Sciences.

Kendall, B., Walsh, N.P., Worth, S.J., Walters, R., Bishop, N.C. & Eston, R. (2003). The effect of exercise-induced muscle damage on neutrophil function. *Journal of Sports Sciences*, **21**, 325-326

## 3.1 Abstract

Oestrogen has been suggested as a skeletal muscle protectant. It has also been shown that oestrogen could play a role within the inflammatory response associated with exercise-induced muscle damage (EIMD). The purpose of this study was to investigate gender differences in EIMD, following an eccentrically biased exercise of the legs, and to determine if oestrogen could in any way attenuate both functional and inflammatory markers associated with EIMD. Twelve healthy, untrained, but recreationally active volunteers participated in this investigation, six males (age 23.5 ± 3.6yrs, ht 181.5 ± 4.9 cm, mass 79 ± 8.5 kg) and six females (age 21.8 ± 3.6yrs, ht 163.6 ± 5.7cm, mass 57.6 ± 6.4kg). Baseline measures of soreness, CK, isometric strength, jump height and mid-thigh circumference were assessed prior to exercise and every 24 hours for 7 days following exercise. In addition to this, leukocyte counts (neutrophil, monocytes, lymphocytes), elastase concentration (stimulated with LPS and unstimulated), C-reactive protein and in a small sub-sample (n = 4) oxidative burst activity was assessed prior to exercise, 2 h after exercise and on days 1, 3, 5 and 7. The exercise protocol designed to induce damage, consisted of 6 sets of 30 lunges on each leg, followed by 10 sets of 10 counter movement jumps. A mixed model repeated measures ANOVA [gender x time] revealed changes in all functional variables and CK activity across time, confirming that muscle damage was successfully induced. In addition, neutrophil count was significantly elevated 2 h after exercise ($F_{(GG)1.4,\ 14.1} = 21.8$, $P<0.05$). Gender differences were found in soreness ($F_{1,10} = 7.6$, $P<0.05$), with females reporting significantly more soreness than males and in mid-thigh circumference ($F_{1,10} = 4$, $P<0.01$), where males showed significantly more swelling than females. C-reactive protein, oxidative burst activity and total elastase concentration showed no significant changes across the testing

period or significant gender differences. LPS stimulated elastase showed a significant increase 2 h (1059 ± 397μg/l vs 2122 ±915 μg/l) after exercise returning to baseline by day 1, however, this significant effect was removed when expressed per neutrophil (222 ± 72 fg/cell vs 199 ± 53 fg/cell). When unstimulated elastase was expressed per neutrophil a significant gender difference was observed, with females showing less elastase concentration/neutrophil ($F_{5,50} = 3.1$, $P<0.05$). The data suggest that gender differences in functional measures of exercise-induced muscle damage are questionable. However, the smaller changes in leg circumference in the females and reduced elastase/neutrophil may suggest inflammation is smaller in this group, although the non-response of C-reactive protein does suggest the inflammatory response is minimal in both genders.

## 3.2 Introduction

Exercise-induced muscle damage (EIMD) is associated with unaccustomed, physical activity, especially eccentric activities (Newham et al., 1983; Armstrong, 1984; Clarkson & Newham 1994), and prolonged metabolically demanding activities (Ostrowski et al., 2000; Dawson et al., 2002). Such skeletal damage has long been associated with delayed-onset muscle soreness (DOMS), which generally manifests itself eight to 24 hours after the exercise bout and peaks between 24 and 48 hours later. Additionally, this specific type of muscle damage has been associated with a loss in dynamic muscle function. For example Byrne and Eston (2002 a, b) have shown that following an eccentric exercise protocol both strength, vertical jump performance and maximal intensity exercise performance are reduced in the days following the exercise.

Mechanical factors are closely associated with the initiation of the muscle damage (certainly with eccentrically biased exercise). However, secondary damage may have metabolic origins. See Chapter 1 for a more detailed discussion of the muscle damage and repair model.

### 3.2.1 Inflammatory response associated with exercise-induced muscle damage

In addition to the functional decrements and associated soreness, EIMD may also result in an inflammatory response (Smith at al., 1989; Cannon et al., 1990; 1994; Fielding et al., 1993; Pizza et al., 1995). Inflammation is characterised by the movement of fluid, plasma proteins and leukocytes into tissues, in response to injuries, infections or antigens (MacIntyre et al., 1995). The production of plasma proteins is referred to as the acute phase response and is characterised by changes in

liver metabolism towards the increased production of a variety of 'generic' antimicrobial proteins, such as C-reactive protein (MacIntyre et al., 1995).

Leukocytes, primarily neutrophils and monocytes/macrophages are thought to perform a wide range of functions during the inflammatory response associated with EIMD (Tidball, 1995; Clarkson & Sayers, 1999). These may include the attack and breakdown of debris, the removal of this cellular debris and potentially regeneration of cells.

Neutrophils, which constitute 60% of circulatory leukocytes, are speculated to be the first cell to infiltrate damaged muscle fibres (Smith, 1991; Tidball, 1995; Abrams, 1997). Although increases in both circulatory neutrophils (Smith et al., 1989; Cannon et al., 1990; 1994) and accumulation of neutrophils in skeletal muscle tissue (Fielding et al., 1993) have been reported in humans following EIMD, the role of these cells in the damage and repair cycle is poorly understood (MacIntyre et al., 1995).

### 3.2.2 Free radicals and reactive oxygen species

By definition a free radical is any chemical species, capable of independent existence that has at least one unpaired electron. This characteristic makes a free radical particularly reactive, searching to pair its electronic status by extracting one electron from another molecule (Guiliani & Cestaro, 1997).

In the generally accepted fluid-mosaic model of biological membranes there is an important interaction between proteins and lipid. Essential to this interaction are

polyunsaturated fatty acids (PFA), found in the membrane and believed to contribute to the fluid characteristics of the membrane. However, PFA are very susceptible to radical attack, resulting in lipid peroxidation and changes in the fluidity of the membrane, potentially leading to a 'leaky' membrane with loss of intracellular enzymes (Jenkins, 1988). In human studies, there is some evidence to suggest a relationship between lipid peroxidation and loss of muscle cell membrane integrity following unaccustomed or prolonged eccentrically biased muscular activation (Kanter et al., 1988; Maughan et al., 1989; Eston et al., 1996). However, Saxton et al. (1994) found no evidence for the involvement of oxygen free radical species in EIMD, although, they acknowledged that there were potential flaws with potentially insensitive measures and small sample size.

### 3.2.3 Oestrogen, exercise-induced muscle damage and inflammation

Potential gender differences in exercise-induced muscle damage have been a focus of investigation for the past 20 years or so. These investigations have used *in vivo*, *in vitro*, animal and human studies, with equivocal results. It is believed that oestrogen, due to its antioxidant capacity, also giving rise to a membrane stabilising property and a gene regulatory effect, may have the potential to reduce exercise-induced muscle damage through these properties. For example, the antioxidant properties may act to 'quench' free radicals.

An area which has received little attention is the potential effect that oestrogen may have upon the inflammatory response associated with EIMD. Very few studies have examined this secondary aspect of EIMD in terms of gender differences or hormonal influences and even fewer have used human models of EIMD.

Komulainen at al. (1999) investigated the time course of structural changes within the muscle following downhill running in rats. They showed that while general histopathological changes in both genders were essentially similar, they occurred more slowly and to a lesser extent in the female rats compared to the males.

This slower time course was also demonstrated by St Pierre Schneider et al. (1996). They investigated the time course and concentration of leukocyte invasion in injured soleus muscle of male and female mice. The invasion of white blood cells began on day 1 after injury in both genders and diminished in males by day 5, remaining evident in females after day 7. It was found that at the maximal leukocyte invasion on day 1, male muscle fibre sections were characterised by a greater concentration of acid phosphatase-positive cells than muscle sections taken from females. The results of this study suggest a slower more prolonged time course of EIMD in female mice compared to males.

Tiidus and Bombardier (1999) investigated post-exercise tissue myeloperoxidase (MPO) activity (marker of neutrophil activation) in male and female rats which were treated and untreated with oestrogen. They observed that both gender and oestrogen administration affected post-exercise tissue MPO activity, by significantly attenuating this response. A further investigation utilising ovariectomised rats treated with oestrogen, provided additional evidence for the relationship between oestrogen and MPO activity. It was also observed that oestrogen significantly attenuated neutrophil number and calpain-like activity one hour after the exercise (Tiidus et al., 2001).

In terms of human studies, the literature is sparse. Stupka et al. (2000) investigated direct (biopsies) and indirect markers of muscle damage in humans following an eccentric exercise protocol. It was found that following exercise there was increase in the number of areas with focal and extensive myofibrillar damage and that this was essentially similar in males and females. However, men showed a significantly higher number of bcl-2-positive inflammatory cells in their muscles compared to women. Furthermore, and the number of LCA-positive inflammatory cells tended ($p=0.052$) to increase in men compared to women. Thus, while initial damage was similar in the two groups, the inflammation in the female subjects was significantly reduced.

However, these observations are equivocal. MacIntyre et al. (2000) found that following an eccentric exercise protocol, while there was an increased presence of neutrophils in the exercised quadriceps muscle of both men and women at 2h and 4h post exercise, the women showed a heightened neutrophil response, with a higher neutrophil concentration.

Therefore, the purpose of this investigation was to further investigate and understand the potential gender differences in the inflammatory response associated with EIMD in human participants.

### 3.2.4 Research hypotheses

The following hypotheses were investigated in the present study:

a) Males will be more susceptible to a bout of exercise-induced muscle damage compared to females, due to the protective effect of oestrogen;

b) Females will show lower levels of inflammation compared to males, due to inhibition of this response by oestrogen.

## 3.3 Method

### 3.3.1 Participants and design

Twelve healthy, untrained, but recreationally active volunteers participated in this investigation, six males (age 23.5 ± 3.6yrs, ht 181.5 ± 4.9 cm, mass 79 ± 8.5 kg) and six females (age 21.8 ± 3.6yrs, ht 163.6 ± 5.7cm, mass 57.6 ± 6.4kg). All volunteers completed an informed consent form and pre-test health questionnaires. The study was approved by the Ethics committee of the School of Sport, Health and Exercise Sciences at the University of Wales, Bangor.

The muscle group studied was the knee extensors. Baseline measures of isometric strength (assessed at angles of 10°, 20° and 80°, where full extension is equal to 0°), jump height, creatine kinase activity, soreness, mid-thigh circumference, white blood cell counts (neutrophils, lymphocyes, monocytes, eosinophils and basophils), elastase concentration (both unstimulated and lipopolysaccharide-stimulated) and C-reactive protein, were assessed prior to the exercise protocol which was designed to induce EIMD. Cell counts, elastase concentration and C-reactive protein were assessed again at 2 hours post, and at days 1, 3, 5 and 7 post exercise. All other criterion measures were assessed every 24 hours until day 7 after the damaging exercise protocol. In a small subset (two males and two females) oxidative burst activity was measured at baseline and at days 1, 3 and 5 post-exercise.

Participants were required to attend the laboratory on at least three occasions prior to commencing the study. This was to ensure thorough familiarisation with the equipment that would assess functional measures of EIMD (jump height and isometric strength), to reduce the training effect which we have found to be associated with such procedures.

### 3.3.2.1 Soreness

Soreness was reported using a visual analogue scale (VAS). Participants were asked to squat and to move a slider along the scale according to their perception of pain during this action. It was explained to the participants that the scale should be seen as a continuum, with *'no soreness'* at one end and *'the worst soreness ever'* at the other end. On the other side of this scale was a numerate scale running from 0-10 allowing the researcher to quantify the participant's response.

### 3.3.2.2 Creatine Kinase

Plasma creatine kinase (CK) activity was determined from a fingertip blood sample. A warm fingertip was cleaned with a sterile alcohol swab and allowed to dry. Capillary puncture was made with a softclix lancet and a sample of whole fresh blood (30μl) was pipetted from a capillary tube onto the test strip and analysed for CK activity via a colorometric assay procedure (Reflotron, Boehringer Mannheim, Lewes, UK). This system uses a plasma separation principle, which is incorporated in the reagent carrier on the test strip.

### 3.3.2.3 Isometric Strength

Participants were required to perform maximal voluntary isometric contractions of the knee extensors at 10°, 20° and 80° knee flexion on a Kin-Com isokinetic dynamometer (500H, Cattecx, Chattanooga, TN, USA). The testing positions were obtained by entering full knee extension (0°) as a reference value into the Kin-Com visual display. The reproducibility of this method was checked on each testing occasion by noting the Kin-Com angle display when the lever arm was at true 90° (determined by spirit level). The pre-test angle display at true 90° was used as the criterion. If any difference existed, the process was repeated until the criterion was achieved. Participants were required to perform three sub-maximal and one maximal practice repetitions as warm-up at each testing angle. Three maximal contractions of three seconds duration were performed at each joint angle with a one-minute rest period between repetitions. The highest average score from the three contractions was recorded and is reported in this investigation.

### 3.3.2.3 Jump Height

Counter-movement jump height was assessed. From a standing position participants were required to squat down and jump up as high as possible. Participants were allowed to use their arms to perform the jump to make the movement more ecologically sound. The simple apparatus consisted of a belt, which participants fastened around their waists, with cord connecting the belt to a mat on which the participants stood and had to land on, for the jump to be valid. The cord was made taut and zeroed. Height jumped was given in cm by a digital reader on the belt (Jump MD, Takei Scientific Instruments, Japan).

### 3.3.2.4 Mid-thigh Circumference

Mid-thigh circumference [midway between the inguinal crease and the proximal border of the patella] was assessed using an anthropometric tape measure. A permanent marker was used to mark reference points to allow repeated measurements across the test period.

## 3.3.3 Blood sampling and handling

Participants were required to attend the laboratory in a fasted state and requested to refrain from any strenuous physical activity for 24 hours preceding the baseline assessment and across the testing period. Additionally, participants were requested to avoid alcohol, caffeine and medication 24 hours prior to blood sampling. On days when blood sampling was required (base, day 1, day 3, day 5 and day 7) participants were required to sit quietly for 10 minutes prior to sampling. Blood samples were obtained by venepuncture from an antecubital vein. Three separate vacutainer tubes (Beckton Dickenson, Oxford, UK), one containing lithium heparin (approx 7 ml) and two containing EDTA (approx 4 ml) were used for blood collection. One EDTA sample was stored at 4°C and analysed within 16 hours for haematological analysis. 800 µl of the heparinised whole blood sample was used for the oxidative burst analysis and 1 ml was used in the degranulation procedure. The remaining heparinised blood and EDTA tube were centrifuged (3000 rpm for 10 minutes) within 15 minutes of sampling. Plasma was removed and immediately stored at 40 °C.

### 3.3.3.1 White blood cell counts

Differential leukocyte counts (total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils and basophils) were measured using a coulter counter (Beckman instruments, Fullerton, CA).

### 3.3.3.2 Plasma elastase and lipopolysaccharide-stimulated neutrophil degranulation

An ELISA kit (Merck, Lutterworth, UK) was used for the determination of the elastase concentration in plasma before and after treatment with bacterial lipopolysaccharide (LPS) stimulant (Sigma, Poole, UK). The principle of the ELISA assay is: in the first step of incubation, the elastase in the sample binds to the antibodies attached to the wall of the tubes. In the second stage of the incubation, antibodies marked with alkaline phosphate (AP) were added, which bind to the complex. After washing out the excess AP-marked antibodies, the enzyme activity of the immuno-complexed AP was measured photometrically at 405 nm. For the neutrophil degranulation assay, 1 ml aliquots of heparinised blood were added to eppendorf tubes containing 50 µl LPS, gently mixed, then incubated in a water bath for 1 hour (exactly). When the incubation period had elapsed, the tubes were centrifuged (13000 rpm for 1 minute) and the supernatant was frozen at 40 °C. Elastase concentration from the stimulated blood was performed as previously explained, and as reported in Blannin et al. (1997).

### 3.3.3.3 C-Reactive protein

An ELISA assay was used to asses C-reactive protein concentration. The principle is similar to that described in the elastase assay.

### 3.3.3.4 Oxidative burst activity

Bursttest (Orpegen Pharma, Heidelberg, Germany) allows the quantitative determination of leukocyte oxidative burst in heparinised whole blood. It determines the percentage of phagocytic cells which produce reactive oxidants and their enzymatic activity.

Heparinised whole blood was incubated with e-coli (as a stimulant) at 37°C, a sample without stimulus served as a negative background control. Upon stimulation, granulocytes and monocytes produce reactive oxygen metabolites (superoxide anion, hydrogen peroxide, hypochlorous acid) which destroy bacteria inside the phagosome. Formation of the reactive oxidants during the oxidative burst can be monitored by the addition and oxidation of DHR 123. The reaction is then stopped by addition of a lysing solution, which removes erythrocytes and results in a partial fixation of leukocytes. After one washing step with washing solution, DNA staining solution is added to exclude aggregation artifacts of bacteria or cells. The percentage of cells having produced reactive oxygen radicals were then analysed as well as their mean fluorescence intensity (enzymatic activity). Analysis was via flow cytometry (FACScan, Becton Dickinson, UK). The mean channel number of fluorescence intensity of activated neutrophils (FI) was used as an index of oxidative burst and phagocytic activity. The percentage of positive cells was counted to express the rate of neutrophils producing ROS.

### 3.3.4 Exercise protocol

The exercise protocol designed to induce EIMD consisted of 6 sets of 30 lunges on each leg followed by 10 sets of 10 counter movement jumps. During the lunges one

complete set of lunges on both legs were performed continuously (30 lunges on left followed immediately by 30 lunges on the right). A two minute break was given between sets. During the jumps, 10 continuous counter-movement jumps were performed with a one minute break between sets. Participants were strictly observed to make sure each movement was performed fully and correctly, also to ensure any stress placed on the knee due to incorrect form was alleviated.

### 3.3.5 Statistical analysis

Mixed model repeated measures (gender x time) analysis of variance were applied to the data. The number of levels on the repeated measures depended on the variable, for example soreness was assessed at baseline and then every 24 hours for seven days, resulting in 8 levels, whereas elastase concentration was assessed at baseline, then 2h post exercise, day 1, day3, day 5 and day 7 following the exercise procedure, resulting in 6 levels.

The assumption of sphericity was tested by the Mauchly Test of Sphericity. Any violations of this assumption were corrected by using the Greenhouse-Geisser adjustment to raise the critical value of F, by adjusting degrees of freedom, as indicated by $_{(GG)}$. Statistical significance was set at the 0.05 alpha level. Main effects for time were further investigated by pairwise comparisons, with the Bonferonni adjustment technique applied to eliminate the problem of an inflated Type 1 error risk. Significant gender x time interactions were further investigated using an adjusted Tukey's HSD post hoc procedure as described by Stevens (1996). The mean and standard deviations for each variable across the testing period and between each group are presented in Appendix 1.

## 3.4 Results

### 3.4.1 Soreness

Reported soreness on the visual analogue scale showed a significant main effect for time ($F_{(GG)2.8,\ 28.4} = 61.3$, $P<0.01$). Soreness was higher in both men and women on day 1 and remained elevated until day 4 after the exercise protocol. A main effect for gender was also found ($F_{1,10} = 7.6$, $P<0.05$). However, no gender x time interaction was found. As can be seen in Figure 3.1, the main effect for gender was due to the higher reported soreness in the females compared to the males.

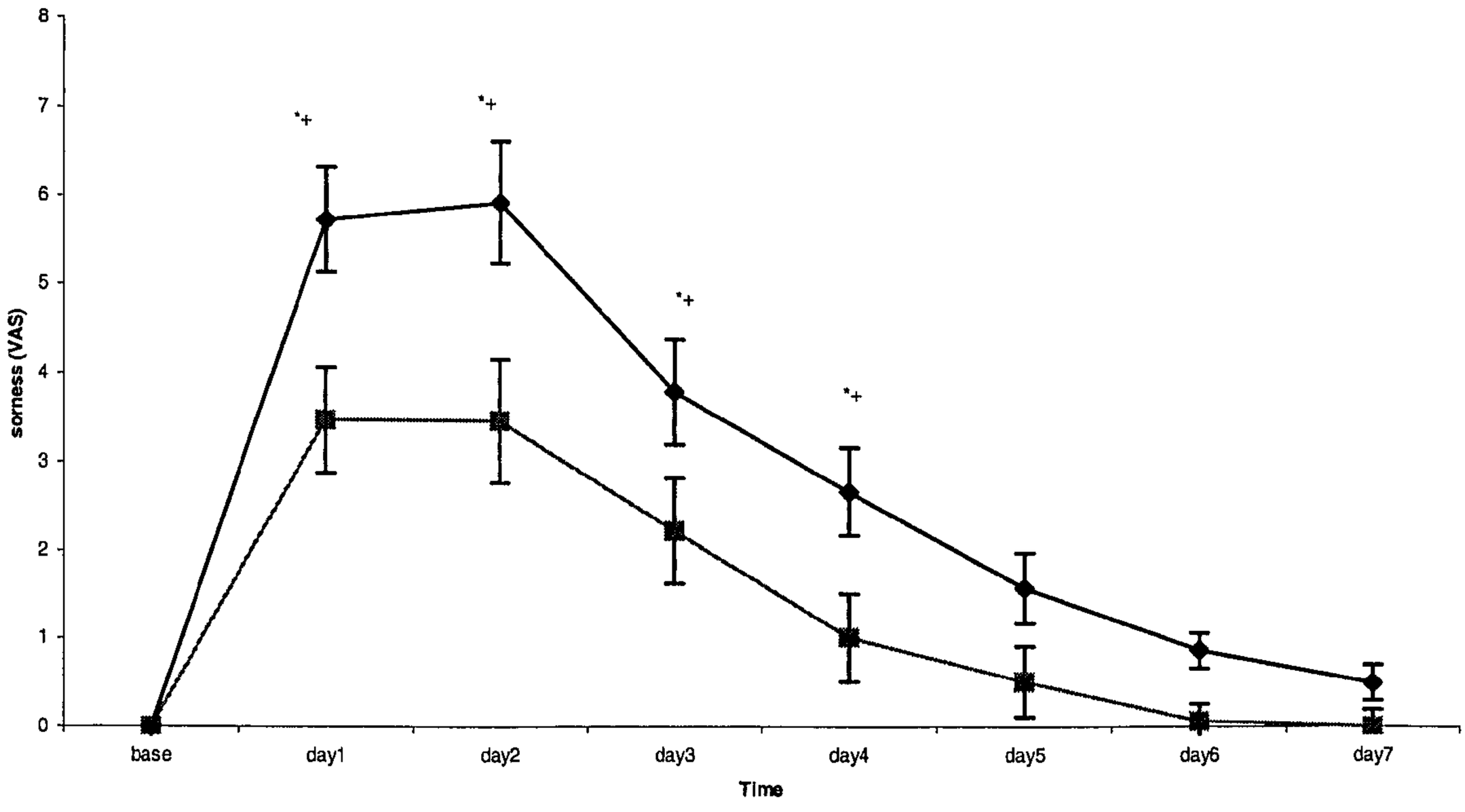



**Figure 3.1**:Incidence of soreness after an eccentrically biased exercise in men ( ▪ ) and women (♦) (mean ± S $\overline{x}$ ).* significantly ($P<0.05$) different from baseline, + significant ($P<0.05$) difference between groups.

### 3.4.2 Creatine Kinase

When CK was analysed in terms of absolute values a significant main effect for time was found ($F_{(GG)1.7,\ 17.7} = 13.2$, $P<0.00$). The main effect for gender approached significance ($P=0.07$) as did the gender x time interaction (P=0.1). As previous research has almost consistently shown a gender difference in CK, with males

showing higher concentrations, both at rest and following various exercise protocols (Meltzer, 1971; Shumate et al., 1979; Berg & Keul, 1981; Rogers et al., 1985; Amelink & Bär, 1986; Bär et al., 1988; Van der Meulan et al., 1991; Reijneveld et al., 1994; Dumke, 1996), there is justification to use one-tailed analysis, with α set at 0.1. In this case, both the main effect for gender and the interaction of gender x time are significant. However, as is common practice with CK, the values were log transformed due to the large amount of variance (violation of homogeneity of variance) associated with this variable. On doing so the main effect for time was maintained ($F_{(GG)2.6,\ 25.8} = 22.3$, $P<0.01$), with CK increasing significantly from baseline on day 1 after the exercise protocol and staying elevated until day 4. The main effect for gender was maintained if analysed with one tail analysis (i.e. $\alpha = 0.1$) ($F_{1,10} = 3.4$, $P = 0.096$), with males shower a higher concentration than females, but the gender x time interaction was now lost. Figure 3.2 illustrates these findings.

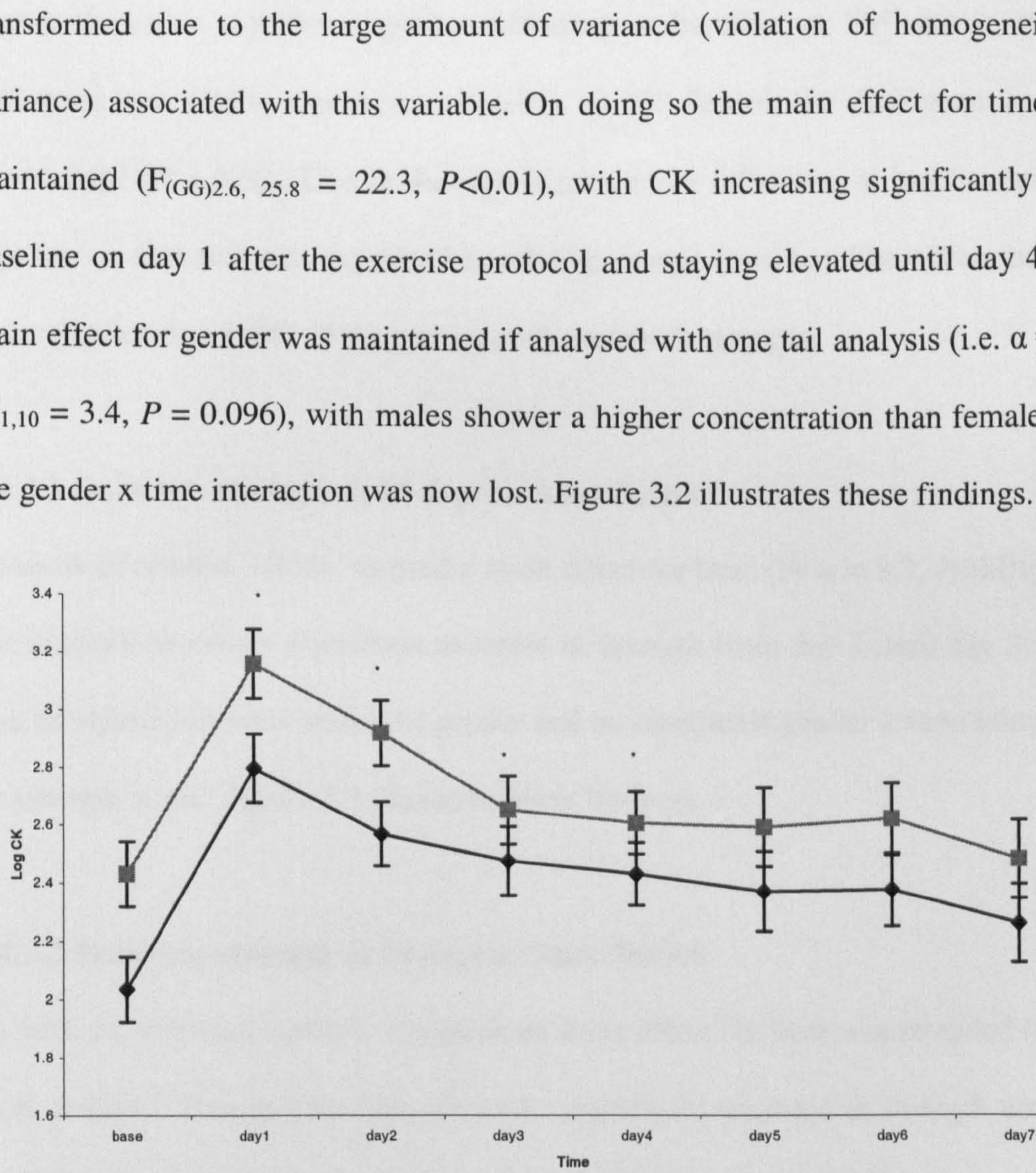



**Figure 3.2**:Log creatine kinase concentration after an eccentrically biased exercise in men ( ▪ ) and women (♦ )(mean ± S $\bar{x}$ ).* significantly ($P<0.05$) different from baseline

### 3.4.3 Isometric strength

A one way analysis of variance was calculated on baseline values to determine if a significant difference existed between males and females in isometric strength, to determine if the data should be expressed in terms of relative change from baseline. Due to the fact that there is a strong physiological argument for men being stronger than females $\alpha$ was set at 0.1, making the analysis one tailed, not two tailed. All angles showed a significant gender difference at baseline, at 20° and at 80° the difference was highly significant ($P<0.01$), at 10° flexion the difference was less pronounced ($P = 0.08$). Due to the significant gender difference at baseline analysis was run on data that was expressed as relative change from baseline, where baseline was considered as 100% of the participant's isometric strength.

#### 3.4.3.1 Isometric strength at 10 degrees knee flexion

Analysis of relative values, showed a main effect for time ($F_{7,70} = 8.3$, $P<0.01$). Post hoc analysis showed a significant decrease in strength from day 1 until day 3. There was no significant main effect for gender and no significant gender x time interaction on strength at 10°. Figure 3.3 illustrates these findings.

#### 3.4.3.2 Isometric strength at 20 degrees knee flexion

As with the previous variable, a significant main effect for time was revealed ($F_{7,70} = 12.8$, $P<0.01$). Post hoc analysis showed a significant decrease in strength until day 4, with a second drop on day 6 post exercise. There was no significant main effects for gender, or significant gender x time interactions found. Figure 3.4 illustrate these findings

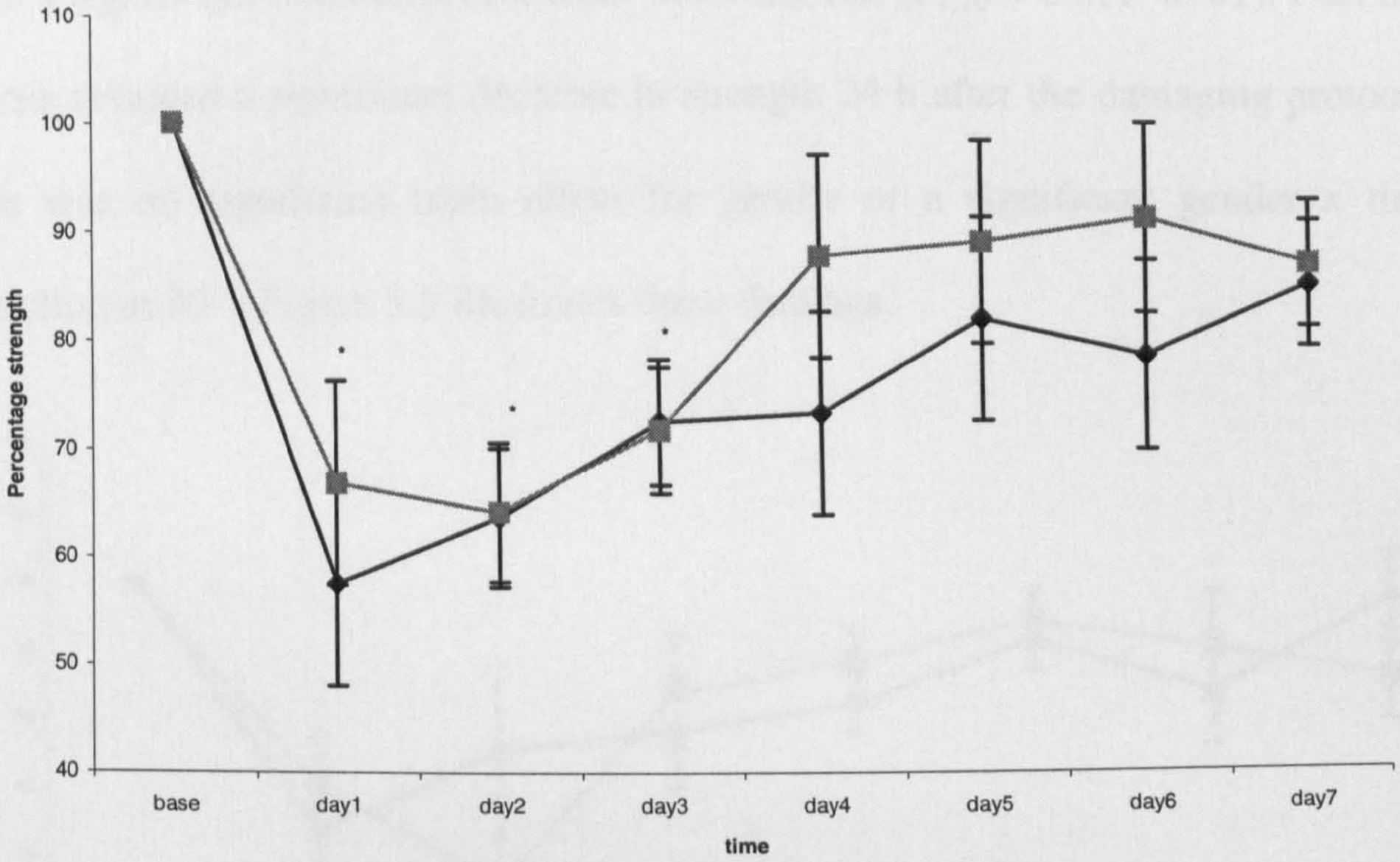



**Figure 3.3**: Relative change in baseline isometric strength at 10 degrees knee flexion after an eccentrically biased exercise in men ( ▪ ) and women (♦ )(mean ± S $\overline{x}$ ).* significantly ($P$<0.05) different from baseline

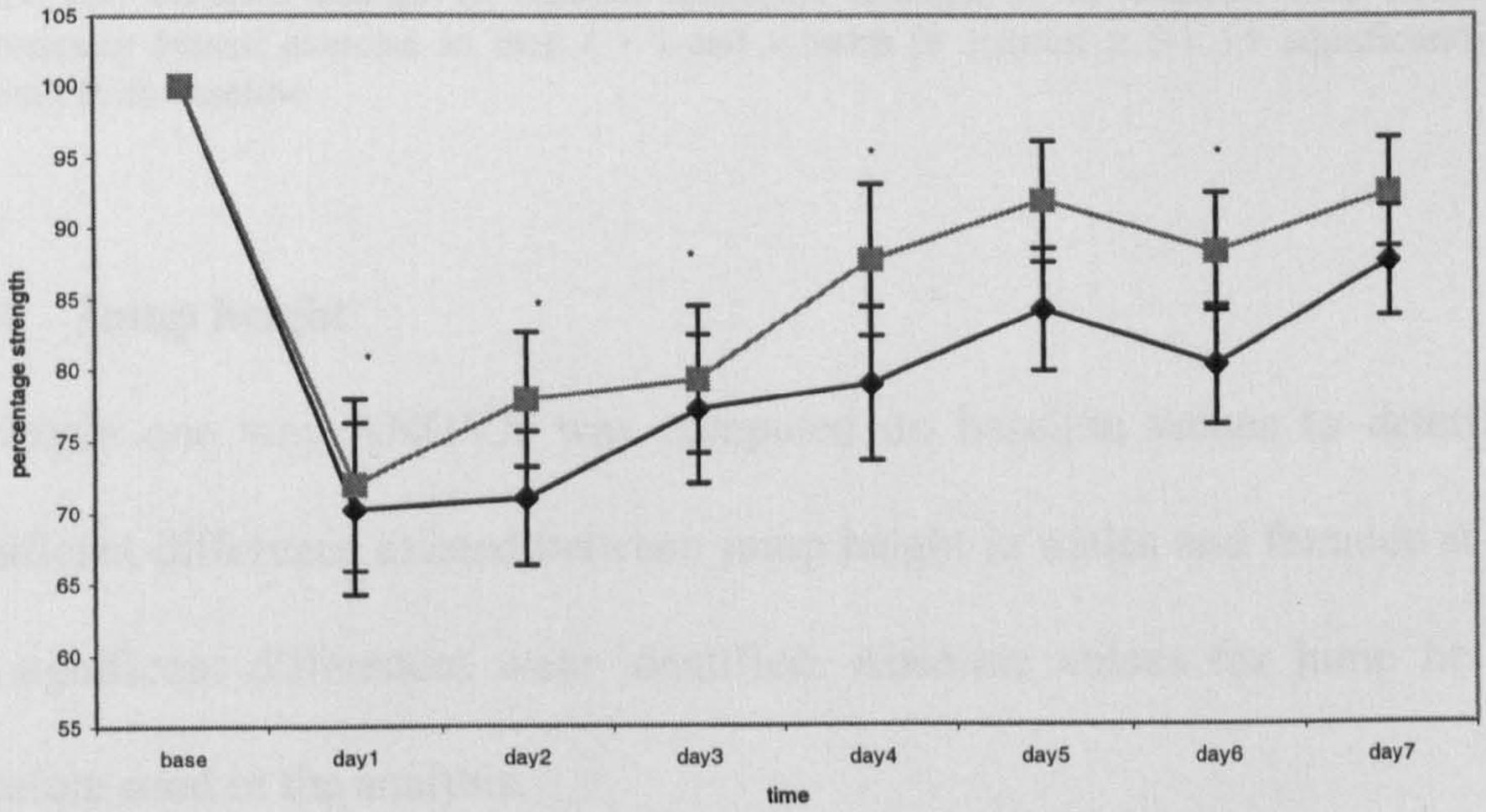



**Figure 3.4**: Relative change in baseline isometric strength at 20 degrees knee flexion after an eccentrically biased exercise in men ( ▪ ) and women (♦ )(mean ± S $\overline{x}$ ).* significantly ($P$<0.05) different from baseline

### 3.4.3.3 Isometric strength at 80 degrees knee flexion

Again a significant main effect for time was observed ($F_{7,70} = 6.3$, $P<0.01$). Post hoc analysis revealed a significant decrease in strength 24 h after the damaging protocol. There was no significant main effect for gender or a significant gender x time interaction at 80 °. Figure 3.5 illustrates these findings.

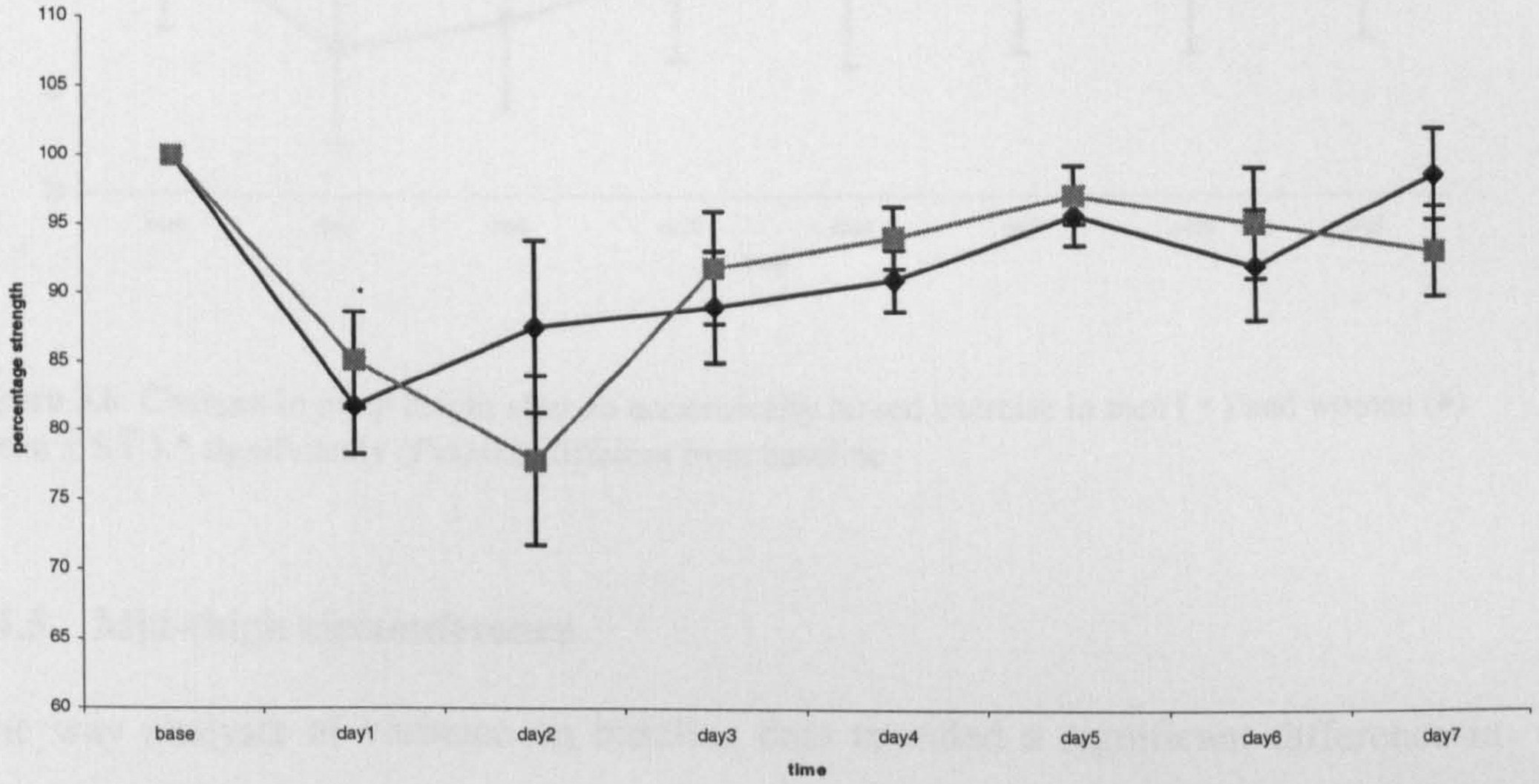



**Figure 3.5**: Relative change in baseline isometric strength at 80 degrees knee flexion after an eccentrically biased exercise in men ( ▪ ) and women (♦ )(mean ± $S\bar{x}$ ).* significantly ($P<0.05$) different from baseline

## 3.4.4 Jump height

A simple one way ANOVA was computed on baseline values to determine if a significant difference existed between jump height in males and females at baseline. No significant differences were identified. Absolute values for jump height were therefore used in the analysis.

A main effect for time on jump height was identified ($F_{7, 70} = 6.2$, $P<0.01$). Post hoc analysis revealed a significant decrease in jump height on day 1 and day 2 following the exercise protocol. There were no significant differences between gender or a significant gender x time interaction. Figure 3.6 illustrates these findings.

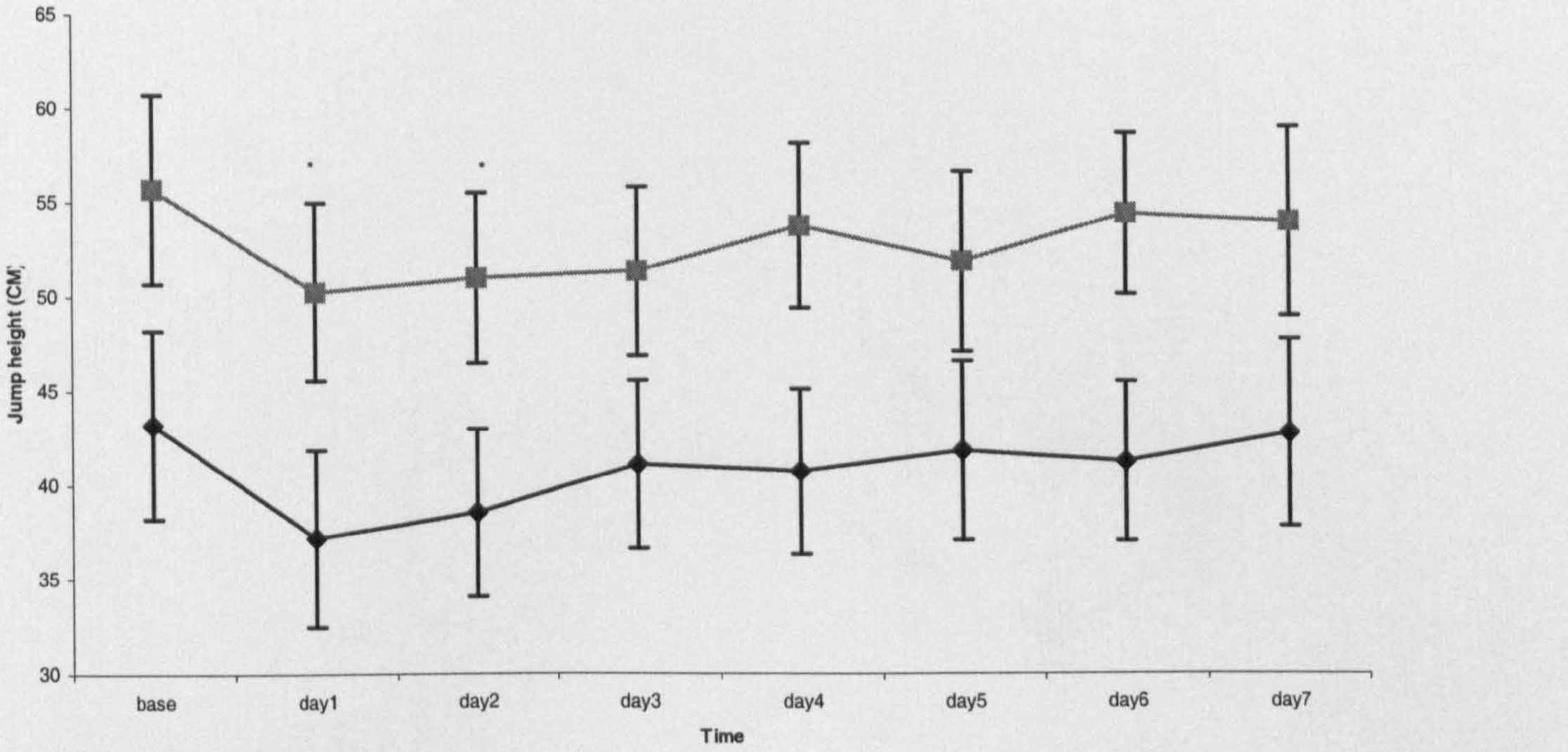



**Figure 3.6**: Changes in jump height after an eccentrically biased exercise in men ( ▪ ) and women (♦) (mean ± S $\overline{x}$ ).* significantly ($P$<0.05) different from baseline

## 3.4.5 Mid-thigh circumference

One way analysis of variance on baseline data revealed a significant difference in circumference between males and females, with males showing significantly larger circumferences than females. Therefore, the data were transformed into relative change from baseline.

There were main effects for time ($F_{7,70}$ = 4.0, $P$<0.01), with significant increases in circumference, and for gender ($F_{1,10}$ = 11.9, $P$<0.01), with larger increases seen in males. The gender x time interaction was not statistically significant. However, as can be seen in Figure 3.7, males and females did appear to respond differently, and the main effect for gender supports such a finding. The small sample size and relatively small increases in circumference may have reduced the power of the investigation and made statistical significance difficult to achieve.

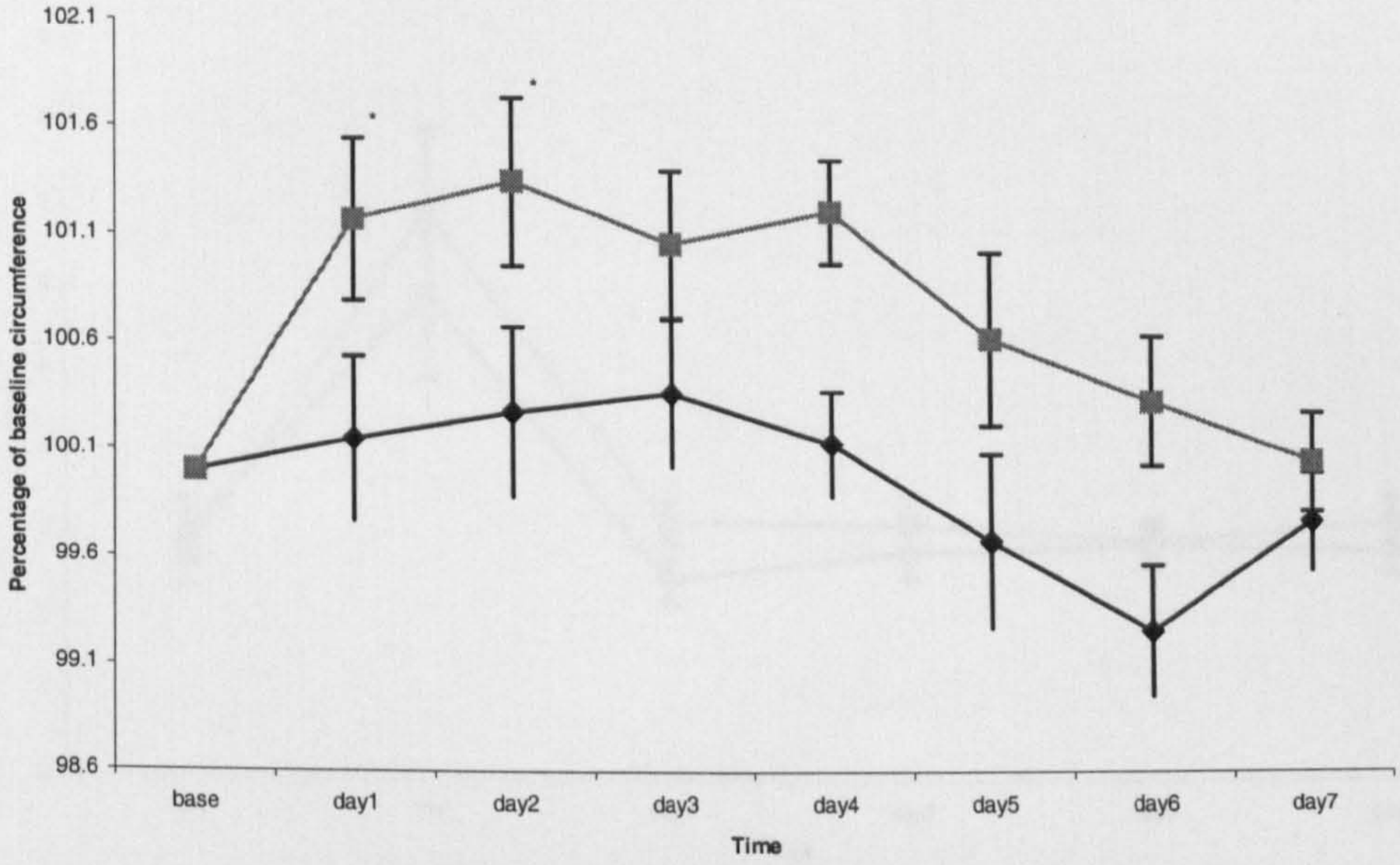



**Figure 3.7**: Percentage change of mid-thigh circumference after an eccentrically biased exercise in men ( ▪ ) and women (♦ )(mean $\pm$ S $\overline{x}$ ).* significantly ($P$<0.05) different from baseline

## 3.4.6 White blood cell counts

Total leukocyte count (neutrophils, lymphocytes, monocytes, eosinophils and basophils combined) showed a significant main effect for time ($F_{(GG)1.7,16.8}$ = 18.8, $P$<0.01), with an expected significant increase in the count 2 h after the exercise protocol. There were no significant main effects for gender nor a significant gender x time interaction. Analysis of leukocyte subunits demonstrated that the significant increase at 2 h post exercise could be accounted for by the significant increase in neutrophil count ($F_{(GG)1.4,\ 14.1}$ = 21.8, $P$<0.01) as no other subunit changed significantly across the testing period. There were no significant main effects for gender and no significant gender x time interactions in any of the leukocyte sub cells.

Figure 3.8 illustrates the increase in neutrophil count 2 h post

exercise.

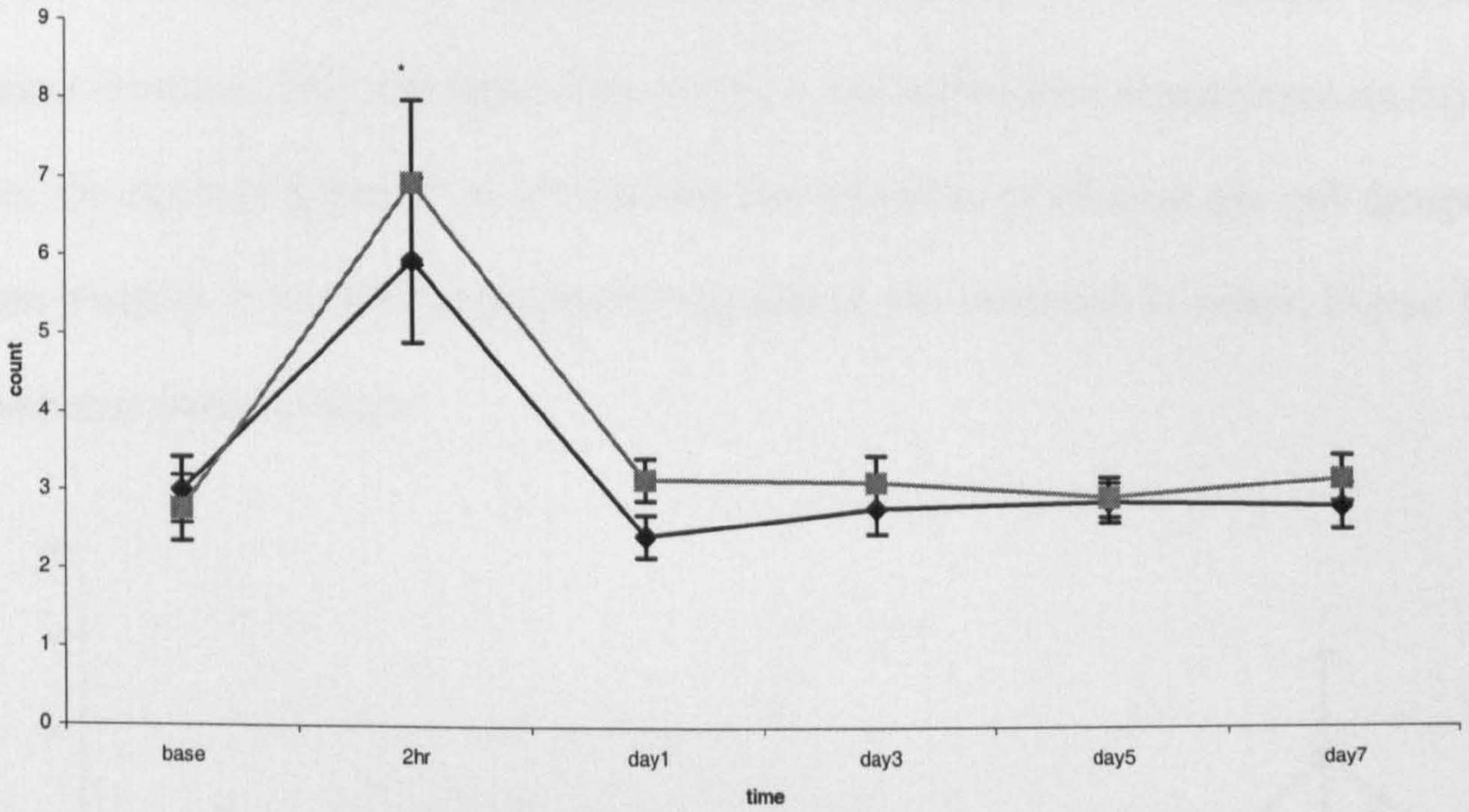



**Figure 3.8**: Neutrophil count ($10^9$/l) after an eccentrically biased exercise in men ( ▪ ) and women (♦) (mean ± S $\overline{x}$ ).* significantly ($P$<0.05) different from baseline

### 3.4.7 Elastase concentration

There were no significant changes in elastase concentration across the testing period ($F_{5,50} = 0.97$, $P = 0.45$) and no gender differences. Upon stimulation with LPS, there was a significant main effect for time ($F_{(GG)1.8,\ 17.8} = 9.2$, $P$<0.01), with a significant increase in elastase 2 h post exercise (1059 ± 397µg/l vs 2122 ± 915 µg/l). There were no gender differences in elastase. When LPS-stimulated elastase was expressed per neutrophil, no significant changes were seen across the testing period. When plasma elastase (i.e. no stimulation) was expressed per neutrophil, a significant main effect for time was observed ($F_{5,\ 50} = 3.3$, $P$<0.05), with significant decreases 2 h post exercise. This would indicate that the neutrophils were not producing as much elastase 2 h post exercise. Additionally a significant gender x time interaction ($F_{5,50}$ = 3.1, $P$<0.05) was observed. An adapted Tukeys post hoc test was used to probe this interaction further. It was found that at baseline no significant differences existed in

the elastase concentration, as expressed per neutrophil, in males and females. However, on day 1 after the exercise protocol the elastase concentration per cell was greater in males. This was again seen on day 5 and approached significance on day 7 after the exercise protocol. In general, the concentration of elastase per cell dropped from baseline in females across the testing period, but increased in males. Figure 3.9 illustrates these findings.

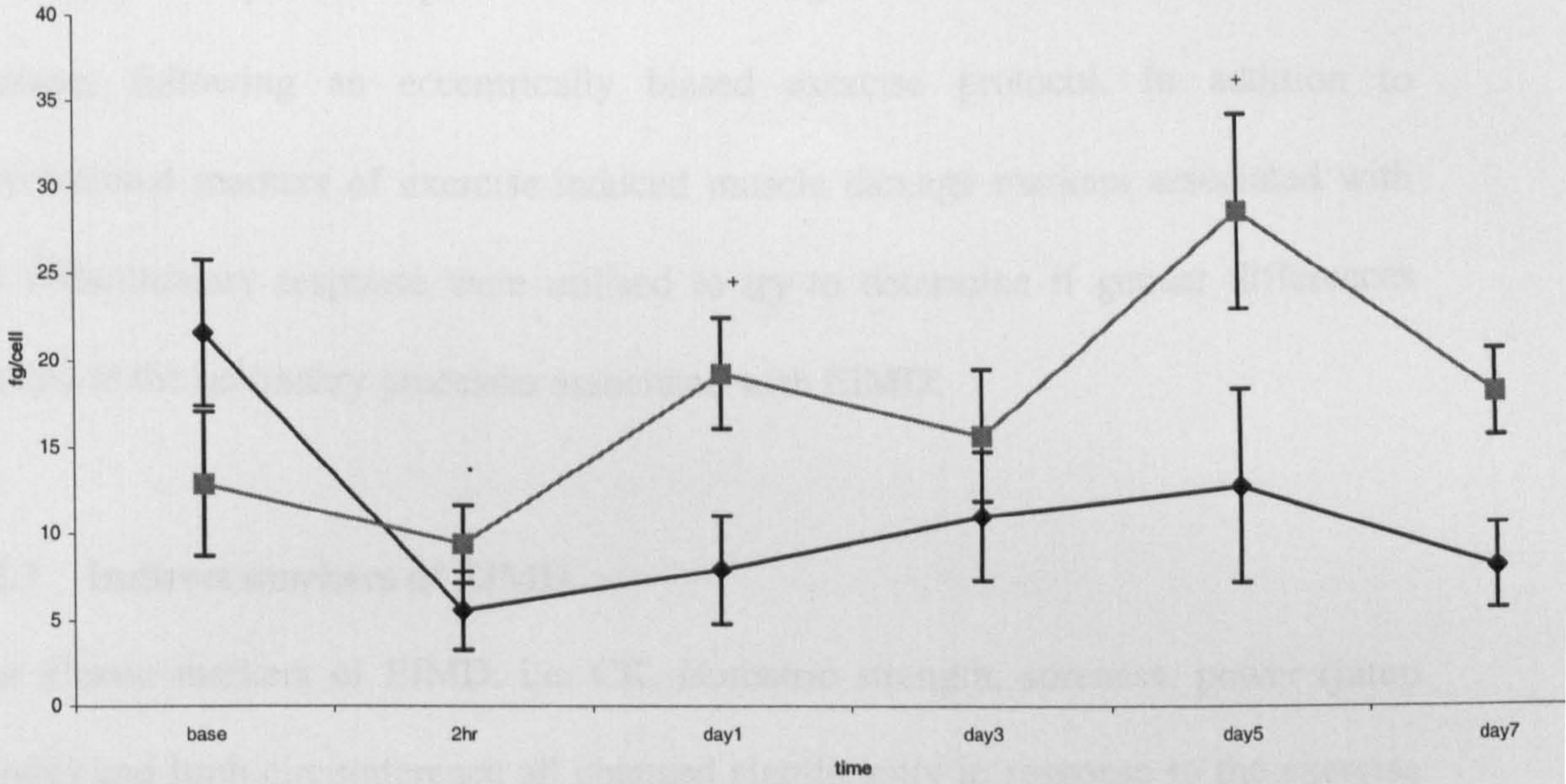



**Figure 3.9**: Elastase concentration per neutrophil after an eccentrically biased exercise in men ( ▪ ) and women (♦ )(mean ± S $\bar{x}$ ).* significantly ($P$<0.05) different from baseline, + significant ($P$<0.05) difference between groups.

### 3.4.8 C-reactive protein

C-reactive protein, a marker of acute phase response was undetectable across the testing period with the exception of four samples. Participant 5 showed heightened concentration on days 1, 3 and 5 (29.3, 26.6, 17.3 mg/l) and participant 12 on day 3 (19.4 mg/l).

### 3.4.9 Oxidative burst activity

Oxidative burst activity, did not change significantly across the testing period and did not differ between the two groups. However, it should be noted that this data were based on four subjects only (2 males and 2 females).

## 3.5 Discussion

This study attempted to explore the influence of gender on exercise-induced muscle damage, following an eccentrically biased exercise protocol. In addition to conventional markers of exercise-induced muscle damage markers associated with the inflammatory response were utilised to try to determine if gender differences existed in the secondary processes associated with EIMD.

### 3.5.1 Indirect markers of EIMD

The classic markers of EIMD, i.e. CK, isometric strength, soreness, power (jump height) and limb circumference all changed significantly in response to the exercise protocol. The changes were all in the direction that would be expected if significant EIMD had been induced. Of these markers, CK, soreness and mid-thigh circumference all demonstrated significant gender differences.

#### 3.5.1.1 Creatine Kinase

The gender difference found in creatine kinase in this investigation is not surprising. Gender differences in this protein have been well documented (Meltzer, 1971; Shumate et al., 1979; Berg & Keul, 1981; Rogers et al., 1985; Amelink & Bär, 1986; Bär et al., 1988; Van der Meulan et al., 1991; Reijneveld et al., 1994; Dumke, 1996) with females showing less CK activity than males in various conditions. In fact, the

gender difference found in this protein appears to have been one of the main stimuli in investigating the potential gender differences in exercise-induced muscle damage, over 20 years ago.

As well as the documented gender differences in CK following exercise, hormonal manipulation in animals has shown that the presence of oestrogen has the ability to reduce CK activity following exercise, in both females and males. Loss of oestrogen in females through ovariectomy attenuates this effect (Bär et al., 1988). The results of the hormonal manipulation studies suggest that the difference is due to oestrogen rather than due to gender differences in skeletal muscle mass.

Creatine kinase has been criticised as a poor marker of EIMD (Warren et al., 1999). Thus, this alone does not allow any strong conclusions to be drawn. Such a finding may indeed be due to reduced levels of muscle damage in the female muscle, therefore resulting in less CK entering the blood. Alternatively, as oestrogen is known to act in similar ways to cholesterol (Wiseman et al., 1993; Wiseman & Quinn 1994), in terms of stabilising cell membranes, it is possible that the female cell membrane has the potential to reduce the flux of CK across the membrane and into the blood system, without a concurrent reduction in muscle damage. Confirmation of a gender difference from other markers of EIMD is definitely needed.

### 3.5.1.2 Soreness

As has previously been shown in chapter 2, the perception of soreness is very complex. In this investigation it was found that the increases in CK and mid-thigh circumference were greater in males, which could perhaps indicate that the level of

EIMD was more severe in males. However, reported soreness in females was greater than that of their male counterparts.

If soreness was a fair gauge of damage, one might have expected that males would have reported more soreness than females. Why this was not found is difficult to determine. The first problem is that the relationship between soreness and markers of EIMD has not been investigated to any extent. Thus, while inflammatory processes may be expected to activate pain receptors through chemical, temperature and pressure stimulation, what is not known is whether this results in greater pain production than if damage had been induced and inflammation had been suppressed (e.g. by the presence of oestrogen), slowing down the repair process. The sensation of DOMS (delayed onset muscle soreness) is not well understood. However, the delay in soreness associated with EIMD suggests that inflammatory processes are indeed involved. Therefore, one would expect that males would report as much, if not more soreness than females.

Fillingim and Ness (2000) review the multifaceted area of gender differences in pain perception, which includes both animal and human studies, with sections on the menstrual cycle. They suggested that women are more sensitive to pain stimuli. This may explain why females, in the current investigation reported more soreness, despite the extent of damage in females being the same, if not less than males. However, the evidence from the studies is equivocal, with reports of females showing both higher and lower pain thresholds than males across various stimulants and conditions.

Barsky et al. (2001) reviewed somatic symptom reporting by females. They reported that females are more likely to report more intense, more numerous and more frequent bodily symptoms than men. These differences are seen in community samples, in medical patients and from adolescence to the elderly. They indicated that men may label and describe the same noxious sensation differently. Women may be more aware of and more attentive to weak or diffuse bodily stimuli which men don't perceive. In support of this, previous research suggests that women have greater bodily vigilance and awareness (Lieban, 1985; Warner, 1995).

Biological differences may not provide sufficient explanation for the perception of soreness, Barsky et al. (2001) believe that psychological factors, for example socialisation are very important. The socialisation process, which begins in earlier childhood, may profoundly influence bodily experiences and the willingness to disclose and communicate distress. They reported that male children are taught to be less expressive about illness and discomfort, to be more stoical and use denial. If this is learned from a young age they may be reticent to give a true indication of the extent to which they are feeling discomfort. This may be another factor which would explain why in our investigation the soreness reported by the males, is less than that reported by the females, despite receiving as much if not more damage to their muscles.

A final consideration, which has not been investigated to any extent and certainly not in terms of reported pain is the potential effect the gender of the experimenter has upon the participant. The experimenter in this investigation was female. It is possible that the females felt more relaxed divulging the extent of their muscle soreness to a

female experimenter, but that males felt less comfortable. This may need be a consideration for future investigations as should the appropriateness of soreness as an accurate gauge of EIMD.

### 3.5.1.3 Mid-thigh Circumference

There was less swelling of the mid-thigh in the female participants compared to the males. The limited swelling in females, may be due to less inflammation. Because no biopsies were taken in the current investigation, we can only surmise that the marked increase in circumference in the male participants was due to inflammatory processes, possibly as the result of more extensive damage, but certainly resulting from higher levels of cellular infiltration.

## 3.5.2 Inflammatory markers

The inflammatory response associated with EIMD has been shown to vary between male and females. General inflammation has been shown to occur more slowly (St Pierre Schneider et al., 1996; Komulainen et al., 1999) and to a lesser extent (St Pierre Schneider et al., 1996; Komulainen et al., 1999; Tiidus & Bombardier 1999; Stupka et al., 2000) in females. In addition, specific events associated with the inflammatory response are moderated by oestrogen, for example $Ca^{++}$ homeostasis (Prakash et al., 1999; Jovanovic et al., 2000), calpain (Tiidus et al., 2001) and cytokines (Pottratz et al., 1994; Chao et al., 1995; Angstwurm et al., 1997; Schwarz et al., 2000). Therefore, suppressed swelling in this investigation, which quite reasonably would suggest reduced inflammation, may be due to the properties of oestrogen. However, it should be born in mind that C-reactive protein, a marker of the acute phase response remained undetectable in the majority of participants. In

addition, no gender differences in circulating neutrophils were seen and neutrophil counts were not elevated in either group in the days following the exercise protocol, only at 2 h post exercise. The C-reactive protein and neutrophil response may suggest that the inflammation was minimal in both groups.

### 3.5.2.1 Neutrophil Count

In this investigation we used circulatory parameters to estimate an inflammatory response. Warren et al. (1999) have criticised the use of circulatory markers to estimate EIMD. They explained that even if muscle damage is present, the extent of the damage cannot be correctly estimated by any types of markers. This is based upon the fact that the concentration of any substance, present in the blood is simply a reflection of the difference between release and uptake by different tissues. At sites of inflammation, leukocytes (primarily neutrophils) decelerate their transit. If the stimulus persists, the rolling leukocytes become activated, adhere firmly and migrate across the endothelium (Celi et al., 1997). The neutrophil-endothelium adhesion is a reversible process and with mild insults there may be intravascular accumulation of neutrophils and without sufficient stimulus the neutrophil migration from the blood vessels and into the tissues will not occur (MacIntyre, 1995).

In this investigation it was found that at two hours post exercise there was a significant increase in neutrophil count in both groups. This is not necessarily evidence of inflammation. During exercise the release of cortisol, adrenaline and other factors lead to demargination of neutrophils from the bone marrow and into circulation. Previous research into EIMD and the inflammatory response has observed a heightened neutrophil count in the first few hours following the exercise

protocol (Smith et al., 1989; Pizza et al., 1995; Malm et al., 1999). What has not been shown is whether this is simply due to exercise hemodynamics and demargination of neutrophils, or whether factors associated with EIMD are in fact acting as chemotactants for neutrophils. Pizza et al. (1995) cited Weiss (1989) and Evans and Cannon (1991) in explaining that circulatory levels of neutrophils peak at 1-2h following injury or infection and generally have a half-life of less than 10h. If the insult in our investigation was only mild, it is possible that the initial increase in neutrophils was sufficient to cope with the level of damage, thus explaining why the neutrophil count had returned to baseline as early as day 1. With regard to exercise hemodynamics and demargination, Pizza et al. (2001) showed the above response following a single bout of damage-inducing exercise. However, in a repeated bout this neutrophil response was not seen, suggesting that damage was the stimulus rather than the act of exercise.

It is clear that without the more invasive procedure of biopsies, it would not be possible to determine if the neutrophils were stimulated enough to enter the injury site. It would generally be fair to assume however, that if the neutrophils were primed (by factors associated with EIMD) more neutrophils would have been generated in the days following the exercise protocol. There is a vicious circle associated with inflammation. Damage evokes a cascade of events, with neutrophils producing oxygen radicals which cause more damage, and attract further neutrophils and eventually macrophages. The latter have been shown to be vital to the repair process, especially $ED2^{+}$ macrophages. In this investigation no significant changes were found in the circulatory macrophage count, again suggesting no stimulus for

these cells. It should be noted however that muscle cell infiltration was not assessed and specific macrophage subsets were not investigated in this study.

### 3.5.2.2 Elastase

In an attempt to understand and follow the time course of EIMD and determine if inflammatory differences existed between males and females, we also assessed elastase concentration (both unstimulated and challenged with LPS), reported as both total concentration and expressed per neutrophil. Elastase is found in the granules of neutrophils and release of these granular enzymes has been used as an indicator of neutrophil activation.

The general finding in this part of the investigation was that elastase concentration did not differ across the testing period. Gleeson et al. (1998) found that following a severe electrically stimulated exercise-induced muscle damage protocol, there was only a small increase in elastase concentration. They believed that this could be explained by one of two things a) there was an increase in neutrophil activation, i.e. the neutrophils had been primed by any number of factors associated with EIMD and this had resulted in the release of their granular enzymes, including elastase into the circulation, or alternatively b) this increase was simply due to an increase in the number of circulatory neutrophils, which in turn resulted in an increase in elastase concentration. Our investigation did not replicate such a finding, probably due to the less severe nature of the protocol used. Not only did we find no significant changes in elastase across the testing period, no gender difference were found.

When elastase concentration was expressed as elastase per neutrophil a very interesting gender difference was discovered. It was found that elastase concentration per neutrophil was significantly lower in the females compared to the males. The meaning and consequence of such a finding is rather difficult to determine from the measures of the current investigation. Such a finding may be due to a protective effect of oestrogen. However, the markers used in this investigation only hint at a gender difference in damage and inflammation (elevated CK activity and increased mid-thigh circumference in males) and total plasma elastase did not change significantly across the testing period in either groups, which suggests minimal inflammatory stimulus. One could suggest that the male neutrophil is being primed by one of many inflammatory/damage induced factors (disturbances in $Ca^{2+}$ homeostasis and calpain production and increases in proinflammatory cytokines), whereas the female neutrophil is lying in a more dormant state, having not been exposed to such stimuli. This may explain why we found a reduced elastase concentration on a per cell basis in the female participants.

Alternatively, it could be suggested that the female neutrophil is simply less effective. Elastase production per cell is a measure of neutrophil function. Therefore, it may be argued that given a similar level of EIMD in males and females, the female may be compromised by less effective neutrophils. Further evidence from this investigation, suggests that this simply is not the case. When the blood is challenged with LPS there is a significant increase in elastase 2 h post exercise, which returns to baseline by day 1, with no gender differences in this response. When this is expressed on a per cell basis, no significant changes are found in the elastase produced per cell across the testing period and more importantly no gender

differences were found. This would suggest that the female neutrophil is more than capable of activation if challenged and is not immunocompetent. The LPS stimulation results in the same activation response in both groups, which does suggest that the reason the unstimulated plasma elastase concentration per cell is lower in females is due to a lack of priming.

This finding is of interest at several levels. Firstly, the role of oestrogen in EIMD is not well understood. Oestrogen is an antioxidant with membrane stabilising properties and a gene regulatory effect. Whether these properties are in fact positive or inhibitory is yet to be discovered. If upon the same stimulation as males, female neutrophil activation occurs to the same extent as in their male counterparts, it would suggest that if a threat was present, one of the most important cells involved in the inflammatory process is not compromised and would function well. This is based solely on the elastase response from this investigation. Whether the oxidative burst capacity and elastase are compatible is not answered in this investigation e.g. the neutrophils have responded but is the action of their content inhibited by antioxidants? Unfortunately, the lack of information from this investigation on oxidative burst activity will not allow this to be answered, but certainly warrants further investigation.

Secondly, if neutrophils are capable of activation and the properties of oestrogen do not interfere at this level of the muscle damage and repair cycle, at what level are they acting? What processes have been inhibited to stop the neutrophils being primed in the current investigation?

Thirdly and finally, what consequence does this inhibition have in terms of the repair and regeneration process? Does this leave the female muscle more prone to a second bout of EIMD due to poor regeneration from a primary insult. If the neutrophils are not active, but the level of muscle damage is comparable between the groups does this result in slower removal of debris and regeneration in the female muscle?

### 3.5.3 Summary and conclusion

This investigation has generated a number of unanswered questions. It still remains unclear if oestrogen offers protection against exercise-induced muscle damage. What is clear is that males and females do respond differently to the same exercise protocol. This initially is important for anyone who is wishing to perform research in the area of exercise-induced muscle damage. Secondly, the question of whether soreness is an objective gauge of muscle damage has yet again been questioned.

Due to the small sample size, and lack of direct measures of muscle damage in this study, it is difficult to determine whether oestrogen reduces inflammation associated with EIMD. The study provides further confirmation that CK concentration is lower in female participants following a bout of exercise-induced muscle damage. In addition to this finding, we have also shown that swelling, an indirect marker of inflammation is lower in the females and that elastase production per cell is also lower in the female participants. Whilst it is recognised that this area of research requires considerably more extensive investigation, it is one of the first human studies which suggests that women may have an inhibited inflammatory response despite demonstrating similar levels of damage to males on other markers.

# CHAPTER 4

# EXERCISE-INDUCED MUSCLE DAMAGE AND THE REPEATED BOUT EFFECT IN MALES AND FEMALES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#### 4.5.3 Summary and conclusions

## 4.1 Abstract

Evidence suggests that oestrogen may reduce inflammation, possibly through its antioxidant properties. It has been shown that inhibited inflammation (through anti-inflammatories) following exercise-induced muscle damage can result in less protection following a repeated bout of exercise-induced muscle damage. Therefore, the purpose of the current investigation is to determine if females (through the action of oestrogen) are less protected from a repeated bout of damage inducing exercise compared to males. Twenty healthy, untrained but recreationally active volunteers participated in this investigation (eleven males (age 19.9 ± 1.3 yrs, ht 175.5 ± 5.6 cm, mass 77.4 ± 6.51 kg) and nine females (age 19.1 ± 1.3 yrs, ht 168.8 ± 6.3 cm, mass 67.1 ± 9.3 kg). Baseline measures of soreness, CK activity, isometric strength and isokinetic function (torque, force, work done and index of fatigue) were assessed at baseline prior to exercise and every 24 hours following exercise for 4 days. The exercise consisted of 6 sets of 30 lunges on both legs and 10 sets of 10 counter movement jumps. A mixed model repeated measures ANOVA [gender (2) x time (5) x bout (2)] revealed all measures showed a significant main effect for time ($P<0.05$), indicating that the exercise procedure had been successful in inducing damage. All measures, but mean peak force showed a main effect for bout, whereby changes in markers of EIMD were less pronounced in the repeated bout when compared to the original. Gender differences were small, although soreness approached significance ($P = 0.09$), with females reporting more soreness than males. In addition, a significant gender x time x bout interaction was observed for the index of fatigue. Males and females responded with a pronounced difference in this index during bout one, but almost identically in bout two. In conclusion, this study provides no evidence that female skeletal muscle is more susceptible to a second bout of muscle

damage. However, it does, provide more evidence for gender difference in the response to EIMD.

## 4.2 Introduction

Exercise-induced muscle damage (EIMD) has long been an associated symptom of activities containing a large component of eccentrically-biased exercise, especially unaccustomed eccentric exercise. This effect is well documented, but still relatively poorly understood. The symptoms of EIMD can last for up to 10 days after the initial exercise insult. As well as decrements in strength and muscle function, other symptoms include the characteristic delayed-onset muscle soreness (DOMS) and swelling of the damaged limb. More recently, research has focussed on the potential inflammatory and secondary events associated with EIMD.

### 4.2.1 Inflammation and repair following exercise-induced muscle damage

Inflammation is characterised by the movement of fluid, plasma proteins and leukocytes into the affected tissue. At least two cell populations respond to muscle injury: inflammatory cells involved in the removal of cellular debris and myogenic cells involved in replacement of the damaged muscle (Tidball, 1995). Cells involved in inflammation include leukocytes, primarily neutrophils and monocytes. These cells are involved in the attack and breakdown of debris (neutrophils and macrophages), removal of debris (neutrophils and macrophages), and regeneration of cells (macrophages). Many complex events and interacting cells are involved during inflammation. A crucial, but poorly understood stage during this process is the stimulation of satellite cells to divide which leads to the eventual repair of the muscle. However, it appears that invasion of an injury site by macrophages, specifically ED2+ cells, is an essential prerequisite for regeneration, possibly through activation of satellite cell division (Jones & Round, 1990).

It would seem that if the process of inflammation results in the eventual repair and regeneration of the muscle (primarily orchestrated by ED2+ macrophages), then inhibition of such processes may negate repair and slow down the regeneration of the muscle and return to normal function. For a more detailed discussion of the inflammatory response associated with EIMD see chapter 1.

### 4.2.2 Oestrogen and inhibition of inflammation

Oestrogen is believed to play a significant role within the muscle damage and repair cycle. The female sex steroid is an antioxidant, with membrane stabilising and gene regulatory effects. It is believed that through these interacting properties, oestrogen has the potential to inhibit many factors associated with EIMD. For example, it has been shown that oestrogen has the potential to reduce and/or slow down the inflammatory response associated with EIMD (St Pierre Schneider et al., 1996; Komulainen at al., 1999; Tiidus and Bombardier, 1999; Stupka et al., 2000; Tiidus et al., 2001). In addition to these studies that have shown that oestrogen may play a role in general inflammation, many studies have shown that oestrogen can both 'up-regulate' and 'down-regulate' specific events associated with the damage-inflammation-repair cycle, for example cytokine activity (Pottratz et al., 1994; Chao et al., 1995; Angstwurm et al., 1997; Schwarz et al., 2000). Thus, the role of oestrogen is a complex one. A more detailed description of the properties of oestrogen and its potential role during inflammation can be found in chapter one.

What has yet to be determined is whether the potential inhibitory effect of oestrogen, both in terms of muscle damage and secondary processes, is in fact positive or

negative. If inflammation is such an important process, then inhibition may well leave the muscle compromised, with poor regeneration and repair.

### 4.2.3 The repeated bout effect

A further dimension of exercise-induced muscle damage is the so called 'repeated-bout effect'. This refers to the apparent protective effect that one bout of exercise-inducing muscle damage has upon a second bout of the same type of exercise. The reduction in symptoms of exercise-induced muscle damage following a second bout of a similar protocol is well documented, e.g., see review by McHugh et al. (1999), but is poorly understood. Three adaptive mechanisms have been proposed to explain this phenomenon: the neural theory; the connective tissue theory; and the cellular theory.

The neural theory is based upon the selective recruitment of fast-twitch fibres following the initial unaccustomed bout of exercise. These fibres are more prone to EIMD and fatigue (McHugh et al., 1999; Lapointe et al., 2002; Byrne & Eston, 2002b). Following the repeated bout there is a shift to recruiting more slow twitch fibres which are less susceptible to EIMD (McHugh et al., 1999; Lapointe et al., 2002). The cellular and connective tissue theories purport that adaptation proceeds from successful muscle repair and regeneration of contractile and structural components (Lapointe et al., 2002). These investigators reported that inflammation, by its very nature, must be involved in this adaptive process, as the primary purpose for inflammation is to remove debris from a site of injury and stimulate the repair process. This further suggests that inhibition of the complex cascade of events

associated with inflammation may well compromise the repair and regeneration of a damaged muscle.

Lapointe et al. (2002) investigated the adaptive response associated with two bouts of EIMD in rats. The proposed hypotheses were that the adaptive effect would be observed even when voluntary recruitment was bypassed (possibly negating the neural theory) and inhibition of inflammation would reduce the adaptive response (supporting the cellular theory). It was found that adaptation following EIMD could not be accounted for by neural adaptation, due to the presence of a repeated bout effect even when the neural recruitment strategy was bypassed. However, when inflammation was suppressed with the use of Diclofenac (an anti-inflammatory drug) a significant impairment in the repeated bout effect was found. They concluded that adaptation was likely to be mediated by strengthening of muscle structural/cellular elements and that inflammation was important for this process.

Stupka et al., (2001) investigated the cellular adaptation to repeated eccentric exercise-induced muscle damage. In addition to this, they also investigated gender differences and to our knowledge are the first to investigate gender differences in the repeated bout effect associated with EIMD in humans. They hypothesised that changes in ultrastructural damage, inflammatory cell infiltration, and markers of proteolysis in skeletal muscle would come about as a result of a repeated bout of EIMD. The particular focus in their study was with regard to the intracellular pathways responsible for proteolysis (such as the protease calpain). Interestingly, their results showed that despite the apparent protective effect of bout 1 as indicated by the reduced CK and force deficit responses in bout 2, there were no differences in

the amount of Z disk streaming between bouts. This may be explained by the length of time between bouts (5½ weeks) and that biopsies may confound such an investigation due to the invasive nature of the procedure (inducing a local inflammatory response). Gender differences were not the main focus of their investigation and as such were not investigated to any extent. However, there were reported differences in neutrophil count, with elevated levels in females following bout two. The extended period between bouts may also have diluted any gender differences in the extent of regeneration, which may have been seen in the first few weeks following the initial insult.

As inflammation is an important process within the muscle damage and repair cycle, including evidence which demonstrates that inhibition of inflammation, with an anti-inflammatory drug, reduces the repeated bout effect (Lapointe et al., 2002), it is of interest to determine how oestrogen, with its own potential anti-inflammatory properties, can affect the regeneration of damaged muscle. Therefore, the purpose of this investigation was to use the repeated bout effect as a marker of repair and regeneration and to compare differences between males and females. The time between bouts was two weeks, to address potential problems associated with the Stupka et al. (2001) investigation with regard to gender differences. If, as demonstrated by Lapointe et al. (2002) that reduced inflammation substantially reduces the repeated bout effect and oestrogen exerts an anti-inflammatory effect within the muscle damage and repair cycle, it is feasible that the repeated bout effect will be reduced in female participants. That is, the amount of protection afforded from the initial bout of eccentric exercise, will be less in women.

### 4.2.4 Research hypotheses

The following hypotheses were proposed in the present study:

a) The extent of exercise-induced muscle damage following a second bout of eccentric exercise will be greater in females.

## 4.3 Methods

### 4.3.1 Participants and design

Twenty healthy, untrained but recreationally active volunteers participated in this investigation (eleven males (age 19.9 ± 1.3 yrs, ht 175.5 ± 5.6 cm, mass 77.4 ± 6.51 kg) and nine females (age 19.1 ± 1.3 yrs, ht 168.8 ± 6.3 cm, mass 67.1 ± 9.3 kg). All participants signed informed consent forms and pre-test health questionnaires. All procedures had previously been approved by the Ethics committee of the School of Sport, Health and Exercise Sciences at the University of Wales, Bangor.

Baseline measures of isometric knee extensor strength (assessed at angles of 20°, 40° and 80°, where full extension is equal to 0°), isokinetic functional measures (e.g. fatigue index and peak torque) at 60° sec $^{-1}$, creatine kinase activity and soreness, were assessed prior to the exercise protocol designed to induce EIMD. These criterion measures were then reassessed every 24 hours until day 4 after the damaging exercise protocol.

Participants were required to attend the laboratory on at least three occasions prior to commencing the study. This was to ensure thorough familiarisation with the equipment used to assess functional measures of EIMD, to reduce the training effect we have found to be associated with such procedures.

### 4.3.2 Soreness

Soreness was reported using a visual analogue scale (VAS). Participants were asked to squat and to move a slider along the scale according to their perception of pain during this action. It was explained to the participants that the scale should be seen as a continuum, with *'no soreness'* at one end and '*the worst soreness ever*' at the other end. On the other side of this scale was a numerate scale running from 0-10 allowing the researcher to quantify the participant's response.

### 4.3.3 Creatine Kinase

Plasma creatine kinase (CK) activity was determined from a fingertip blood sample. A warm fingertip was cleaned with a sterile alcohol swab and allowed to dry. Capillary puncture was made with a Softclix lancet and a sample of whole fresh blood (30μl) was pipetted from a capillary tube onto the test strip and analysed for CK activity via a colorometric assay procedure (Reflotron, Boehringer Mannheim, Lewes, UK). This system uses a plasma separation principle, which is incorporated in the reagent carrier on the test strip.

### 4.3.4 Isometric Strength

Participants were required to perform maximal voluntary isometric contractions of the knee extensors at 20°, 40° and 80° knee flexion on a Kin-Com isokinetic dynamometer (500H, Cattecx, Chattanooga, TN, USA). The testing positions were obtained by entering full knee extension (0°) as a reference value into the Kin-Com visual display. The reproducibility of this method was checked on each testing occasion by noting the Kin-Com angle display when the lever arm was at true 90° (determined by spirit level). The pre-test angle display at true 90° was used as the

criterion. If any difference existed, the process was repeated until the criterion was achieved. Participants were required to perform three sub-maximal and one maximal practice repetitions as warm-up at each testing angle. Three maximal contractions of three seconds duration were performed at each joint angle with a one-minute rest period between repetitions. The highest average score (across 3 seconds) from the three contractions was recorded and is reported in this investigation.

### 4.3.5 Muscle function and isokinetic strength at 60° sec $^{-1}$

Muscle function was assessed on the isokinetic dynamometer. Participants were required to perform 30 maximal isokinetic (60° sec $^{-1}$) concentric contractions of the quadriceps across a range from 80° of flexion to 20° flexion, these angles were determined in relation to full extension corresponding to 0°, as for isometric strength. Participants were fully familiarised with this procedure to ensure proficiency during the test. Verbal encouragement was given throughout to ensure each contraction was maximal throughout the test protocol. From this procedure the Kin-Com determines a fatigue index (where 100% corresponds to no fatigue), peak torque, mean peak force and total work done. This allowed a more in-depth understanding of how muscle damage could affect other aspects of muscle function in addition to the decrements seen in isometric strength. The reason for this is two fold, 1) the use of isokinetic measurements is more ecologically sound for the sporting arena when compared to isometric contractions and 2) we have generally observed that when isometric strength has returned to baseline, many participants continue to complain of functional decrements and just not feeling '100%' during exercise.

### 4.3.6 Exercise protocol

The exercise protocol designed to induce EIMD consisted of 6 sets of 30 lunges on each leg followed by 10 sets of 10 counter movement jumps. During the lunges one complete set of lunges on both legs were performed continuously (30 lunges on left followed immediately by 30 lunges on the right). A two minute break was given between sets. During the jumps, 10 continuous counter-movement jumps were performed with a one minute break between sets. Participants were strictly observed to make sure each movement was performed fully and correctly, also to ensure any stress placed on the knee due to incorrect form was alleviated.

### 4.3.7 Statistical analysis

A mixed model gender (2) x time (5) x bout (2) repeated measures analysis of variance (ANOVA) was applied to the data. The assumption of sphericity was tested by the Mauchly Test of Sphericity. Any violations of this assumption were corrected by the Greenhouse-Geisser adjustment to raise the critical value of F, by adjusting degrees of freedom, as indicated by $_{(GG)}$. Statistical significance was set at the 0.05 alpha level. Main effects for time were further investigated by pairwise comparisons, with the Bonferonni adjustment technique applied to eliminate the problem of an inflated Type 1 error risk. Significant interactions were further investigated using an adjusted Tukey's HSD post hoc procedure as described by Stevens (1996). The mean and standard deviations for each variable across each bout and between each group are presented in Appendix 1.

## 4.4 Results

### 4.4.1 Soreness

Reported soreness on the VAS showed a significant main effect for time ($F_{4,72}$ = 155.4, $P$<0.01). Soreness increased significantly compared to baseline at 24 h post exercise. This remained significantly elevated throughout the testing period. The time x gender interaction was not significant. A main effect for bout was observed ($F_{(1,\ 18)}$ = 39.9, $P$<0.01), whereby the soreness reported following the second bout was significantly lower than that reported following the first bout of exercise. The time x bout interaction was also significant ($F_{(GG)2.6,47.6}$ = 15.3, $P$<0.05). This result is a factor of the repeated bout effect and explains the main effect for bout. Post hoc analysis revealed that at baseline, no significant differences existed between bouts. However on day one, and throughout the remaining testing period, there was a significant difference between the soreness reported following bout one of the exercise compared to bout two, with significantly less soreness reported following the second bout of exercise. The main effect for gender approached significance ($F_{1,18}$ = 3.2, $P$ = 0.09), with females reporting more soreness than males. The bout x gender and time x bout x gender interactions were non significant. This is shown in figure 4.1.

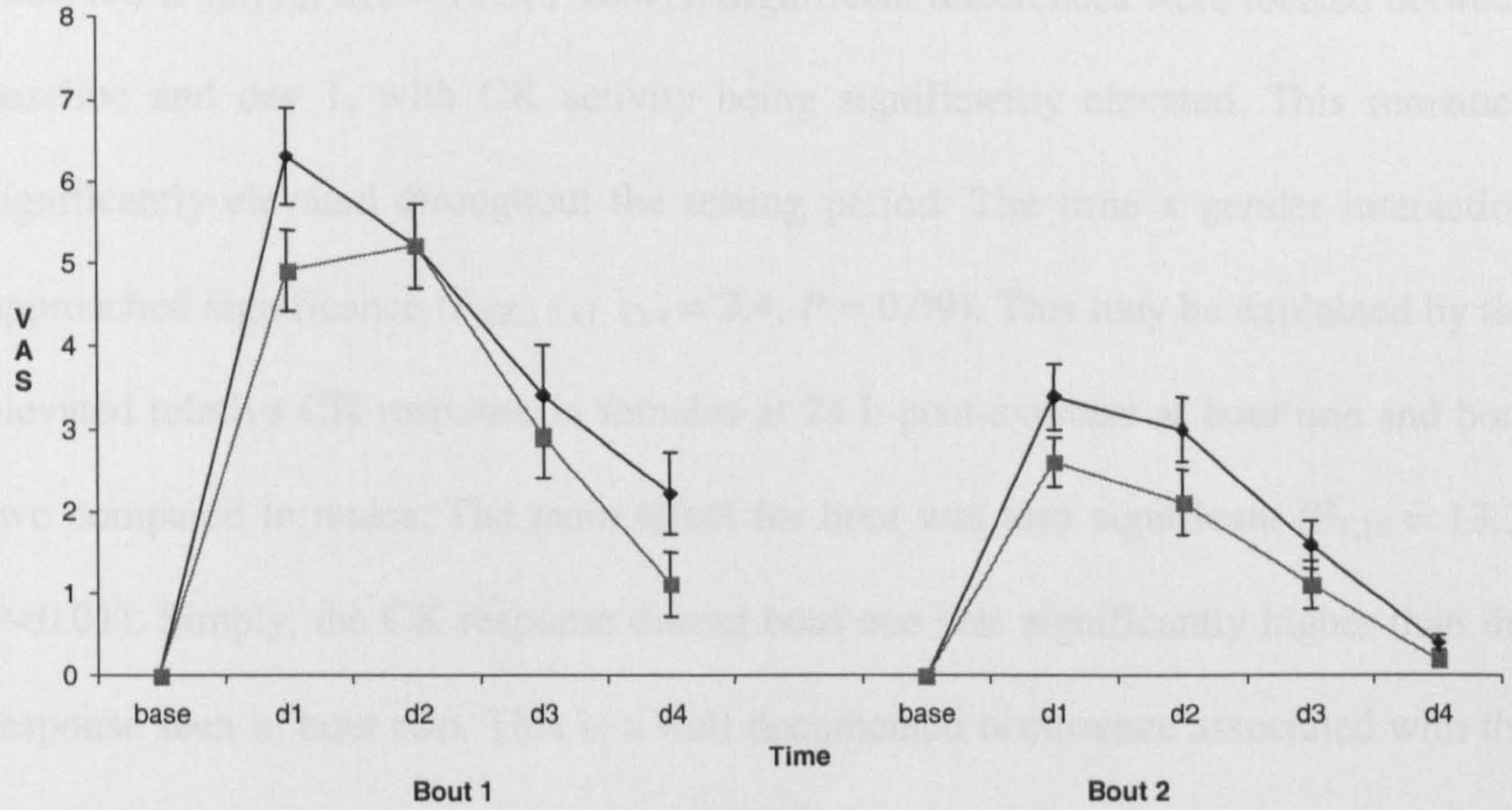


**Figure 4.1**: Incidence of soreness after two bouts of eccentrically biased exercise in men ( ▪ ) and women (♦ )(mean ± S $\overline{x}$ ).

## 4.4.2 Creatine Kinase

A one way ANOVA was applied to the data to determine if significant differences existed between genders at baseline for bout one and bout two. Females showed significantly lower CK activity at baseline than their male counterparts ($P<0.05$). In addition an initial exploration of the data by a three way ANOVA gender (2) x time (5) x bout (2) demonstrated that the common violation of homogeneity of variance which is seen with CK, due to its large inter-participant variability, was only violated at baseline during the first bout of exercise. A common practice with CK, to overcome this problem, is to log transform the data (e.g. Eston & Peters, 1999). This was performed in studies 1 and 2 when such violations occurred. However, due to the minimal deviation away from this assumption in the present study, and because of the baseline differences between genders, it was decided that the data would be relativised and changes would be investigated relevant to baseline.

A three way gender (2) x time (5) x bout (2) ANOVA was applied to the transformed CK data (expressed as percentage change from baseline). A main effect for time was

observed ($F_{(GG)\ 2.41,\ 43.4} = 13.5$, $P<0.01$). Significant differences were located between baseline and day 1, with CK activity being significantly elevated. This remained significantly elevated throughout the testing period. The time x gender interaction approached significance ($F_{(GG)\ 2.41,\ 43.4} = 2.4$, $P = 0.09$). This may be explained by the elevated relative CK response in females at 24 h post-exercise at bout one and bout two compared to males. The main effect for bout was also significant ($F_{1,18} = 13.3$, $P<0.01$). Simply, the CK response during bout one was significantly higher than the response seen in bout two. This is a well documented occurrence associated with the repeated bout effect (Newham et al., 1987; Nosaka et al., 1991; Eston et al., 1996). The time x bout interaction was found to be significant ($F_{(GG)2.4,44.6} = 5.15$, $P<0.01$). This interaction may be explained by the repeated bout effect, with smaller changes from baseline in bout 2 compared to bout 1. Significant differences between bouts were located on day one, with significant differences being maintained throughout the testing period. The CK response was significantly less in bout two compared to bout one. The bout x gender and the time x bout x gender interactions were non significant. Figure 4.2 illustrates these findings.

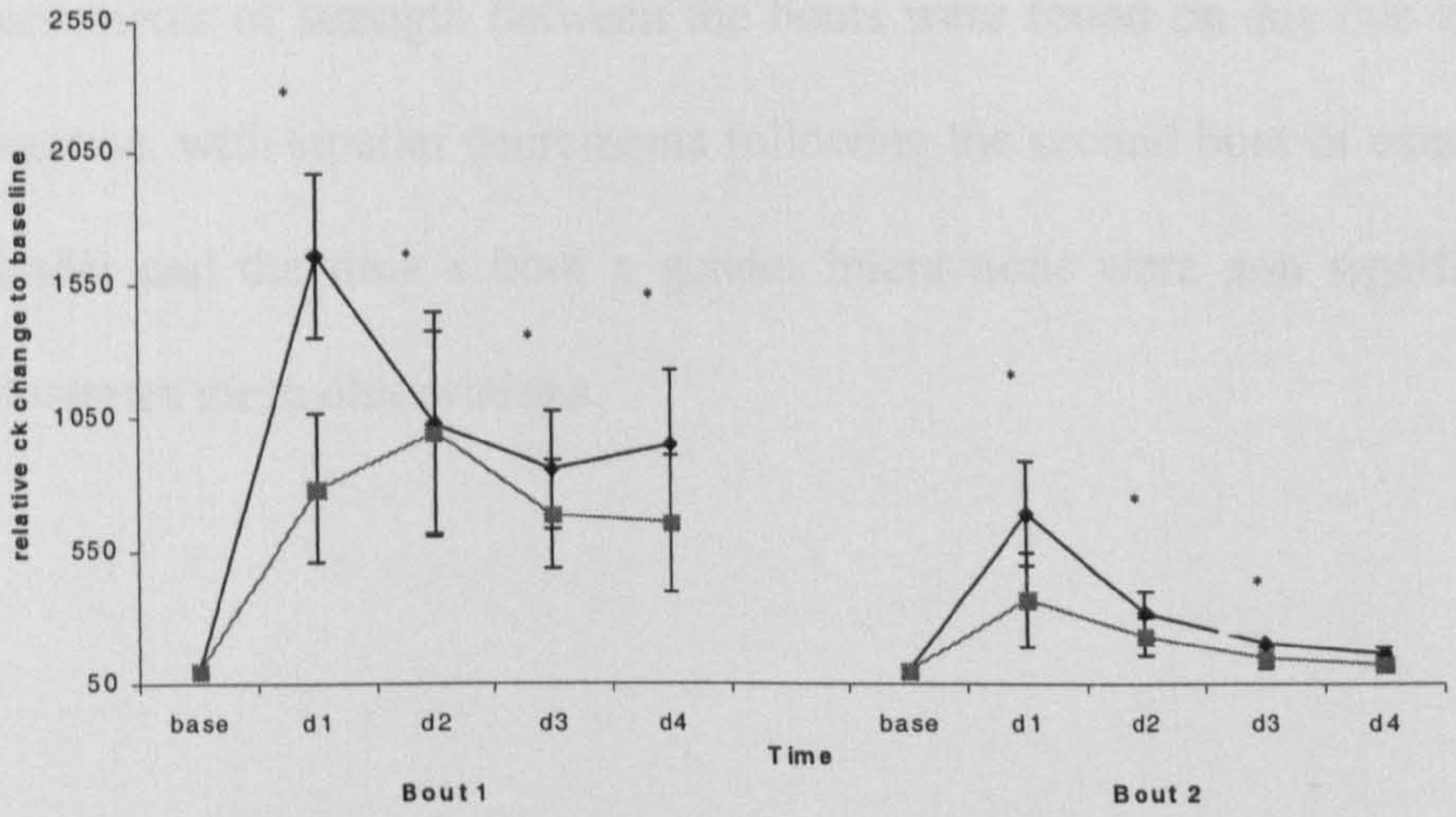


**Figure 4.2**: Changes in relative CK activity after two bouts of eccentrically biased exercise in men (▪) and women (♦ )(mean ± S $\overline{x}$ ). * significantly ($P<0.05$) different to baseline.

### 4.4.3 Isometric strength

A one way ANOVA on all angles was performed to assess whether baseline differences existed between genders at bout one and bout two. As expected, all angles showed significant differences between genders at baseline for both bout one and bout two, with males being significantly stronger than females across the three angles ($P<0.05$). Data were transformed and expressed relative to baseline, where the baseline value was considered to be 100% of isometric strength. The data were then analysed using a gender (2) x time (5) x bout (2) ANOVA.

#### 4.4.3.1 Isometric strength at 20 degrees knee flexion

A main effect for time on strength was observed ($F_{4,72} = 12.5$, $P<0.01$). Significant decrements from baseline strength were seen at day one and day two following the exercise protocol. Returning to baseline levels by day three. The gender x time interaction was non significant. The main effect for bout was significant ($F_{1, 18} = 6.5$, $P<0.05$), whereby, decrements in strength were significantly reduced following the second bout of exercise when compared to the first bout of exercise. The time x bout interaction was also significant ($F_{(GG)\ 2.7,48.7} = 5.4$, $P<0.01$). Significant differences in decrements of strength between the bouts were found on day one and day two post exercise, with smaller decrements following the second bout of exercise. The bout x gender and the time x bout x gender interactions were non significant. Figure 4.3 illustrates these observations.

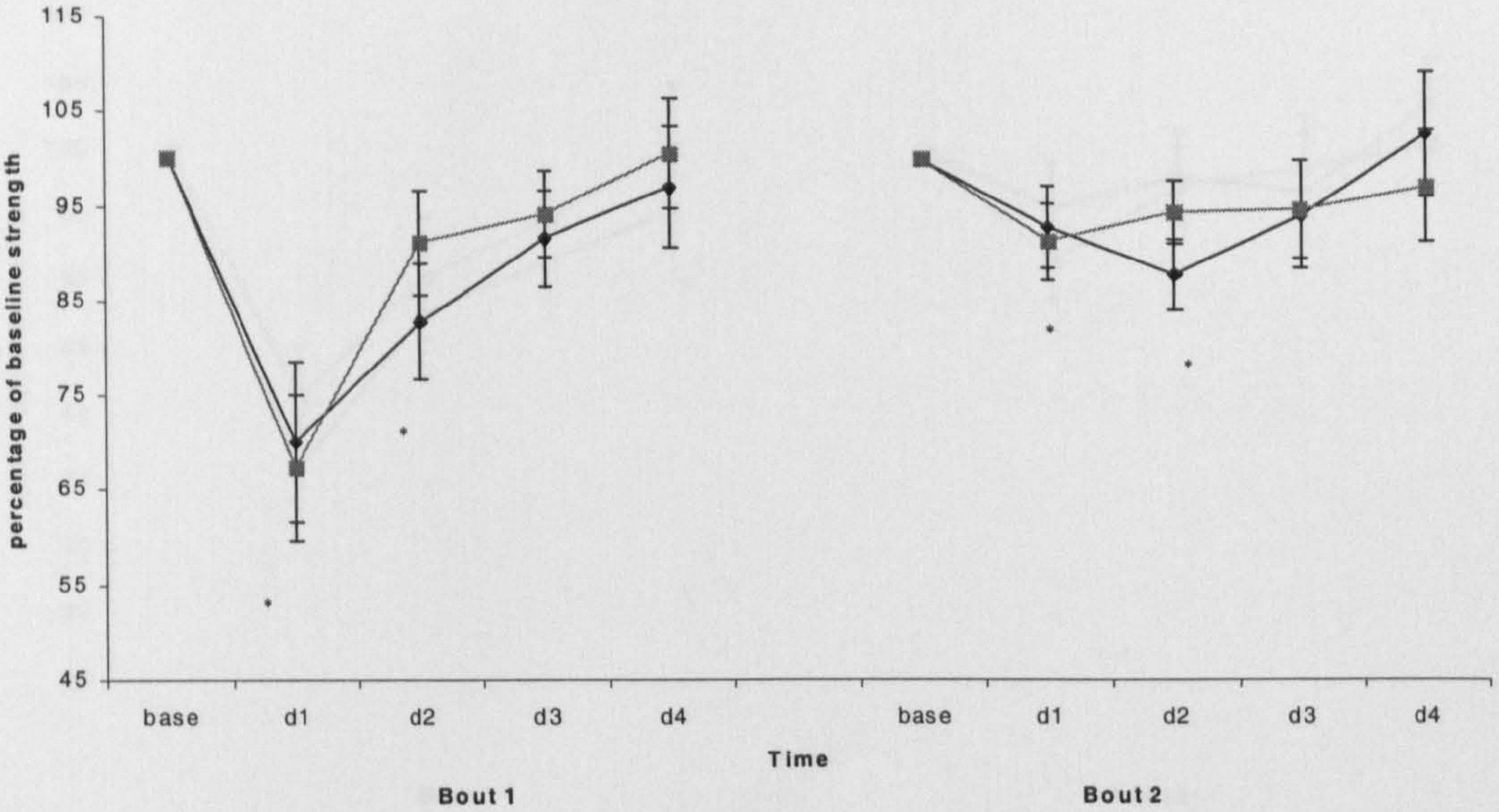


**Figure 4.3.**: Relative change in isometric strength at 20 degrees knee flexion after two bouts of eccentrically biased exercise in men ( ▪ ) and women (♦ )(mean $\pm$ S $\overline{x}$ ). * significantly ($P$<0.05) different to baseline.

## 4.4.3.2 Isometric strength at 40 degrees knee flexion

The main effect for time was significant ($F_{4,72}$ = 14.7, $P$<0.01). Significant decrements in baseline strength were found one day after the exercise protocol. The main effect for bout was significant ($F_{1,\ 18}$ = 5.9, P<0.01). Again, decrements in strength were significantly reduced following the second bout of exercise compared to baseline. The time x bout interaction was significant ($F_{(GG)\ 2.8,\ 51.4}$ = 4.55, $P$<0.01). Significant differences in decrements of isometric strength were found on day one and day two after the bout of exercise, again with smaller decrements in strength found in the second bout compared to the first. The time x gender interaction, bout x gender interaction and time x bout x gender interaction were all non-significant. Figure 4.4 illustrates these observations.

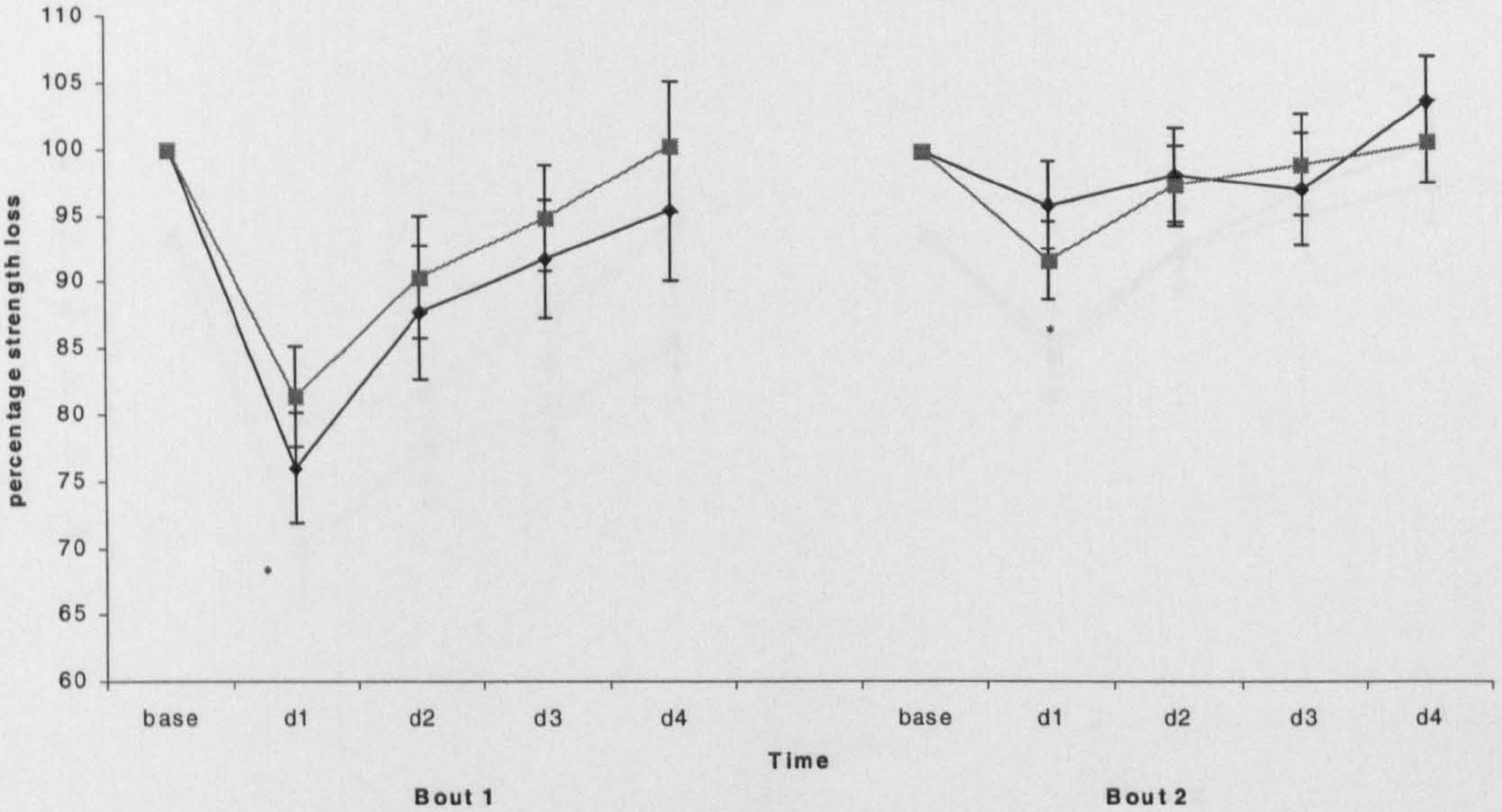


**Figure 4.4**.: Relative change in isometric strength at 40 degrees knee flexion after two bouts of eccentrically biased exercise in men ( ▪ ) and women (♦ )(mean ± S $\overline{x}$ ). * significantly ($P$<0.05) different to baseline.

## 4.4.3.3 Isometric strength at 80 degrees knee flexion

The main effect for time on strength was significant ($F_{4,\ 72}$ = 20.6, $P$<0.01). Significant decrements in baseline strength were found at day one and two following the exercise protocol. The main effect for bout was also significant ($F_{1,18}$ = 14.3, $P$<0.01). Decrements in isometric strength were greater following bout one compared to bout two. The time x bout interaction was also significant ($F_{(GG)\ 3.3,59.2}$ = 6, $P$<0.01) Again, this can be explained by the repeated bout effect and explains the main effect for bout found in this analysis. Significant differences in decrements of strength in the two bouts were found on day one and across the testing period, with smaller decrements found following the second bout of exercise. The time x gender interaction, bout x gender interaction, and the time x bout x gender interaction were all non-significant. Figure 4.5 illustrates these findings.

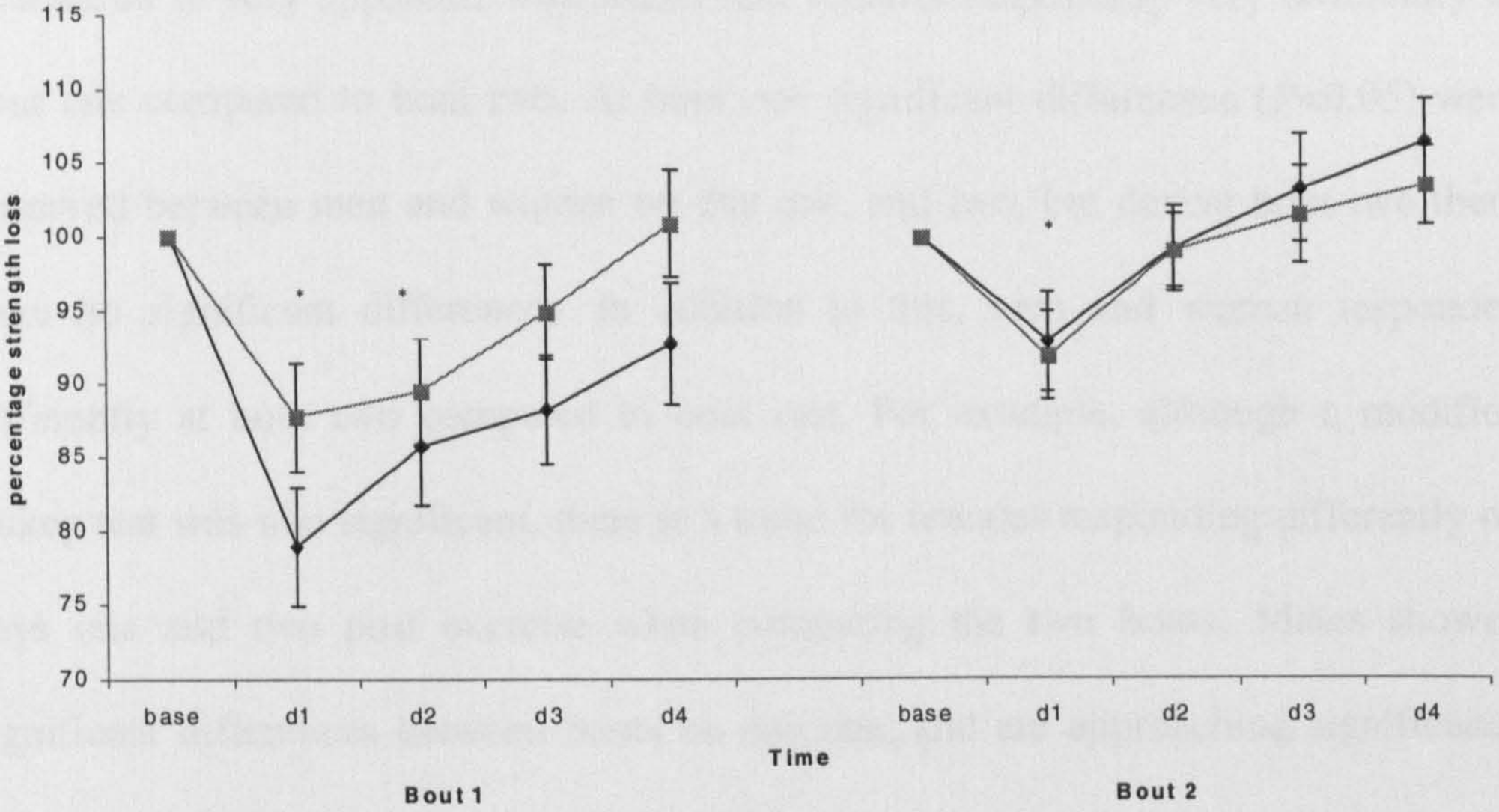


**Figure 4.5**: Relative change in isometric strength at 80 degrees knee flexion after two bouts of eccentrically biased exercise in men ( ▪ ) and women (♦ )(mean ± S $\bar{x}$ ).* significantly ($P$<0.05) different to baseline.

## 4.4.4 Muscle Function

One way ANOVAs on baseline peak torque, fatigue index, mean peak force and work done were performed to determine if gender differences existed for bout one and bout two. These analyses demonstrated that significant differences between genders existed for all variables except the fatigue index at both bouts. Consequently, all variables except the fatigue index, were transformed and expressed relative to baseline.

### 4.4.4.1 Fatigue index

A main effect for time ($F_{4,72}$ = 2.8, $P$<0.05) and a significant time x gender interaction ($F_{4,72}$ = 2.8, $P$<0.05) were observed on fatigue index. Interestingly, the main effect for bout and the interaction for time x bout were non-significant. Neither was the bout x gender interaction. However the time x bout x gender interaction was significant ($F_{4,72}$ = 2.6, $P$<0.05). This interaction is demonstrated in Figure 4.6. The

interaction is very apparent, with males and females responding very differently at bout one compared to bout two. At bout one significant differences ($P<0.05$) were observed between men and women on day one, and two, but during bout two there were no significant differences. In addition to this, men and women responded differently at bout two compared to bout one. For example, although a modified Tukey test was non significant, there is a trend for females responding differently on days one and two post exercise when comparing the two bouts. Males showed significant differences between bouts on day one, and are approaching significance on days two and four post exercise.

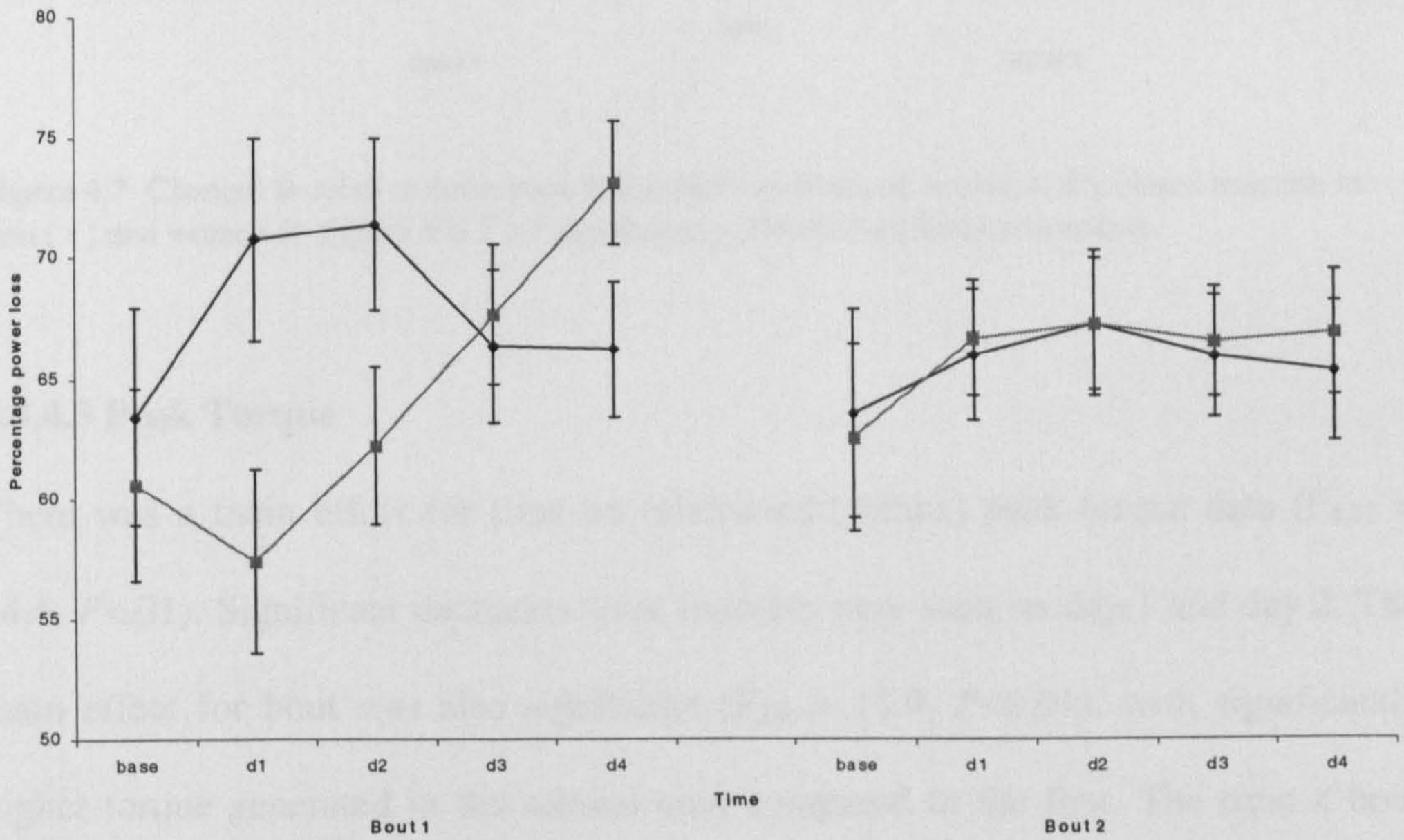


**Figure 4.6**: Changes in fatigue index after two bouts of eccentrically biased exercise in men ( ▪ ) and women (♦ )(mean $\pm$ S $\overline{x}$ ).

### 4.4.4.2 Mean peak force

Relative (%) mean peak force data showed a main effect for time ($F_{(GG)2.4,42.9} = 10.5$, $P<0.01$). Significant differences were observed between baseline and day 1. No other significant results were found (main effect for bout, gender x bout interaction, time x

bout interaction, time x gender interaction, gender x time x bout interaction). These findings are illustrated in Figure 4.7.

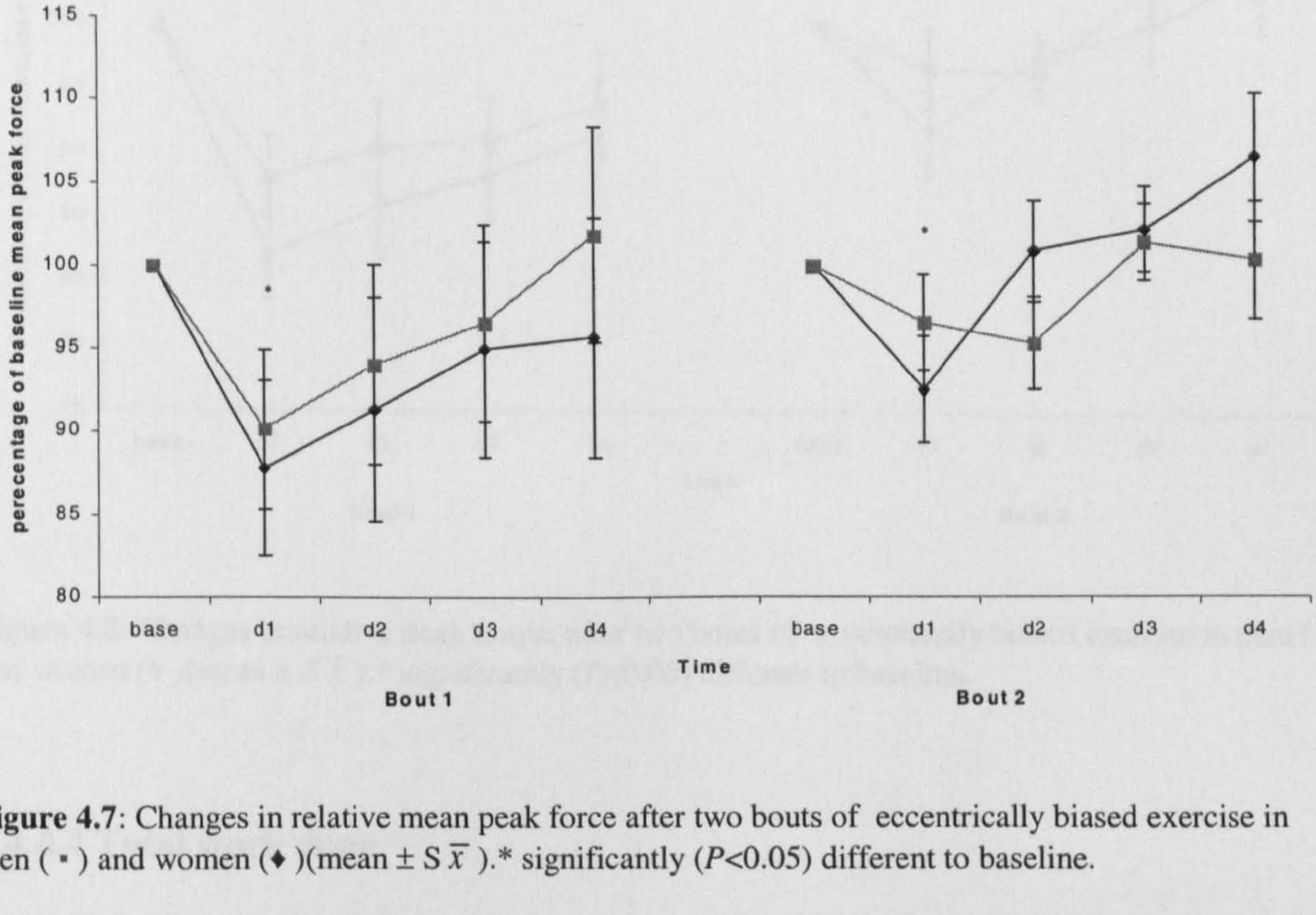


**Figure 4.7**: Changes in relative mean peak force after two bouts of eccentrically biased exercise in men ( ▪ ) and women (♦ )(mean ± S $\overline{x}$ ).* significantly ($P$<0.05) different to baseline.

### 4.4.4.3 Peak Torque

There was a main effect for time on relativised (%max) peak torque data ($F_{4,72}$ = 14.6, $P$<.01). Significant decreases from baseline were seen on day 1 and day 2. The main effect for bout was also significant ($F_{1,8}$ = 11.9, $P$<0.01), with significantly higher torque generated in the second bout compared to the first. The time x bout interaction was found to be significant ($F_{4,72}$ = 4.8, $P$<0.01), with the peak torque being significantly higher during bout 2 on day one and remaining significantly higher compared to bout 1 throughout the testing period. No other significant results were observed. These findings are illustrated in Figure 4.8

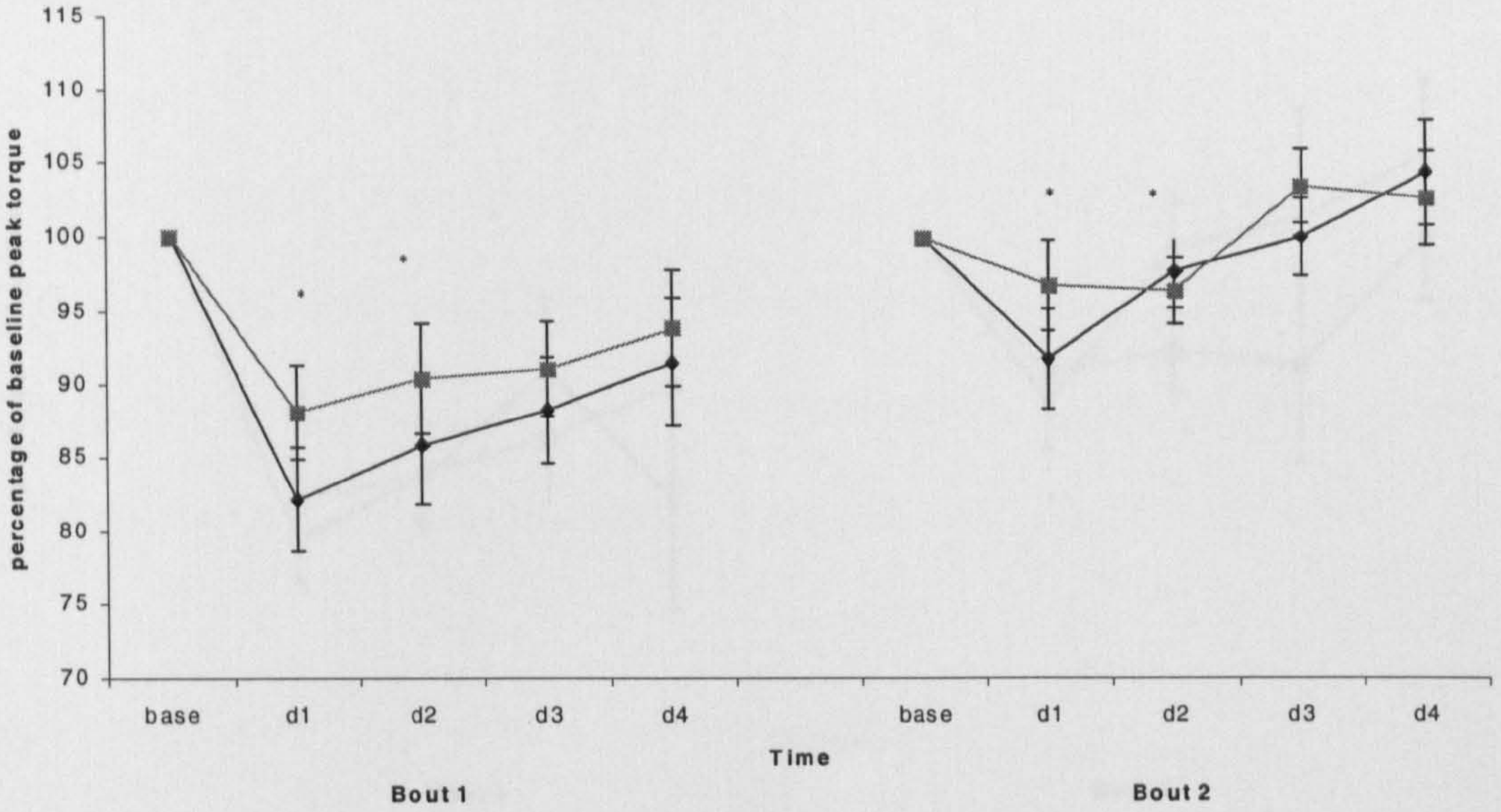


**Figure 4.8**: Changes in relative peak torque after two bouts of eccentrically biased exercise in men (▪) and women (♦ )(mean ± S $\overline{x}$ ).* significantly ($P$<0.05) different to baseline.

## 4.4.4.4 Total work done

Transformed data showed a main effect for time ($F_{(GG)2.6,46.6}$ = 11.8, $P$<0.01), with significant decreases from baseline on day 1 and day 2 and approaching significance on day 3. The main effect for bout was also significant ($F_{1,18}$ = 7.3, $P$<0.05), with decrements in work done being less in the second bout compared to the first bout of exercise-inducing muscle damage. The time x bout interaction approached significance ($F_{(GG)2.4,43.1}$ = 2.4, $P$ = 0.09). This can be explained by the ability of the muscles to perform more work following the second bout of exercise compared to the first. No other significant results were observed. These findings are illustrated in Figure 4.9.

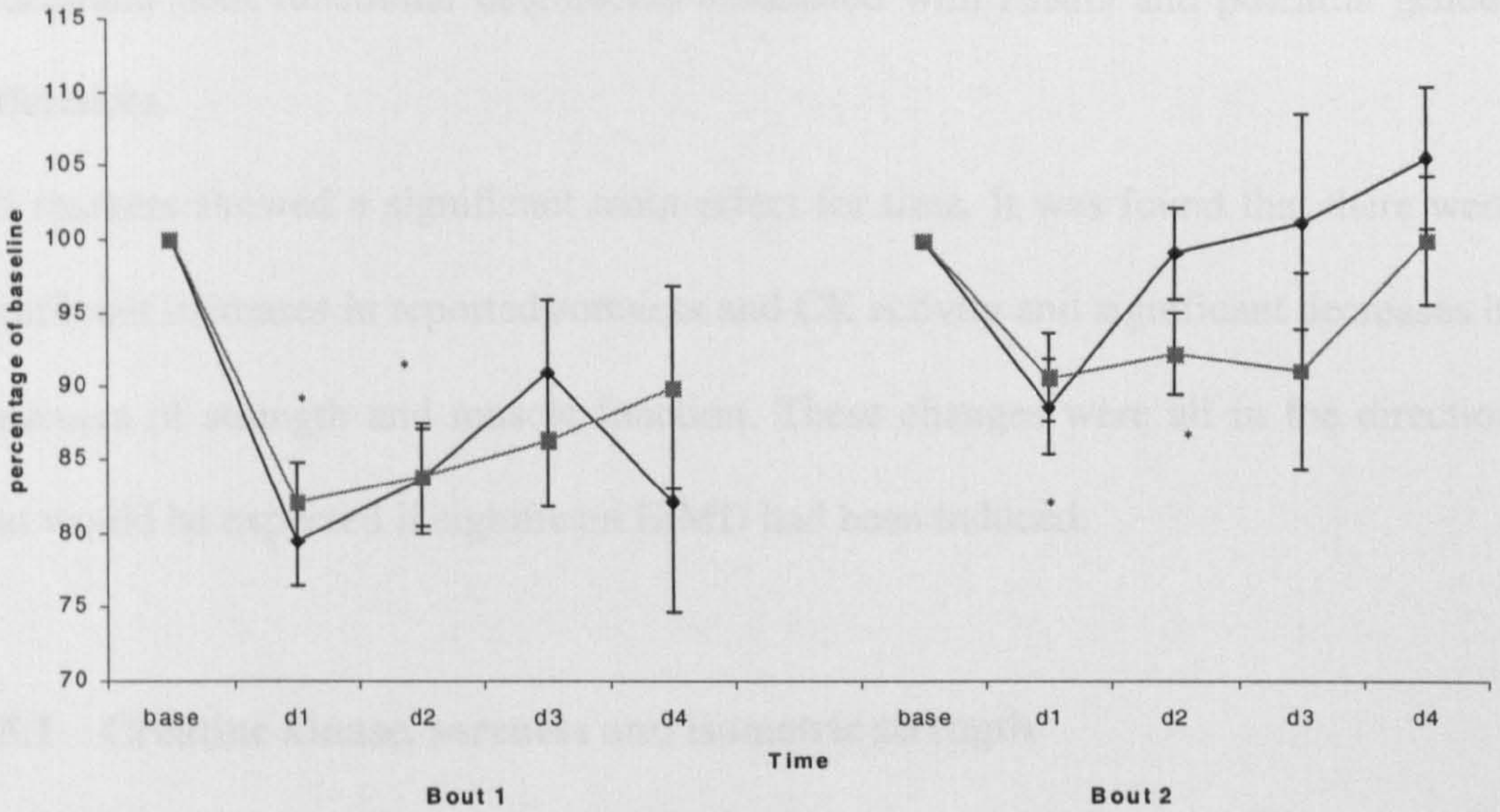


**Figure 4.9**: Changes in relative work done after two bouts of eccentrically biased exercise in men ( ▪) and women (♦ )(mean ± S $\overline{x}$ ).* significantly ($P$<0.05) different to baseline.

## 4.5 Discussion

The purpose of this investigation was to determine if female muscle responded in a similar way to male muscle following a repeated bout of damage-inducing exercise. The rationale for the investigation was stimulated from research which had shown that oestrogen may in some way reduce inflammation (St Pierre Schneider et al.,1996; Komulainen at al.,1999; Tiidus and Bombardier, 1999; Stupka et al., 2000; Tiidus et al., 2001). It was hypothesised that reduced inflammation may result in poor regeneration of the damaged muscle leaving it more susceptible to a second bout of EIMD as previously demonstrated by Lapointe et al. (2002) with the use of anti-inflammatory drugs. This is the first study to focus solely on the potential gender differences in the repeated bout effect. The common markers of creatine kinase, soreness and isometric strength were utilised to investigate the repeated bout effect, but measurements were also taken from isokinetic dynamometry to further

understand both functional decrements associated with EIMD and potential gender differences.

All markers showed a significant main effect for time. It was found that there were significant increases in reported soreness and CK activity and significant decreases in measures of strength and muscle function. These changes were all in the direction that would be expected if significant EIMD had been induced.

### 4.5.1 Creatine kinase, soreness and isometric strength

Creatine kinase, soreness and isometric strength all demonstrated similar patterns of response in the two bouts. It was found that all variables demonstrated a main effect for time, i.e. increases in soreness and CK were found after the damage-inducing exercise, with significant decreases in isomeric strength at all angles. These responses were all significantly reduced following the second bout of exercise (lower reports of soreness, CK activity and decrements in isometric strength). This response following a repeated bout of exercise-inducing muscle damage is well documented (McHugh et al., 1999). Nosaka et al. (2001) reported that the repeated bout effect could last for up to 6 months!

In addition to these findings, there was a trend for a gender difference in soreness ($P = 0.09$), with males reporting less soreness than females. This has been found in a previous investigation in our laboratory and has been discussed and examined in further detail in chapter 4. This result calls in to question the objectiveness of soreness as a gauge of muscle damage.

Interestingly, transformed creatine kinase showed a trend for gender differences across time, but not in the direction one might expect. It was found that transformed CK showed relatively greater activity in females on day 1 post exercise compared to males. While gender differences in CK is not surprising, the direction of the difference in this investigation is a surprise. Lower CK activity in females has been well documented (Meltzer, 1971; Shumate et al., 1979; Berg & Keul, 1981; Rogers et al., 1985; Amelink & Bär, 1986; Bär et al., 1988; Van der Meulan et al., 1991; Reijneveld et al., 1994; Dumke, 1996) and in fact appears to have been the initial stimulus in investigating potential gender differences in EIMD. Hormonal manipulation of oestrogen in ovariectomised and male rats have demonstrated that the presence of oestrogen can reduce CK activity (Bär et al., 1988), which would negate the obvious suggestion that differences in muscle mass could be held responsible.

It is difficult to surmise why this investigation observed a greater relative increase in CK in females following EIMD. If differences in muscle mass were accountable for the differences seen in CK then one would expect that when values were transformed relative to baseline, CK values would respond similarly. However, as discussed in previous chapters, CK has been criticised as a poor marker of EIMD and as such should always be used in conjunction with other variables. In addition to this it should be born in mind that the gender x time interaction was only approaching significance and that the absolute data still showed higher CK values in males compared to females, which is consistent with previous literature.

Of the above variables (soreness, CK and isometric strength) none showed a significant difference between genders in terms of the repeated bout effect. These results suggest that the muscle is not compromised, in terms of repair, in the female participants. The exercise protocol used in this investigation was the same as used in study 2 (chapter 4), where potential differences in terms of inflammation were seen between genders, with inflammation being reduced in females. Thus, while markers of inflammation were not used in this investigation it was assumed the genders would behave similarly as to those in the previous study (chapter 4), with reduced inflammation in female participants. Already there is a flaw to this assumption given that CK response did in fact differ between those who participated in the previous investigation compared to those who participated in the present investigation. This may indirectly suggest that the response of the genders in the two studies differed, although caution is always taken when using CK as discussed previously.

### 4.5.2 Isokinetic functional measures of exercise-induced muscle damage

Peak torque (relativised) and total work done (relativised) showed similar patterns of statistical significance to the previous variables (CK, soreness and isometric strength), with decreases following the initial damage. In addition they demonstrated the repeated bout effect, with lower reductions seen following the second bout of damage-inducing exercise. As with previous variables, no gender differences in terms of the repeated bout effect were seen in either of these measures. The results suggest that the female muscle is in no way compromised in a repeated bout of exercise, from poor regeneration in the primary bout.

A significant reduction in mean peak force in the days following the exercise protocol was also found. Interestingly, this variable did not show any other statistically significant effects. This suggests that whatever adaptations occurred following one bout of EIMD, they did not affect the mean (dynamic) peak force associated with muscle damage. Reductions in this variable are just as pronounced in bout two as they were in bout one.

The fatigue index showed a very interesting interaction, as illustrated in Figure 4.6. Fatigue can be defined as an inability to maintain the required or expected force or power output (Edwards, 1981). Research with regard to fatigue following EIMD is limited, especially with respect to the repeated bout effect. Damaged muscle has been shown to both maintain a pre damage fatigue index (Davies & White, 1981) and has also been shown to be less fatiguable following EIMD (Balnave & Thompson, 1993).

Research from our laboratories support the latter finding, with muscle being significantly less fatiguable under both isometric and dynamic conditions for over three days following eccentric exercise (Byrne & Eston, 2002b). This finding was explained in relation to fibre type recruitment, which may also help explain the results of the current investigation, particularly with regard to the repeated bout effect. Research suggests that type II (fast twitch) fibres are selectively recruited during unaccustomed eccentric exercise and therefore are the fibres selectively damaged (Friden et al., 1983; Jones et al., 1986; Lieber & Friden, 1988; Lieber & Friden, 1992; MacPherson et al., 1996). If this is the case, the contribution from these fibres to a post damage fatigue protocol would be diminished. It would be expected

that type 1 (slow twitch) fibres would be recruited, which would result not only in less power generation (as was seen in the current investigation with reduced peak torque on days one and two in bout one), but also with the muscle showing higher resistance to fatigue. As can be seen from figure 4.6, this may hold true for the female participants, but males do not show a significant reduction in fatigue until day 3 in the current investigation. However, Twist (PhD thesis) has shown similar findings in male participants following exercise-induced muscle damage. He showed that the rate of fatigue (during a cycle ergometer protocol) was significantly lower 48 h after exercise and not at 24 h.

A further consideration which may have implications for this study is the potential gender differences in fibre type. It has been reported that males have more type II fibres than females (Liljedahl et al ., 1996), although this was not assessed during the current investigation. Discussion with the participants suggested an almost equal spread of those who were predisposed to explosive activities (presumably with a predominance of type II fibres) and those who were predisposed to more prolonged aerobic activities (predominance of type I fibres) across genders. In addition to this there were no significant differences between genders in the baseline fatigue index, where we may have seen a higher susceptibility to fatigue in males if they had a greater proportion of fast twitch fibres.

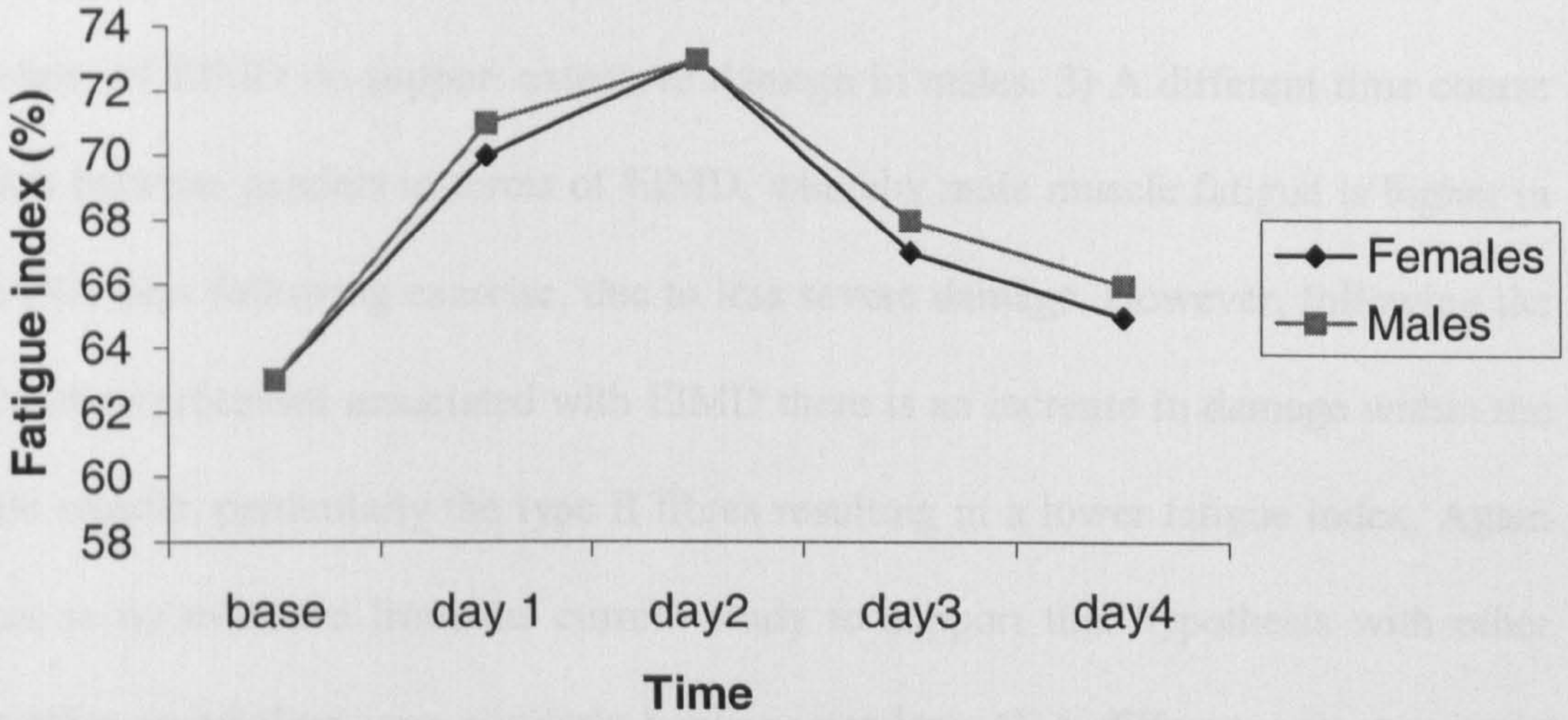


**Figure 4.10**: Proposed fatigue index, following a bout of exercise-induced muscle damage, based on similar fibre type between genders and previous research.

In view of this observation, we may have expected to see results similar to those shown in Figure 4.10, but this was not the case. Females were less susceptible to fatigue on day one and two following EIMD, returning to baseline by day three which is similar to the findings of Byrne & Eston (2002b). The male muscle however does not significantly differ from baseline until day three and day four following the damaging exercise, where it then shows an elevated resistance to fatigue, by which time the peak torque and the mean peak force have returned to baseline.

An explanation for such findings is difficult. Several hypotheses can be put forward 1) despite familiarisation the baseline measure is not true and in actual fact baseline fatigue could have been lower. I do not feel this is the case, I was confident in the familiarisation process. 2) damage in the male muscle fibres was not sufficient enough to affect the fatigue index and the muscle itself was 'learning' how to cope with the fatigue protocol, with more effective recruitment of all fibre types in the final days of testing. This could only be verified with a control group undergoing the

same fatigue procedure without the presence of EIMD. In addition to this, other markers of EIMD do support extensive damage in males. 3) A different time course exists between genders in terms of EIMD, whereby male muscle fatigue is higher in the first days following exercise, due to less severe damage. However, following the secondary processes associated with EIMD there is an increase in damage within the male muscle, particularly the type II fibres resulting in a lower fatigue index. Again there is no evidence from the current study to support this hypothesis with other variables responding very similarly between genders. 4) A difference does exist in terms of fibre type between the genders, with males having more type II fibres. If this were the case, following the damaging exercise males may still have 'undamaged' but still highly fatiguable type II fibres to activate during the fatigue protocol and across time there is a training effect (learning to recruit type I fibres, reducing fatigue). Again, this is not supported by the repeated bout as learning does not appear to occur (no significant changes in the index across the testing period). Clearly this requires further investigation.

Understanding the response in the males is further complicated when the repeated bout is taken into account. The findings from the repeated bout effect show no significant changes in fatiguability of the muscle across time or between genders. This could lend support to the 'neural theory' of adaptation. This theory predicts that the initial damage is the result of a high stress on a small number of active type II fibres. Following the repeated bout there is an increase in motor unit activation which distributes the contractile stress over a larger number of active fibres (both type I and II) (McHugh et al., 1999). If this was the case, one would expect the first bout to show decreases in fatiguability in the days following exercise due to the

selective damage of type II fibres, leaving type I fibres to 'cope' with the protocol. During the second bout the muscle should maintain the fatigue index due to a switch in the fibre type activation during the damage protocol, allowing type II undamaged fibres to be activated during the fatigue protocol. This is seen in the female participants, but not in the male.

If, as had been suggested earlier, the males had received less damage (hypothesis 2), or could still recruit type II fibres (hypothesis 3) and the fatigue index decreased across time due to a training effect, this would have been seen in the second bout. This was not the case, which negates these hypotheses and prompts more thorough investigation.

### 4.5.3 Summary and conclusion

The current investigation provides no evidence that the female muscle is more susceptible to a second bout of EIMD through poor regeneration following the primary bout. This either a) negates oestrogen's strong anti-inflammatory properties, as one would expect a similar response to that of Lapointe et al. (2002), where anti-inflammatory drugs were shown to markedly reduce the repeated bout effect; b) a further dimension/mechanism of protection is involved to ensure the female muscle is not compromised. As there is considerable evidence which shows the potential for oestrogen to inhibit inflammation this warrants further investigation. For example, oestrogen has been shown to increase vascular endothelial growth factor, thus promoting endothelial cell proliferation (Gargett et al., 2002). Such a mechanism could provide a protective effect that ensured the female skeletal muscle was not subject to compromised repair and regeneration. The study suggests that males and

females may respond differently to EIMD (fatiguability of muscle) and prompts further investigation.

# CHAPTER 5

# CONCLUSIONS AND FUTURE DIRECTIONS


## 5.1 General summary

## 5.2 New contributions to the literature

### 5.2.1 Intra-female differences

### 5.2.2 Gender differences in soreness

### 5.2.3 Gender differences in markers of inflammation

### 5.2.4 Gender differences in the repeated bout effect associated with exercise-induced muscle damage

## 5.3 Study limitations

### 5.3.1 Oestrogen not measured

### 5.3.2 Peripheral markers of inflammation utilised above direct markers

### 5.3.3 Sample size and statistical power

### 5.3.4 Volitional measures of strength

## 5.4 General conclusion and future directions

## 6.1 General summary

This thesis has focused on the potential role that oestrogen may play within the exercise-induced muscle damage and repair cycle. It has attempted to describe the positive and negative effects that may be attributed or linked to the action of oestrogen during the cycle of events associated with EIMD. Chapter 1 contains a more detailed discussion of the potential interacting factors proposed to be involved during exercise-induced muscle damage (EIMD) commencing with the initial insult to the skeletal muscle and ending with the eventual full regeneration and repair. The area of EIMD is a complex one, with limited understanding in regard to many mechanisms, particularly the secondary processes involved in EIMD. Therefore, how and where any additional factors, for example oestrogen, interact with the events associated with EIMD is very difficult to determine and requires extensive research. Given the enormity of this area of research, this thesis focussed on specific questions relating to the action and effects of oestrogen during EIMD. There is a progression through the thesis in an attempt to develop upon the current research findings and understanding within this area.

The purpose of the first study was to determine if oestrogen played a role within the muscle damage and repair cycle and, to what extent did endogenous fluctuations in oestrogen and exogenous administration of oestrogen have on EIMD. Muscle damage was investigated using simple markers, during two phases (high and low oestrogen) within the menstrual cycle of healthy eumenorrheic females. A comparison was also made between this group and oral contraceptive pill users, to determine if prolonged supplementation with exogenous oestrogen could reduce any symptoms of EIMD.

The above study observed limited intra female differences associated with oestrogen levels and EIMD. The second study then focussed on the potential gender differences associated with EIMD, with a focus on secondary processes associated with EIMD. The final study investigated whether gender differences existed in terms of the repair and regeneration of the muscle following a bout of damage inducing exercise.

## 5.2 New contributions to the literature

### 5.2.1 Intra female differences (chapter 2)

Study 1 investigated potential differences in the extent of damage across high and low oestrogen phases of the menstrual cycle. To our knowledge, this has not previously been investigated, despite the evidence that oestrogen may play a role in EIMD and the fact that this hormone shows large fluctuations across the menstrual cycle.

From this study there is no evidence to suggest that the fluctuations in oestrogen seen across the menstrual cycle affect any symptoms associated with EIMD. This is important in progressing the knowledge of the role of oestrogen in EIMD further for several reasons. Firstly, the levels of oestrogen in the low phase of the menstrual cycle is comparable to the levels found in males. However, differences in markers of EIMD, e.g., CK, have been observed between males and females. This may suggest that the presence of oestrogen is an important factor in determining the response. Oestrogen levels taken at a snap shot in time, for example across different phases of the menstrual cycle, can not convey information with regard to susceptibility to EIMD during that specific phase. This finding is in line with research that has shown that ovariectomised rats show greater levels of protection in terms of EIMD when

compared to sexually immature rats. Despite having the source of oestrogen removed, the prior prolonged exposure to oestrogen has offered additional protection, which slowly declines with time (Amelink and Bär, 1986).

The results from this study also have implications for the design of future investigations into EIMD. The relatively large fluctuations in oestrogen across the menstrual cycle do not appear an important moderating factor which needs consideration in designing future investigations. This is important when so many mechanisms are not clearly understood in the area of EIMD and control of confounding variables is important.

In addition to observing the effects of the menstrual cycle phase on EIMD, the effects of oral contraceptive use on EIMD was also assessed in this first investigation. Only three studies have previously investigated the effects of exogenous oestrogen taken for contraceptive use and EIMD. The findings of these studies are contradictory. Carter et al. (2001) found that oral contraceptive use could attenuate the CK response following a bout of eccentric exercise when compared to eumenorrheic females, but that no differences existed between perceived soreness. However, Thompson et al. (1997) found the only difference to exist between these two groups was the associated pain following a bout of damage inducing exercise. This is concurrent with the findings of the present study. Finally, Savage and Clarkson (2002) reported that the only variable to differ between users and non-users of oral contraceptives was isometric strength. In their study oral contraceptive users showed a slower return to baseline values. This is discussed in more detail in chapter 2.

Of great interest and requiring further investigation is the potential role that oestrogen may play in the perception of soreness associated with EIMD. The findings from this investigation confirm the research of Thompson et al. (1997). They observed that oral contraceptive users reporting significantly lower values of soreness when compared to the non-users following a bout of EIMD. The implication of this is that exogenous oestrogen may in someway modulate pain perception, with possible analgesic properties. This observation is of particular interest in relation to subsequent contradictory findings from studies two and three. In these studies males reported significantly lower soreness levels than females, suggesting that oestrogen may not provide a protective effect. However, what clearly requires clarification is the potential confounding variable of differences in gender specific personality traits (chapter 3). A further factor worthy of consideration and possibly further investigation are the potential personality differences between females who choose to take the oral contraceptive pill compared to those who do not, especially within the student population, where the latter is certainly a minority.

### 5.2.2 Gender differences in soreness (chapter 3 and 4)

Observations from these studies tend to suggest that there is a difference in the soreness associated with EIMD reported by males when compared to females (chapter three main effect for gender $P<0.05$, chapter four main effect for gender approached significance $P = 0.09$). The research on gender differences in the perception of soreness following EIMD is limited. Stupka et al. (2001) did not use soreness as a gauge of muscle damage when assessing gender differences. Rinard et al. (2000) showed no significant differences in soreness between genders following exercise, although the participant selection procedure is questionable for this

investigation, making generalisation very difficult. MacIntyre et al (2000) did show a gender x time interaction on soreness although did not probe the interaction to determine where differences occurred. Their data suggest a very different response in males and females. In their study, females reported lower levels of soreness in the immediate post-exercise period, whilst males reported lower levels of soreness in the latter post-exercise period.

The findings of our investigation suggest that such differences in pain perception, with limited differences in other markers of EIMD, need to be taken into account when carrying out research into muscle damage in humans. This would hold true whether gender is a factor or not, as groups would need to be matched for gender to ensure it was not a confounding variable with regards to the soreness response. This finding is also of importance especially when taken in conjunction with the findings of the first investigation as they strongly bring into question the utility of using VAS scales and pain perception as an objective gauge of EIMD.

### 5.2.3 Gender differences in markers of inflammation (chapter 3)

The second study used both simple and more complex markers of EIMD to determine if gender differences existed in terms of the secondary response to EIMD. Research has shown that oestrogen has the potential to both 'up-regulate' and 'down-regulate' the inflammatory response. The role of oestrogen in both general inflammation and specific events involved in inflammation is discussed in more detail in chapter one. However, limited research has been carried out on humans with regard to EIMD and gender differences in inflammation (see chapters 1, 3 and 4 for more specific literature).

The present study showed that changes in leg circumference were smaller in females, which could be taken as an indirect marker of reduced inflammation. A more intriguing and potentially influential finding of this investigation is the potential gender difference which existed in terms of elastase concentration per cell. Elastase concentration following EIMD in humans has not been widely investigated and no study has previously looked at total stimulated and unstimulated elastase concentration and stimulated and unstimulated elastase expressed on a per cell (neutrophil) basis.

The results suggest that oestrogen does not effect total elastase concentration, nor does it effect total stimulated elastase concentration. However, when elastase is assessed on a per cell basis a gender difference exists, with a lower response in females. The findings may suggest the following explanations, for example, 1) the neutrophils in the female participants are less active than the male neutrophils 2) the neutrophils in the female subjects have not been primed due to lack of stimulus 3) there is reduced damage in the female participants which accounts for the lack of stimulation 4) the female neutrophil is immunocompetent when compared to the male and 5) given the ability of the female neutrophil to become primed and as equally active as the male (no differences following LPS stimulation), a mechanism associated with EIMD in the female has been 'switched off' possibly due to the properties of oestrogen.

### 5.2.4 Gender differences in the repeated bout associated with EIMD

The final investigation attempted to assimilate the information together from previous research and the second study which suggests that oestrogen inhibits

inflammation. If this is true, it is possible that the female response to a second bout of EIMD may be compromised. The female muscle may be more susceptible to a second bout of EIMD, due to poor regeneration from the first bout of exercise. This is the first study which has focussed on gender differences in the repeated bout effect associated with EIMD. Stupka et al. (2001) included gender as an aspect of a repeated bout investigation with no significant findings. Their investigation included a 5½ week gap, which may have allowed thorough regeneration of the female muscle. Findings from the current investigation provide further understanding of the cycle of events associated with the early stages of repair in males and females following EIMD.

The results showed no differences between men and women for the repeated bout effect, except in terms of the fatigue index. It is difficult to determine if this can be attributed to differences in the initial level of damage in bout one, or due to differences in inflammation, or whether it is an indication of differences in selective muscle type recruitment. This study adds to our understanding of muscle damage and the repeated bout effect, but also further supports gender differences with regard to EIMD. It would appear that there are differences in the fatigue profile in men and women after an initial bout of EIMD but not after a repeated bout. A more detailed discussion of this can be found in chapter 4.

## 5.3 Study limitations

Each study has highlighted where limitations occur, this sections attempts to develop these and to discuss further general limitations of the theses.

### 5.3.1 Exact quantities of oestrogen unknown

Assumptions are made throughout the investigations that group (pill vs non pill and male vs female) differences are accounted for by differences in oestrogen. This is obviously a gross assumption. However, there are also problems associated with measuring oestrogen. During the early phase of the menstrual cycle oestrogen levels have been shown to be comparable to men. However, during such a phase oestrogen may still afford protection to the female muscle due to prolonged exposure to this sex hormone. Animal studies have shown that the protective effect of oestrogen tends to continue following ovariectomisation, despite a fall in circulating levels of oestrogen. This declines with time (Amelink & Bär, 1986). In addition, oral contraceptive users have lower levels of endogenous oestrogen (which is what an assay would have to assess to compare groups), but higher levels of exogenous oestrogen (Thompson et al., 1997; Savage & Clarkson 2002). Therefore, such assessment would be confounded by the above considerations. With regard to chapter 2 and the assessment of phase of cycle, oestrogen assessment would have been useful to confirm that oestrogen was at its highest and at its lowest during the respective phase of the cycle.

### 5.3.2 Peripheral markers of inflammation utilised above direct markers

Warren et al. (1999) reported that peripheral markers of EIMD were a shadow of what was really happening. However, biopsies are invasive by nature and also have associated problems. They allow only a snap shot in time, where time course in males and females could well be different. In addition to this, biopsies have associated inflammatory problems, inducing their own response.

### 5.3.3 Sample size and statistical power

Power is the ability of a test to correctly reject a false null hypothesis (Stevens, 1996). Power is dependent on four factors 1) The α level set by the researcher, 2) The difference between the mean values being compared, 3) The standard deviations of the groups, determining the spread of the curves and 4) The sample size of all groups (Stevens, 1996). Only α and the sample size are under the researcher's control. The means and standard deviations contribute to what is known as the effect size (eta squared). Huck (2000) stated that an inverse relationship exists between sample size and the probability of a type II error (failing to reject a false null hypothesis). That is, if power is high, the chances of not rejecting a false null hypothesis are low, whereas if power is low, the chances of reaching a fail to reject decision are high. There is also a direct relationship between sample size and the probability of rejecting a false null hypothesis. That is, a study with an insufficient sample size will probably lead to a fail to reject decision (Stevens, 1996). Therefore it is desirable for researchers to use sample sizes that are large enough to give their statistical tests adequate power to detect important and noteworthy deviations from the null hypothesis and make it unlikely that an important finding is missed due to a type II error.

Due the relatively unknown nature of this research area and the poor reporting of power and effect size in the literature it would have been difficult to predict the sample size (*a priori*) needed for the studies. In addition to this, according to Stevens (1996) as of yet no work has been completed to allow such predictions to be made with a repeated measures mixed design. Prediction equations are available for t tests, correlations, chi square, one way and factorial ANOVAs and more recently for a repeated measures design, but confined to a single sample.

In chapter 3 a main effect for time on thigh circumference was observed, as was a main effect for gender. The data are illustrated in Figure 3.7, surprisingly, no significant interaction was observed. The effect size for time was 0.28 and for the time x group interaction it was 0.09, both are very small effect sizes. Therefore, the sample size needed to be a lot larger when dealing with such small effects. As explained previously, the design of this investigation does not allow for a prediction equation to be utilised to determine the necessary sample size. However, if we look at where an interaction is most likely e.g. day 1 the prediction of sample size from a t test (Vincent, 1995) could be utilised. This gives a value of 26.5, therefore approximately 27 participants would be needed in both groups where such a small effect size is present, this could be used as a guideline for future investigations to ensure type II errors are not committed.

### 5.3.4 Volitional measures of strength

In all studies volitional measures of strength have been utilised. This requires that participants are motivated and are performing to their maximum ability. This can obviously be criticised because motivation may vary not only between males and females and possibly between oral contraceptive users and non-users, but also across a testing period or a repeated bout, especially when exercise-induced muscle damage and associated soreness is a further factor. As explained by Baltzopoulos and Gleeson (2001) voluntary contractions are flawed with human input. There is however, an ecological argument for volitional strength measures. If the response we see is going to be applicable to sporting performance, then perhaps the volitional input is more important than an electrically stimulated response, which is less ecologically sound.

In chapter 4 isokinetic assessments of functional loss were assessed, again through volitional input on the participants part. This assessment was made at 60°sec$^{-1}$, the equivalent of 1.05 rad sec$^{-1}$. James et al. (1994) evaluated the problems associated with measuring the concentric force-velocity characteristics of human knee extensor muscles. They concluded that stimulated contractions were the most accurate and reliable measurement, but that voluntary contractions were an acceptable alternative provided the angular velocity was less than 3.1 rad sec$^{-1}$. The use of voluntary isometric contractions were criticised. Therefore, future research will focus more on isokinetic assessment and should definitely consider the use of electrical stimulation. As the understanding of this area is poor, while electrical stimulation may not be ecologically sound, it could develop a better understanding of potential gender differences in response to exercise-induced muscle damage.

## 5.4 General conclusion and future research

So far the contributions to the literature have been based on each individual study and not taken as a whole thesis. In addition, the contributions have been across the board of the muscle damage research area and not just specific to oestrogen and muscle damage. Unfortunately the conclusions with regard to the role of oestrogen and muscle damage aren't strong conclusions, due in part to the above limitations, but also to the complexity of this area as a whole. However, the thesis does direct future research in this area through what has been discovered. What can be concluded is that intra-female differences in markers of EIMD are limited and do not appear to relate to the actual breakdown and repair of the muscle. Gender differences in terms of EIMD are more complex. It would appear that the majority of markers assessing muscle function do not show differences between males and females,

possibly negating the role of oestrogen and certainly reducing its impact within the sporting arena.

However, changes in circumference of the damaged limb (chapter 3), differences in CK activity and lower levels of elastase/cell (chapter 3) suggest a difference in the secondary processes between these two groups, which is intriguing. A further aspect of this, is that following a short break (two weeks between damage inducing exercises) the potential inhibition of inflammation within the female muscle does not appear to compromise repair (see chapter 1and figure 1.2), if the repeated bout effect is a fair assessment of such mechanisms. However, as is shown in chapter 3, the female muscle is more than capable of reacting (LPS stimulated neutrophils are just as active in females as males) if stimulated, this lends itself to the suggestion that the reason changes in circumference are smaller in females compared to males and elastase produced per cell is lower is because the stimulus for inflammation is simply not as large as in males. What is beyond this thesis is where oestrogen may play a role in reducing inflammation and how repair and regeneration are ensured in skeletal muscle.

The main and obvious future direction is to improve on the research contained within this thesis, by addressing the limitations highlighted above. Males and females may respond differently to EIMD, but it is difficult to determine the exact mechanism, when the effect size and sample size are both small. It would be fair to suggest that due to the small differences found in the samples of these studies, the implications within the sporting arena for fit, healthy young adults appear to be minimal. However, soreness as a gauge of damage does warrant future investigation, to ensure

symptom reporting is as accurate as it needs be. Muscle soreness/damage has, for example, been linked with overtraining. Despite the limited applied implications, within sport and exercise for fit, healthy individuals, the determination of the exact role and mechanism that oestrogen plays within the muscle damage and repair cycle is important and may have implications in understanding processes within both cardiac and smooth muscle. Additionally, there are implications for post-menopausal females where oestrogen is significantly reduced.

As discussed in chapter 4, there is strong evidence that oestrogen inhibits inflammation. However, it would appear that this does not affect the female muscle undergoing a repeated bout of damage-inducing exercise. Therefore, it would appear that a further mechanism may be in place to ensure this is not the case. This would obviously make evolutionary sense, to ensure that female muscle is resilient to future damage. The understanding of this additional potential role of oestrogen is important. As suggested in chapter 4, this may be due to an up-regulation of growth factors (Gargett et al., 2002) to ensure optimal regeneration. Alternatively, Duarte et al. (1994) have shown that suppression of leukocytes actually leads to more oxidative stress within the muscle. It is postulated that inhibited leukocyte invasion results in poor scavenging of damaged cells, resulting in even more oxidative stress. How this in turn would effect the muscle is unknown. One could suggest that this is almost the muscle's own defence. Oxidative stress would begin to breakdown the debris, allowing the repair of muscle. Alternatively, how would debris be removed from the muscle? Would this set up a cascade of events destroying both healthy and damaged cells? Clearly, the processes warrant future investigation.

An area of recent interest are heat shock proteins. These proteins are considered vital to the survival of any organism through their involvement within a stress response (Thompson et al., 2002). It is believed that these proteins protect against stress-induced protein denaturation and aid the elimination of damaged proteins (Welch, 1992). Heat shock proteins are also believed to play a role within cardiac muscle and are thought to afford protection against cardiac disease (Paroo et al., 2002a). Interestingly, oestrogen has been shown to attenuate post-exercise heat shock proteins in skeletal muscle (Paroo et al., 2002b). This adds an additional aspect to the muscle damage and repair cycle, not yet considered within this thesis, but which warrants further investigation.

Research in heat stress proteins and muscle damage may be applied/developed in the aging population. In such a population muscle damage is more prolific, especially following exercise, but repair is less effective. Understanding the mechanisms of muscle damage, exercise-induced or otherwise, is important for such a population. How and where heat stress proteins can play a role, needs to be incorporated within such developments.

# CHAPTER 6

## REFERENCES

Ahmed, S.A., Hissong, B.D., Verthelyi, D., Donner, K., Becker, K., & Karpuzoglo-Sahin, E. (1999). Gender and risk of autoimmune diseases: possible role of estrogenic compounds. *Environmental Health Perspectives*, **107**, 681-686.

Amandusson, A., Hallbeck, M., Hallbeck, A.L., Hermanson, O., & Blomqvist, A. (1999). Estrogen-induced alterations of spinal cord enkaphalin gene expression. *Pain*, **83**, 243-248.

Amandusson, A., & Blomqvist, A. (2001). Estrogen receptors can regulate pain sensitivity. *Lakartidningen*, **15**, 1774-1778.

Amelink, G.J., & Bär, P.R. (1986). Exercise-induced muscle protein leakage in the rat: effects of hormonal manipulation. *Journal of the Neurological Sciences*, **76**, 61-68.

Amelink, G.J., van der Kallen, C.J.M., Wokke, J.H.J., & Bär, P.R. (1990). Dantrolene sodium diminishes exercise-induced muscle damage in he rat. *European Journal of Pharmacology*, **179**, 187-192.

Angstwurm, M.W.A., Gärtner, R. & Ziegler-Heitbrock, H.W.L (1997). Cyclic plasma IL-6 levels during normal menstrual cycle. *Cytokine*, **9**, 370-374.

Armstrong, R.B. (1984). Mechanisms of exercise-induced delayed onset muscle soreness; a brief review. *Medicine and Science in Sports and Exercise*, **16**, 529-538.

Armstrong, R.B. (1990). Initial events in exercise-induced muscular injury. *Medicine and Science in Sports and Exercise*, **22**, 429-435.

Armstrong, R.B., Warren, G.L., & Warren, J.A. (1991). Mechanisms of Exercise-

Induced muscle fibre injury. *Sports Medicine*, 12, 184-207.

Armstrong, A.L., Oborne, J., Coupland, C.A., Macpherson, M.B., Bassey, E.J., & Wallace, W.A. (1996). Effects of hormone replacement therapy on muscle performance and balance in post-menopausal women. *Clinical Science*, **91**, 685-690.

Ayres, S., Tang, M.,& Subbiah, M.T.R. (1996). Estradiol 17β as an antioxidant: some distinct features when compared with common fat-soluble antioxidants. *Journal of Laboratory and Clinical Medicine*, 367-375.

Ayres, S., Abplanalp, W., Liu, J.H., & Subbiah, M.T.R. (1998). Mechanisms involved in the protective effect of estradiol-17β on lipid peroxidation and DNA damage. *American Journal of Physiology*, **274**, E1002-E1008.

Bagby, G.J., Larry, C.D., & Shepherd, R.E. (1996). Exercise and cytokines: spontaneous and elicited responses. In L. Hoffman-Goetz (Ed). *Exercise and Immune Function*. Boca Raton. 55-79.

Balnave, C.D., & Thompson, M.W. (1993). Effect of training on eccentric exercise-induced muscle damage. *Journal of Applied Physiology*, **75**, 1545-1551.

Baltzopoulos, V., & Gleeson, N.P. (2001). Skeletal muscle function. In R. Eston & T. Reilly (Eds). *Kinanthropometry and Exercise Physiology Laboratory Manual: Tests, Procedures and Data. Volume 2: Exercise Physiology* (2nd Ed.). Routledge. 7-35.

Bär, P.R., Amelink, G.J., Oldenberg, B., & Blankenstein, M.A. (1988). Prevention of exercise-induced muscle membrane damage by oestradiol. *Life Sciences*, **42**, 2677-2681.

Bär, P.R., Reijneveld, J.C., Wokke, J.H.J. Jacobs, S.C.J.M., Bootsma, A.L. (1997). Muscle damage induced by exercise: nature, prevention, and repair. In

S.Salmon (Ed). *Muscle Damage*. Oxford Medical Publications.

Bär, P.R.,& Amelink, G.J. (1997). Protection against muscle damage exerted by oestrogen; hormonal or antioxidant action? *Biochemical Society Transactions*, **25**, 50-54.

Barret-Connor, E. & Bush, T. (1991). Estrogen and coronary heart disease in women. *Journal of the American Medical Association*, **265**, 1861-1865.

Barsky, A.J., Peekna, H.M.,& Borus, J.F. (2001). Somatic symptom reporting in women and men. *Journal of General Internal Medicine*.**16(4)**, 266-75

Beato, M. (1989). Gene regulation by steroid hormones. *Cell*, **56**, 335-344.

Belcastro, A.N., Shewchuk, L.D., & Raj, D.A. (1998). Exercise-induced muscle injury; a calpain hypothesis. *Molecular and Cellular Biochemistry*, **179**, 135-145.

Berg, A., & Keul, J. (1981). Physiological and metabolic responses of female athletes during laboratory and field exercise. *Medicine Sport*, **14**, 77-96

Best, T.M., Fiebig, R., Corr, D.T., Brickson, S., & Ji, L. (1999). Free radical activity, antioxidant enzyme, and glutathione changes with muscle stretch injury in rabbits. *Journal of Applied Physiology*, **87**, 74-82.

Bethea, C.L., Pecins-Thompson, M., Schutzer, W.E., Gundlah, C.,& Lu, Z.N. (1998). Ovarian steroids and serotonin neural function. *Molecular Neurobiology*, **18**, 87-123.

Blannin, A.K., Gleeson, M.G., Brooks, S., & Cave, R. (1997). The effect of endurance training on the bacterially stimulated degranulation of human neutrophils in vitro. *Journal of Sports Sciences*, **15**, 38.

Boveris, A., & Chance, B. (1973). The mitochondrial generation of hydrogen peroxide. *Biochemical Journal*, **128**, 617-630.

Bowles, D. Torgan, C., Ebner, S., Kehrer, J., Ivy, J., & Starnes, J. (1991). Effects of acute submaximal exercise on skeletal muscle vitamin E. *Free Radical Research Communications*, **14**, 138-143.

Brooke-Wavell, K., Prelevic, G.M., Bakridan, C., & Ginsburg, J. (2001). Effects of physical activity and menopausal hormone replacement therapy on postural stability in postmenopausal women: a cross sectional study. *Maturitas*, **37(3)**, 167-172.

Bruunsgaard, H., Galbo, H., Halkjaer-Kristensen, J., Johansen, T.L., MacLean, D.A., & Pedersen, B.K. (1997). Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *Journal of Physiology*, **499**, 833-841.

Buckley-Bleiler, R., Maughan, R.J., Clarkson, P.M., Bleiler, T.L., & Whiting, P.H. (1989). Serum creatine kinase activity after isometric exercise in pre-menopausal and post-menopausal women. *Experimental Aging Research*, **15**, 195-198.

Bunt, J. (1990). Metabolic actions of estradiol: significance for acute and chronic exercise response. *Medicine and Science in Sports and Exercise*, **22**, 286-290.

Bush, T.L., Barret-Conner, E., & Cowan, L.D. (1987). Cardiovascular mortality and non-contractile estrogen use in women; results from the lipid research clinics program follow up study. *Circulation*, **75**, 1002-1009.

Byrd, S.K. (1992). Alterations in the sarcoplasmic reticulum, a possible link to exercise-induced muscle damage. *Medicine and Science in Sports and Exercise*, **24**, 531-536.

Byrne, C., & Eston, R. (2002a). Maximal intensity isometric and dynamic exercise performance after eccentric muscle actions. *Journal of Sports Sciences*, **20**, 951-959.

Byrne, C., & Eston, R. (2002b). The effect of exercise-induced muscle damage on isometric and dynamic knee extensor strength and vertical jump performance. *Journal of Sports Sciences*, **20**, 417-425.

Byrne. C., & Eston, R., & Edwrads, R.H.T. (2001). Characteristics of isometric and dynamic strength loss following eccentric exercise-induced muscle damage. *Scandinavian Journal of Medicine and Science in Sports*, **11**, 134-140.

Cannon, J.G., Orencole, S.F., Fielding, R.A., Meydani, M., Meydani, S.N., Fiatarone, M.A., Blumberg, J.B.,& Evans WJ.(1990) Acute phase response in exercise: interaction of age and vitamin E on neutrophils and muscle enzyme release. *American Journal of Physiology*. **259(6 Pt 2)**, R1214-R1219.

Cannon, J.G., Meydani, S.M., Fielding, R.., Fiatarone, M., Meydani, M., Farhangmehr, M., Orencole, S., Blumberg, J., & Evans, W. (1991). Acute phase response in exercise II: associations between vitamin E, cytokines, and muscle proteolysis. *American Journal of Physiology*, **260**, R1235-R1240.

Cantini, M., & Carraro, U. (1995). Macrophage-released factor stimulates selectively myogenic cells in primary muscle culture. *Neuropathology and Experimental Neurology*, **54**, 121-128.

Carter, A., Dobridge, J.,& Hackney, A.C. (2001). Influence of estrogen on markers of muscle tissue damage following eccentric exercise. *Fiziol Cheloveka*, **27**, 133-137.

Caulin-Glaser, T., Watson, C.A., Pardi, R.,& Bender, J.R. (1996). Effects of 17β-estradiol on cytokine-induced endothelial cell adhesion molecule expression. *Journal of Clinical Investigation*, **98**, 36-42.

Celi A, Lorenzet R, Furie B,& Furie B.C. (1997) Platelet-leukocyte-endothelial cell interaction on the blood vessel wall. *Seminars in Hematology*. **34(4)**, 327-35

Chao, T.Z., Van Alten, P.J., Greager, J.A., & Walters, R.J. (1995). Steroid sex hormones regulate the release of tumor necrosis factor by macrophages. *Cellular Immunology*, **160**, 43-49.

Child, R.B., Saxton, J.M., & Donnelly, A.E. (1998). Comparison of eccentric knee extensor muscle actions at two muscle lengths on indices of damage and angle-specific force production in humans. *Journal of Sports Sciences*, **16**, 301-308.

Chisholm, G. (1991). Antioxidants and atherosclerosis: a current assessment. *Clinical Cardiology*, **12**, 125-130.

Cid, M.C., Kleinman, H.K., Grant, D.S., Schnaper, H.W., Fauci, A.S., & Hoffman, G.S. (1994). Estradiol enhances leukocyte binding to tumor necrosis factor (TNF)-stimulated endothelial cells via an increase in TNF-induced adhesion molecules E-selectin, intercellular adhesion molecule type 1, and vascular cell adhesion molecule type 1. *The Journal of Clinical Investigation*, **93**, 17-25.

Clarkson, P.M., Byrnes, W.C., McCormick, K.M., Turcotte, L.P. & White, J.S. (1986). Muscle soreness and serum creatine kinase activity following isometric, eccentric, and concentric exercise. *International Journal of Sports Medicine*, **7**, 152-155.

Clarkson, P.M., Nosaka, K., & Braun, B. (1992). Muscle function after exercise-induced muscle damage and rapid adaptation. *Medicine and Science in Sports and Exercise*, **24**, 512-520.

Clarkson, P.M., & Newham, D.J. (1994). Associations between muscle soreness, damage and fatigue. In S.C, Gandevia, R.M. Enoka, A.J. McComas, D.G. Stuart, & C.K. Thomas (Eds). *Fatigue: Neural and Muscular Mechanisms*. New York, Plenum, 457-469.

Clarkson, P.M., & Sayers, S.P. (1999). Etiology of exercise-induced muscle damage.

*Canadian Journal of Applied Physiology*, **24**, 234-248.

Cleak, M.J., & Eston, R. (1992a). Delayed onset muscle soreness: mechanisms and management. *Journal of Sports Sciences*, **10**, 325-341.

Cleak, M.J., & Eston, R. (1992b). Muscle soreness, swelling, stiffness and strength loss after intense eccentric exercise. *British Journal of Sports Medicine*, **26**, 267-272.

Cogan, R., & Spinnato, J.A. (1986). Pain and discomfort thresholds in late pregnancy. *Pain*, **27**, 63-68.

Crane, F.L., Sun, I.L., Clark, M.G., Grebing, C., & Low, H. (1985). Transplasma-membrane redox systems in growth and development. *Biochimica et Biophysica Acta*, **811**, 233-264.

Davies, K.J.A., Quintanilha, A.T., Brooks, G.A., & Packer, L. (1982). Free radicals and tissue damage produced by exercise. *Biochimica et Biophysica Research Communications*, **107**, 1198-1205.

Davies, C.T.M. & White, M.J. (1981). Muscle weakness following eccentric work in man. *Pflugers Archives*, **392**, 168-171.

Dawson, B., Henry, G.J., Goodman, C., Gillam, I., Beilby, J.R., Ching, S., Fabian, V., Dasig, D., Morling, P.,& Kakulus, B.A. (2002). Effect of Vitamin C and E supplementation on biochemical and ultrastructural indices of muscle damage after a 21 km run. *International Journal of Sports Medicine*, **23(1)**,10-15.

Dawson-Basoa, M.E., & Gintzler, A.R. (1996). Estrogen and progesterone activate spinal kappa-opiate receptor analgesic mechanisms. *Pain*, **64**, 607-615.

Dawson-Basoa, M.E., & Gintzler, A.R. (1998). Gestational and ovarian sex steroids antinociception: synergy between spinal κ and δ opioid systems. *Brain Research*, **794**, 61-67.

Degitz, K., Lian-Jie, L., & Caughman, S.W. (1991). Cloning and characterization of the 5' transcriptional regulatory region of the human intercellular adhesion molecule 1 gene. *Journal of Biological Chemistry*, **266**, 14024-14030.

Dina, O.A., Aley, K.O., Isenberg, W., Messing, R.O., & Levine, J.D. (2001). Sex hormones regulate the contribution of PKC and PKA signalling in inflammatory pain in the rat. *European Journal of Neuroscience*, **13**, 2227-2233.

Dumke, C.L. (1996). Protective mechanism of estradiol on eccentrically induced muscle damage. Thesis. International Institute for Sport and Human Performance, University of Oregon.

Duncan, C.J. (1978). Role of intracellular calcium in promoting muscle damage: a strategy for controlling the dystrophic condition. *Experientia*, **34**, 1531-1535.

Edwards, R.H.T. (1981). Human muscle function and fatigue. In Porter and Whelan (Eds). *Human Muscle Fatigue: Physiological Mechanisms*. Pitman Medical, London, pp1-18, (Ciba foundation symposium 82).

Enoka, R.M. (1996). Eccentric contractions require unique activation strategies by the nervous system. *Journal of Applied Physiology*, **81**, 2339-2346.

Esperson, G.T., Elbeak, A., Ernst, E., Toft, E., Kaalund, S., Jersild, C., & Grunnet, N. (1990). Effects of physical exercise on cytokines and lymphocyte subpopulations in human peripheral blood. *APMIS (Copenhagen)*, **98**, 395-400.

Eston RG, Mickleborough J, Baltzopoulos V. (1995). Eccentric activation and muscle damage: biomechanical and physiological considerations during downhill running. *British Journal of Sports Medicine*, **29(2)**, 89-94.

Eston, R.G., Finney, S., Baker, S., & Baltzopoulos, V. (1996a). Muscle tenderness and peak torque changes after downhill running following a prior bout of

isokinetic eccentric exercise. *Journal of Sports Sciences*, **14**, 291-299.

Eston, R., Jackson, M., & Pears, J. (1996b). Association between the production of thiobarbituric reactive substances (malondialdehyde) and markers of muscle damage induced by uphill and downhill running. *Journal of Sports Sciences*, **14**, 80P.

Eston, R., & Peters, D. (1999). Effects of cold water immersion on the symptoms of exercise-induced muscle damage. *Journal of Sports Sciences*, **17**, 231-238.

Evans, W.J., & Cannon, J.G. (1991). The metabolic effects of exercise-induced muscle damage. *Exercise and Sport Sciences Reviews*, **19**, 99-125.

Fielding, R.A., Manfredi. T.J., Ding, W., Fiatarone, M.A., Evans, W.J.,& Cannon, J.G. (1993). Acute phase response in exercise III: neutrophil and IL-1β accumulation in skeletal muscle. *American Journal of Physiology*, **265**, R166-72.

Fillingim, R.B.,& Ness, T.J.. (2000). Sex-related hormonal influences on pain and analgesic responses. *Neuroscience and Biobehaviour*, **24(4)**, 485-501

Friden, J., Sjostrom, M. & Ekblom, B. (1983). Myofibrillar damage following intense eccentric exercise in man. *International Journal of Sports Medicine*, **4**, 170-176.

Friden, J., & Lieber, R.L. (1992). Structural and mechanical basis of exercise-induced muscle injury. *Medicine and Science in Sports and Exercise*, **24**, 521-530.

Font, B., Vial., C., & Goldschmidt, D. (1977). Metabolic levels and enzyme release in the ischaemic myocardium. *Journal of Molecular Medicine*, **2**, 291-297.

Gargett, C.E., Zaitseva, M., Bucak, K., Chu, S., Fuller, P.J., Rogers, P.A. (2002). 17Beta-estradiol up-regulates vascular endothelial growth factor receptor-2

expression in human myometrial microvascular endothelial cells: role of estrogen receptor-alpha and –beta. *Journal of Clinical Endocrinology and Metabolism*, **87(9)**, 4341-4349.

Gaub, M.P., Bellard, M., Scheuer, I., Chambon, P., Sassone-Corsi P. (1990). Activation of the ovalbumin gene by the estrogen receptor involves the fos-jun complex. *Cell*, **63**, 1267-1276.

Gintzler, A.R. (1980). Endorphin-mediated increase in pain threshold during pregnancy. *Science*, **210**, 193-195.

Gintzler, A.R., & Bohan, M.C. (1990). Pain thresholds are elevated during pseudopregnancy. *Brain Research*, **507**, 312-316.

Giuliani A, Cestaro B. (1997). Exercise, free radical generation and vitamins. *European Journal of Cancer Prevention*, **6 Suppl 1**, S55-67.

Gleeson, M., Walsh, N.P., Blannin, A.K., Robson, P.J., Cook, L., Donnelly, A.E., and Day, S.H. (1998). The effect of severe eccentric exercise-induced muscle damage on plasma elastase, glutamine and zinc concentrations. *European Journal of Applied Physiology*, **77**, 543-546.

Gordon, F.T., & Solimon, M.R.I. (1996). The effects of estradiol and progesterone on pain sensitivity and brain opioid receptors in ovariectomized rats. *Hormones and Behavior*, **30**, 244-250.

Gossland, I.F., Wynn, V., Crook, D., & Miller, N.E. (1987). Sex, plasma lipoproteins and atherosclerosis: prevailing assumptions and outstanding questions. *American Heart Journal*, **114**, 1427-1503.

Gruchow, H.W., Anderson, A.J., & Barbarak, J.J. (1988). Postmenopausal use of estrogen and occlusion of coronary arteries. *American Heart Journal*, **115**, 954-963.

Halliwell. B., & Gutteridge, J.M.C. (1985). *Free Radicals in Biology and Medicine.* Claredon Press, Oxford.

Hellsten, Y., Frandsen, V., Orthenblad, N., Sjodin, B., Richter, E.A. (1997). Xanthine oxidase in human skeletal muscle following eccentric exercise: a role in inflammation. *Journal of Physiology*, **498**, 239-248.

Hortobagyi, T., Hill, J.P., Houmard, J.A., Fraser, D.D., Lambert, N.J., & Israel, R.G. (1996). Adaptive responses to muscle lengthening and shortening in humans. *Journal of Applied Physiology*, **80**, 765-772.

Howell, J.N., Chlebourn, G., & Conatser, R. (1993). Muscle stiffness, strength loss, swelling and soreness following exercise-induced injury in humans. *Journal of Physiology*, **464**, 183-196.

Huber, L., Scheffler, E., Poll, T., Zeigler, R., & Dresel, H. (1990). 17β-estradiol inhibits LDL oxidation and cholesteryl ester formation in cultured macrophages. *Free Radical Research Communication*, **8**, 167-175.

Iadermarco, M., McQuillan, J., Rosen, G.,& Dean, D. (1992). Characterization of the promoter for vascular cell adhesion molecule-1 (VCAM-1). *Journal of Biological Chemistry*, **267**, 16323-16329.

Ingalls, C.P., Warren, G.L., Williams, J.H., Ward, C.W., & Armstrong, R.B. (1998). E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions. *Journal of Applied Physiology*, **85**, 58-67.

Imura, H., Fukata, J., & Mori, T. (1991). Cytokines and endocrine function: an interaction between the immune and neuroendocrine systems. *Clinical Endocrinology*, **35**, 107-15

Iwasaki, H., Collins, J.G., Saito, Y., & Kerman-Hinds, A. (1991). Naloxene-sensitive pregnancy-induced changes in behavioural responses to colorectal distension:

pregnancy-induced analgesia to visceral stimulation. *Anesthesiology*, **74**, 927-933.

James, C., Sacco, P., Hurley, M.V.,& Jones, D.A. (1994). An evaluation of different protocols for measuring the force-velocity relationship of the human quadriceps muscles. *European Journal of Applied Physiology and Occupational Physiology*. **68(1)**, 41-47.

Jenkins, R.R. (1988). Free radical chemistry: relationship to exercise. *Sports Medicine*, **5**, 156-120.

Jones, D.A., Newham, D.J., Round, J.M., & Tolfree, S.E. (1986). Experimental human muscle damage: morphological changes in relation to other indices of damage. *Journal of Physiology*, **375**, 435-448.

Jones, D.A., Newham, D.J. & Clarkson, P.M. (1987). Skeletal muscle stiffness and pain following eccentric exercise of the elbow flexors. *Pain*, **30**, 233-242.

Jones, D.A., & Round, J.M. (1990). *Skeletal Muscle in Health and Disease*. Manchester University Press.

Jovanovic, S., Jovanovic, A., Shen, W.K., & Terzic, A. (2000). Low concentrations of 17β-estradiol protect single cardiac cells against metabolic stress-induced Ca2+ loading. *Journal of the American College of Cardiology*, **36**, 948-952.

Kanter, M.M., Lesmes, G.R., Kaminsky, L.A., La Ham-Saeger, J., & Nequin, N.D. (1988). Serum creatine kinase and lactate dehydrogenase changes following an eighty kilometer race. Relationship to lipid peroxidation. *European Journal of Applied Physiology and Occupational Physiology*, **57(1)**, 60-63.

Karlsson, J. (1997). *Antioxidants and Exercise*. Human Kinetics.

Komulainen, J., Koskinen, S.O., Kalliokoski, R. Takala, T.E., & Vihko, V. (1999). Gender differences in skeletal muscle fibre damage after eccentrically biased

downhill running in rats. *Acta Physiologica Scandinavica*, **165**, 57-63.

Kristal, M.B., Thompson, A.C., Abbott, P., Di Pirro, J.M., Ferguson, E,J., & Doerr, J.C. (1990). Amniotic-fluid ingestion by parturient rats enhances pregnancy-mediated analgesia. *Life science*, **46**, 693-698.

Lapointe, B.M., Fremont, P., & Cote, C.H. (2002). Adaptations to lengthening contractions is independent of voluntary muscle recruitment but relies on inflammation. *American Journal of Physiology*, **282**, R323-R329.

Lastayo, P.C., Reich, T.E., Urquhart, M., Hoppeler, H., & Lindstedt, S.L. (1999). Chronic eccentric exercise improvements in muscle strength can occur with little demand for oxygen. *American Journal of Physiology*, **276**, R611-R615.

Lescaudron, L., Peltekian, E., Fontaine-Perus, J., Paulin, D., Zampieri, M., Garcia, L., & Parrish, E. (1999). Blood borne macrophages are essential for the triggering of muscle regeneration following muscle transplant. *Neuromuscular Disorders*, **9**, 72-80.

Lieban, R.W. (1985). Gender and symptom sensitivity: report on a Philippine study. *American Journal of Orthopsychiatry*, **55(3)**, 446-50.

Lieber, R.L., & Friden, J. (1988). Selective damage of fast glycolytic muscle fibres with eccentric contartion of the rabbit tibialis anterior. *Acta Physiologica Scandinavica*, **133**, 97-588.

Lieber, R.L., & Friden, J. (1993). Muscle damage is not a function of muscle force but active muscle strain. *Journal of Applied Physiology*, **74**, 520-526.

Liljedahl, M.E., Holm, I., Sylven, C., Jansson, E. (1996). Different response of skeletal muscle following sprint training in men and women. *European Journal of Applied Physiology and Occupational Physiology*, **74(4)**, 375-383.

Liu, N., & Gintzler, A.A. (2000). Prolonged ovarian sex steroid treatment of male

rats produces antinociception: identification of sex-based divergent analgesic mechanisms. *Pain*, **85**, 273-281.

Lowe, D.A., Warren, G.L., Ingalls, C.P., Boorstein, D.B., & Armstrong, R.B. (1995). Muscle function and protein metabolism after initiation of eccentric contraction-induced injury. *Journal of Applied Physiology*, **79**, 1260-1270.

Macintyre, P.E., & Ready, L.B. (1996). *Acute Pain Management: A Practical Guide.* W.B. Saunders Company LTD. London.

MacIntyre, D.L., Reid, W.D., & McKenzie, D.C. (1995). The inflammatory response to muscle injury and its clinical implications. *Sports Medicine*, **20**, 24-40.

MacIntyre, D.L., Reid, W.D., Lyster, D.M., & McKenzie, D.C. (2000). Different effects of strenuous eccentric exercise on the accumulation of neutrophils in muscle in women and men. *European Journal of Applied Physiology*, **81**, 57-53.

MacPherson, C.D., Schork, A.M., & Faulkner, J.A. (1996). Contraction-induced injury to single permeabilised muscle fibres from fast and slow muscles of the rat following single stretches. *American Journal of Physiology*, **271**, C1438-C1446.

Malm, C., Lenkei, R., & Sjodin, B. (1999). Effects of eccentric exercise on the immune system in men. *Journal of Applied Physiology*, **86(2)**, 461-8.

Martinez-Gomez, M., Cruz, Y., Salas, M., Hudson, R., & Pachecos, P. (1994). Assessing pain thresholds in the rat: changes with estrus and time of day. *Physiology and Behavior*, **55**, 651-657.

Marui, N., Offerman, M., Swerlick, R., Kunsch, C., Rosen, C.A., Ahmad, M., Alexander, R.W., & Medford, R.M. (1993). Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an

antioxidant-sensitive mechanism in human vascular endothelial cells. *Journal of Clinical Investigation*, **92**, 1866-1874.

Maughan, R.J., Donnelly, A.E., Gleeson, M., Whiting, P.H., Walker, K.A.,& Clough PJ. *(1989). Delayed-onset muscle damage and lipid peroxidation in man after a* downhill run. *Muscle and Nerve*, **12(4)**, 332-6.

McArdle, A., & Jackson, M.J. (1997). Intracellular mechanisms involved in skeletal muscle damage. In S.Salmon (ed). *Muscle Damage*. Oxford Medical Publications.

McComas, A.J. (1996). Skeletal Muscle: Form and Function. Human Kinetics.

McCord, J. (1995). Superoxide radical: controversies, contradictions, and paradoxes. *Proceedings of the Society for Experimental Biology and Medicine*, **209**, 112-117.

McHugh, M.P., Connolly, D.A., Eston, R.G., & Gleim, G.W. (1999). Exercise-induced muscle damage and the potential mechanisms for the repeated bout effect. *Sports Medicine*, **27(3)**, 157-170.

Meltzer, H.Y. (1971). Factors affecting serum creatine phosphokinase levels in the general population. The role of race, activity and sex. *Clinica Chimica Acta,* **33**, 165-172.

Merly, F., Lescaudron, L., Rouaud, T., Crossin, F., & Gardahaut, M.F. (1999). Macrophages enhance muscle satellite cell proliferation and delay their differentiation. *Muscle and Nerve*, **22**, 724-732.

Mishra, D.K., Friden, J., Schmitz, M.C., & Lieber, R.L. (1997). Anti-inflammatory medication after muscle injury. A treatment resulting in short-term improvement but subsequent loss of muscle function. *Journal of Bone and Joint Surgery*, **79**, 1270-1271.

Montgomery, K.F., Osborn, L., Hession, C., Tizard, R., Goff, D., Vassallo, C., Tarr, P.I., Bomsztyk, K., Lobb, R., Harlan, J.M., & Pohlman, T.H. (1991). *Activation* of endothelial-leukocyte adhesion molecule 1 (ELAM-1) gene transcription. *Proceedings of the National Academy of Sciences,* **88**, 6523-6527.

Mooradian, A.D. (1993). Antioxidant properties of steroids. *Biochemical Molecular Biology*, **45**, 509-511.

Naessen, T., Linmark, B., & Larsen, H.C. (1997). Better postural balance in elderly women receiving estrogens. *American Journal of Obstetrics and Gynaecology*, **177**, 412-416.

Newham, D.J., Mills, K.R., Quigley, B.M., & Edwards, R.H.T. (1983). Pain and fatigue after concentric and eccentric muscle contractions. *Clinical Science*, **64**, 55-62.

Newham, D.J., Jones, D.A., & Clarkson, P.M. (1987). Repeated high force eccentric exercise: effects on muscle pain and damage. *Journal of Applied Physiology*, **63**, 1381-1386.

Niess, A.M., Dickhuth, H., Northoff, H., Fehrenbach, E. (1999). Free radicals and oxidative stress in exercise-immunological aspects. *Exercise Immunology Review*, **5**, 22-56.

Nosaka, K., Clarkson, P. M., McGuiggin, M. E.,& Byrne, J.M. (1991). Time course of muscle adaptation after high force eccentric exercise. *European Journal of Applied Physiology Occupational Physiology*, **63(1)**, 70-76.

Nosaka, K., & Clarkson, P.M. (1995). Muscle damage following repeated bouts of high force eccentric exercise. *Medicine and Science in Sports and Exercise*, **27**, 1263-1269.

Nosaka, K., Sakamoto, K., Newton, M.,& Sacco, P. (2001).How long does the protective effect on eccentric exercise-induced muscle damage last? *Medicine and Science in Sports and Exercise*, **33(9)**,1490-1495.

Nosaka, K., Newton, M., & Sacco, P. (2002). Response of human elbow flexor muscle to electrically stimulated forced lengthening exercise. *Acta Physiologica Scandinavica*, **174**, 137-145.

Ostrowski, K., Schjerling, P., & Pedersen, B.K.(2000) Physical activity and plasma interleukin-6 in humans--effect of intensity of exercise. *European Journal of Applied Physiology*, **83(6)**,512-5.

Paroo, Z., Haist, J.V., Karmazyn, M., & Noble, E.G. (2002a). Exercise improves postischemic cardiac function in males but not females: consequences of a novel sex-specific heat shock protein response. Circulation Research, 90(8), 911-917.

Paroo, Z., Dipchand, E.S., & Noble, E.A. (2002b). Estrogen attenuates postexercise HSP70 expression in skeletal muscle. *American Journal of Physiology*, **282**, C245-C251.

Pedersen, B.K., Bruunsgaard, H., Klokker, M., Kappel, M., MacLean, D.A., Nielson, H.B. Rohde, T., Ullum, H., & Zacho, M. (1997). Exercise-induced immunomodulation: Possible roles of neuroendocrine and metabolic factors. *International Journal of Sports Medicine*, **18**, S2-S7.

Persky, A.M., Green, P.S., Stubley, L., Howell, C.O., Zaulyanov, L., Brazeau, G.A., Simpkins, J.W. (2000). Protective effect of estrogen against oxidative damage to heart and skeletal muscle in vivo and in vitro. *Proceedings of the Society for Experimental Biology and Medicine*, **223**, 59-66.

Phillips, S.K., Rook, K.M., Siddle, N.C., Bruce, S.A., & Woledge, R.C. (1993).

Muscle weakness in women occurs at an earlier age than in men, but strength is preserved by hormone replacement therapy. *Clinical Science*, **84**, 95-98.

Pizza, F.X., Mitchell, J.B., Davis, B.H., Starling, R.D., Holtz, R.W., & Bigelow, N. (1995). Exercise-induced muscle damage: effect on circulating leukocyte and lymphocyte subsets. *Medicine and Science in Sports and Exercise*, **27**, 363-370.

Pizza, F.X., Baylies, H., & Mitchell, J.B. (2001). Adaptation to eccentric exercise: neutrophils and E-selectin during early recovery. *Canadian Journal of Applied Physiology*, **26**, 245-253.

Pottratz, S.T., Bellido, T., Macharla, H., Crabb, D., & Manolagas, S.C. (1994). 17β-estradiol inhibits expression of human interleukin-6 promoter-reporter constructs by a receptor-dependent mechanism. *Journal of Clinical Investigation*, **93**, 944-950.

Prakash, Y.S., Togaibayeva, A.A., Kannan, M.S., Miller, V.M., Fitzpatrick, L.A., & Sieck, G.C. (1999). Estrogen increases $Ca^{2+}$ efflux from female porcine coronary arterial smooth muscle. *American Journal of Physiology*, **276**, H926-H934.

Pyne, D.B. (1994). Regulation of neutrophil function during exercise. *Sports Medicine*, **17**, 245-258.

Rao, S.S., Ranganekar, A.G., & Saifi, A.Q. (1987). Pain thresholds in relation to sex hormones. *Indian Journal of Physiology and Pharmacology*, **31**, 250-254.

Reijneveld, J.C., Ferrington, D.A., Amelink, G.J., & Bär, P.R. (1994). Estradiol treatment reduces structural muscle damage after exercise in male rats. *Medicine and Science in Sports and Exercise*, **25**, S72.

Rider, V., Foster, R.T., Evans, M., Svenaga, R., & Abdou, N.I. (1998). Gender

differences in autoimmune diseases: estrogen increases calcineurin expression in systemic lupus erythematosus. *Clinical Immunology and Immunopathology*, **89**,171-180.

Riley, J.L., Robinson, M.E., Wise, E.A., & Price, D.D. (1999). A meta-analytic review of pain perception across the menstrual cycle. *Pain*, **81,** 225-235.

Rinard, J., Clarkson, P.M., Smith, L., Grosman, M. (2000). Response of males and females to high-force eccentric exercise. *Journal of Sports Sciences*, **18**, 229-236.

Rogers, M.A., Stull, G.A., & Apple, F.S. (1985). Creatine kinase isoenzyme activities in men and women following a marathon race. *Medicine and Science in Sports and Exercise*, **17**, 679-682.

Ruehlmann, D.O., & Mann, G.E. (1997). Actions of oestrogen on vascular endothelial and smooth muscle cells. *Biochemical Society Transactions*, **25**, 40-45.

Savage, K.J., & Clarkson, P.M. (2002). Oral contraceptive use and exercise-induced muscle damage and recovery. *Contraception*, **66**, 67-71.

Saxton, J.M., Donnelly, A.E., & Roper, H.P. (1994). Indices of free-radical-mediated damage following maximum voluntary eccentric and concentric muscular work. *European Journal of Applied Physiology and Occupational Physiology*, **68(3)**,189-93

Schwarz, E., Schafer, C., Bode, J.C., & Bode, C. (2000). Influence of the menstrual cycle on the LPS-induced cytokine response of monocytes. *Cytokine*, **12**, 413-416.

Seeley, D.G., Cauley, J.A., Grady, D., Browner, W.S., Nevitt, M.C., & Cummings, S.R. (1995). Is postmenopausal estrogen therapy associated with

neuromuscular function or falling in elderly women? Study of osteoporotic fractures research group. *Archives of International Medicine*, **155**, 293-299.

Sen, C.K., Atalay, M., Agren, J., Laaksonen, D.E., Roy, S., & Hanninen, O. (1997). Fish oil and vitamin E supplementation in oxidative stress at rest and after physical exercise. *Journal of Applied Physiology*, **83**, 189-95.

Sen, C.K. (2001). Antioxidant and redox regulation of cellular signalling: introduction. *Medicine and Science in Sports and Exercise*, **33**, 368-370.

Sen, C.K., & Roy, S. (2001). Antioxidant regulation of cell adhesion. *Medicine and Science in Sports and Exercise*, **33**, 377-381.

Shumate, J.B., Brooke, M.H., Carroll, J.E., & Davis, J.E. (1979). Increased serum creatine kinase after exercise: a sex-linked phenomenon. *Neurology*, **29**, 902-904.

Shyamala, G., & Guiot, M.C. (1992). Activation of Kβ-specific proteins by estradiol. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 10628-10632.

Sjöström, M., Friden, J., & Ekblom, B. (1987). Endurance, what is it? Muscle morphology after an extremely long distance run. *Acta Physiologica Scandinavica*, **130**, 513-520.

Smith, L.L., McCammon, M., Smith, S., Chamness, M., Isreal, R.G., & O'Brien, K.F. (1989). White blood cell response to uphill walking and downhill jogging at similar metabolic loads. *European Journal of Applied Physiology*, **58**, 833-837

Smith, L.L. (1991). Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Medicine and Science in Sports and Exercise*, **23**, 542-551.

Smith, L.L., Anwar, A., Fragen, M., Rananto, C., Johnson, R., & Holbert, D. (2000).

Cytokines and cell adhesion molecules associated with high intensity eccentric exercise. *European Journal of Applied Physiology*, **82**, 61-67.

Stampfer, M.J., Colditz, G.A., & Willet, W.C. (1991). Postmenopausal estrogen therapy and cardiovascular disease. *New England Journal of Medicine*, **325**, 756-762.

Stevens, J. (1996). *Applied Multivariate Statistics For The Social Sciences* (3rd Ed). Mahwah, N.J.

St Pierre Schneider, B., Correia, L.A., & Cannon, J.G. (1999). Sex differences in leukocyte invasion in injured murine skeletal muscle. *Research in Nursing and Health*, **22**, 243-251.

Stumpf, W., Sar, M., & Aumuller, G. (1977). The heart: a target organ for estradiol. *Science*, **196**, 319-320.

Stupka, N., Lowther, S., Chorneyka, K., Bourgeois, J.M., Hogben, C., & Tarnopolsky, M.A. (2000). Gender differences in muscle inflammation after eccentric exercise. *Journal of Applied Physiology*, **89**, 2325-2332.

Stupka, N., Tarnopolsky, M.A., Yardley, N.J., & Phillips, S.M. (2001). Cellular adaptation to repeated eccentric exercise-induce muscle damage. *Journal of Applied Physiology*, **91**, 1669-1678.

Subbiah, M., Kessel, B., Agrawal, M., Rajan, R., Abplnalp, W., & Rymaszewski, Z. (1993). Antioxidant potential of specific estrogens on lipid peroxidation. *Journal of Clinical Endocrinology and Metabolism*, **77**, 1095-1097.

Sugioka, K., Shimosegawa, Y., & Nakano, M. (1987). Estrogens as natural antioxidants of membrane phospholipid peroxidation. *FEBS Letters*, **210,** 37-39.

Suzuki, K., Totsuka, M., Nakaji, M.Y., Kudoh, S., Liu, Q., Sugawara, K., Yamaya,

K., & Sato, K. (1999). Endurance exercise causes interaction among stress hormones, cytokine, neutrophil dynamics and muscle damage. *Journal of Applied Physiology*, **87**, 1360-1367.

Taaffe, D.R., Luz Villa, M., Delay, R., & Marcus, R. (1995). Maximal muscle strength of elderly women is not influenced by oestrogen status. *Age and Ageing*, **24**, 329-333.

Talag, T. (1973). Residual muscle soreness as influenced by concentric, eccentric and static contractions. *Research Quarterly*, **44**, 458-469.

Thomas, V.N. (1997). *Pain: its nature and management*. Bailliere Tindall. London.

Thompson, H.S., Hyatt, J.P., De Souza, M.J., & Clarkson, P.M. (1997). The effects of oral contraceptives on delayed onset muscle soreness following exercise. *Contraception*, **56**, 59-65.

Thompson, H.S., Clarkson, P.M., & Scordilis, S.P. (2002). The repeated bout effect and heat shock proteins: intramuscular HSP27 and HSP70 expression following two bouts of eccentric exercise in humans. *Acta Physiologica Scandinavica,* **174**, 47-56.

Thorsson, O., Rantanen, J., Hurme, T., & Kalimo, H. (1998). Effects of nonsteroidal anti-inflammatory medications on satellite cell proliferation during muscle regeneration. *American Journal of Sports Medicine*, **26**, 172-176.

Tidball, J.G. (1995). Inflammatory cell response to acute muscle injury. *Medicine and Science in Sports and Exercise*, **27**, 1022-1032.

Tiidus, P.M., & Houston, M.E. (1993). Vitamin E status does not affect the responses to exercise training and acute exercise in female rats. *Journal of Nutrition*, **R3**, 834-840.

Tiidus, P.M. (1995). Can estrogens diminish exercise induced muscle damage?

*Canadian Journal of Applied Physiology*, **20**, 26-38.

Tiidus, P.M. (1998). Radical species in inflammation and overtraining. *Canadian Journal of Physiological Pharmacology*, **76**, 533-538.

Tiidus, P.M. (1999). Nutritional implications of gender differences in metabolism: estrogen and oxygen radicals: oxidative damage, inflammation and muscle function. In M. Tarnopolsky (Ed). *Gender Differences in Metabolism; Practical and Nutritional Implications*. Boca Raton, Florida.

Tiidus, P.M., & Bombardier, F. (1999). Oestrogen attenuates post exercise myeloperoxide activity in skeletal muscle of male rats. *Acta Physiologica Scandinavica*, **166**, 85-90.

Tiidus, P.M., Holden, D., Bombardier, E., Zajchawski, S., Enns, D., & Belcastro, A. (2001). Estrogen effect on post-exercise skeletal muscle neutrophil infiltration and calpain activity. *Canadian Journal of Physiology and Pharmacology*, **79**, 400-406.

Tornwall, J., Carey, A.B., Fox, R.I., & Fox, H.S. (1999). Estrogen in autoimmunity; expression of estrogen receptors in thymic and autoimmune T Cells. *Journal of Gender Specific Medicine*, **2**, 33-40.

Twist, C. (on going). PhD thesis. The effects of exercise-induced muscle damage on exercise performance.

Van Der Meulen, J.H., Kuipers, H., & Drukker, J. (1991) Relationship between exercise-induced muscle damage and enzyme release in rats. *Journal of Applied Physiology*, **71**, 999-1004.

Van Wynsberghe, D., Noback, C.R. & Carola, R. (1995). *Human Anatomy And Physiology (3rd Ed)*. McGraw-Hill, Inc.

Vincent, W.J. (1995). *Statistics in Kinesiology*. Human Kinetics.

Warner, C.D.(1995) Somatic awareness and coronary artery disease in women with chest pain. *Heart and Lung,* **24(6)**,436-43.

Warren G.L., Lowe, D.A., & Armstrong, R.B. (1999). Measurement tools used in the study of eccentric contraction-induced injury. *Sports Medicine*, **27**, 43-59.

Weiss S.J. (1989). Tissue destruction by neutrophils. *New England Journal of Medicine,* **320(6)**, 365-76

Welch, W.J. (1992). Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. *Physiology Review*, **72**, 1063-1081.

Whipple, B., Josimovich, J.B.,& Komisaruk, B.R. (1990). Sensory threshold during the antepartum, intrapartum, and postpartum periods. *International Journal of Nursing Studies*, **27**, 213-221.

Whiting, K.P., Restall, C.J., Brain, & P.F. (2000). Steroid hormone-induced effects on membrane fluidity and their potential roles in non-genomic mechanisms. *Life Sciences*, **67**, 743-757.

Wilmore, J.H., & Costill, D.L. (1994). *Physiology of Sport and Exercise*. Human Kinetics.

Wiseman H, Quinn P, & Halliwell B. (1993). Tamoxifen and related compounds decrease membrane fluidity in liposomes. Mechanism for the antioxidant action of tamoxifen and relevance to its anticancer and cardioprotective actions? *FEBS Letters,* **330**, 53-6.

Wiseman, H., & Quinn, P. (1994). The antioxidant action of synthetic oestrogens involves decreased membrane fluidity: relevance to their potential use as an anticancer and cardioprotective agents compared to tamoxifen. *Free Radical Research*, **21**, 187-194.

Wiseman, H. (1997). Oestrogens: molecular mechanisms of cardioprotection. *Biochemical Society Transactions*, **25**, 31-32.

Wiseman, H., & O'Reilly, J. (1997). Oestrogen as antioxidant cardioprotectants. *Biochemical Society Transactions*, **25**, 54-59.

Yackzan, L., Adams, C., & Francis, K.T. (1984). The effects of ice massage on delayed onset muscle soreness. *American Journal of Sports Medicine*, **12**, 159-65.

Yagi, K., & Komura, S. (1986). Inhibitory effect of female hormones on lipid peroxidation. In C. Vigo-Pelfrey (Ed). *Membrane Lipid Peroxidation I.* 205-301. Boca Raton.

Yoshikawa, T., & Yoshida, N. (2000). Vitamin E and leukocyte endothelial cell interactions. *Antioxidant Redox Signal*, **2**, 821-825.

Zuckerman, S.H., Ahmari, S.E., Bryan-Poole, N., Evans, G.F., Short, L., & Glasebrook, A.L. (1996). Estriol: a potent regulator of TNF and IL-6 expression in a murine model of endotoxemia. *Inflammation*, **20**, 581-597.

# Appendix 1

Study 1
Measures of exercise-induced muscle damage between pill and non-pill users and within phase 1 and phase 2 of the menstrual cycle following a bout of eccentric exercise of the elbow flexors. Data are mean ± SD

| | Variable | Phase | Base | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|---|---|
| NON-PILL | RAA (°) | 1 | 165.43±5.2 | 160.43±6.5 | 158.71±6.7 | 157.85±6.8 |
| | RAA (°) | 2 | 164.29±5.1 | 159.14±6.2 | 158.43±7.8 | 159.28±5.9 |
| | CK (log IU/l) | 1 | 1.97±0.21 | 2.07±.21 | 2.14±0.26 | 2.51±0.68 |
| | CK (log IU/l) | 2 | 1.82±0.18 | 1.94±.21 | 2.52±0.83 | 2.34±0.75 |
| | ISO (%max) | 1 | 100±0 | 71.67±13.8 | 77.74±15.8 | 80.56±17.5 |
| | ISO (%max) | 2 | 100±0 | 70.55±15.9 | 76.13±15.4 | 83.34±19.0 |
| | SORE (VAS) | 1 | 0±0 | 4.53±1.9 | 4.46±1.9 | 4.11±2.1 |
| | SORE (VAS) | 2 | 0±0 | 4.83±1.3 | 5.39±2.4 | 3.64±2.8 |
| | CIRC (CM) | 1 | 26.45±1.9 | 26.70±2.3 | 27.15±2.7 | 27.19±2.1 |
| | CIRC (CM) | 2 | 26.54±1.9 | 26.63±2.1 | 26.63±1.9 | 27.14±2.2 |
| | | | | | | |
| PILL | RAA (°) | 1 | 166.22±6.8 | 161.22±6.4 | 159.11±7.6 | 160.55±7.1 |
| | RAA (°) | 2 | 162.33±3.2 | 156.22±6.2 | 154.55±9.6 | 156.44±8.2 |
| | CK (log IU/l) | 1 | 1.94±0.14 | 2.07±0.32 | 2.09±0.37 | 2.33±0.49 |
| | CK (log IU/l) | 2 | 1.96±0.27 | 2.06±0.29 | 2.19±0.51 | 2.49±0.63 |
| | ISO (%max) | 1 | 100±0 | 65.86±16.6 | 78.77±12.9 | 84.76±10.1 |
| | ISO (%max) | 2 | 100±0 | 69.99±7.8 | 81.42±15.6 | 84.35±14.9 |
| | SORE (VAS) | 1 | 0±0 | 1.88±1.4 | 2.93±2.2 | 1.30±1.3 |
| | SORE (VAS) | 2 | 0±0 | 2.65±2.3 | 3.02±2.6 | 1.59±1.6 |
| | CIRC (CM) | 1 | 27.89±1.7 | 28.25±1.8 | 28.28±1.8 | 28.07±1.8 |
| | CIRC (CM) | 2 | 27.64±1.9 | 28.03±1.8 | 28.11±1.72 | 28.14±1.94 |

Note: RAA is relaxed arm angle, CK is log CK values, Iso is percentage of maximal isometric strength, Sore is perceived soreness, and Circ is upper arm circumference.

Study 2
Measures of exercise-induced muscle damage and inflammatory response between males and females following a bout of eccentrically biased exercise. Data are mean ± SD

| | | Base | 2 hr | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|---|---|---|
| Females | Sore | 0±0 | | 5.8±1.3 | 6.0±0.9 | 3.8±1.5 | 2.7±1.3 | 1.6±0.9 | 0.9±0.7 | 0.5±0.5 |
| | CK (log) | 2.1±0.2 | | 2.8±0.3 | 2.6±0.2 | 2.5±0.2 | 2.4±0.1 | 2.4±0.3 | 2.4±0.2 | 2.3±0.3 |
| | Iso 10 | 100±0 | | 57.4±28.3 | 63.3±20.0 | 72.1±17.6 | 72.8±24.1 | 81.4±10 | 78.2±1 14.9 | 85.0±12.3 |
| | Iso 20 | 100±0 | | 70.3±15.8 | 71.1±14.0 | 77.2±15.0 | 79.0±17.2 | 84.1±9.5 | 80.2±1 3.9 | 87.5±11.7 |
| | Iso 80 | 100±0 | | 81.9±11.1 | 87.9±8.1 | 89.3±11.6 | 91.2±4.6 | 95.7±4.9 | 92.0±1 2.7 | 98.6±7.8 |
| | Jump | 43.2±10.7 | | 37.2±12.3 | 38.6±10.2 | 41.1±11.9 | 40.7±11 | 41.8±12.6 | 41.3±1 0.7 | 42.9±11.8 |
| | Circ | 100±0 | | 100.1±0.2 | 100.3±0.4 | 100.3±0.6 | 100.1±0.6 | 99.7±1.3 | 99.2±0. 9 | 99.8±0.6 |
| | WBC | 5.8±2 | 8.6±2.5 | 5.2±1.4 | | 5.7±1.1 | | 5.6±1.0 | | 5.6±1.0 |
| | Neut | 3.0±1.4 | 5.9±2 | 2.4±0.8 | | 2.8±0.9 | | 2.8±0.6 | | 2.8±0.7 |
| | Elast | 64.3±47.9 | 56.1±43.8 | 69.5±76 | | 44.1±29.1 | | 99.9±88.6 | | 74.5±44.3 |
| | Elast/cell | 21.7±13.8 | 5.6±4.6 | 8.1±5.5 | | 11.3±5.9 | | 13±7.1 | | 8.3±4.2 |
| | LPS Elast | 922±360 | 1892±477 | 776±455 | | 790±380 | | 907±348 | | 1066±574 |
| | LPS Elast/cell | 205±81 | 209±62 | 206±94 | | 176±75 | | 205±74 | | 234±83 |

| | | Base | 2 hr | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|---|---|---|
| Males | Sore | 0±0 | | 3.5±1.5 | 5.2±2.2 | 2.2±1.4 | 1.0±1.1 | 0.5±1 | 0.0±0.2 | 0±0 |
| | CK | 2.4±0.3 | | 3.2±0.3 | 2.9±0.3 | 2.7±0.4 | 2.6±0.4 | 2.6±0.4 | 2.6±0.4 | 2.5±0.4 |
| | Iso 10 | 100±0 | | 66.6±16.2 | 63.8±9.6 | 71.3±9.8 | 87.3±21.4 | 88.6±31.1 | 90.9±26.6 | 87±16.1 |
| | Iso 20 | 100±0 | | 72±13.5 | 78±8.3 | 79.3±9.9 | 87.6±7.7 | 91.8±11.6 | 88.2±4.6 | 92.4±11.7 |
| | Iso 80 | 100±0 | | 85.3±4.9 | 78.2±19.3 | 92.1±7.3 | 94.2±6.2 | 97.1±5.2 | 95.0±5.9 | 93.0±8.4 |
| | Jump | 55.7±13.8 | | 50.3±10.8 | 51.1±11.7 | 51.4±10.2 | 53.8±10.5 | 51.9±10.9 | 54.6±10.2 | 54.2±12.6 |
| | Circ | 100±0 | | 100.1±0.2 | 101.3±1.3 | 101.2±0.6 | 101.2±0.6 | 100.6±0.5 | 100.3±0.6 | 100.0±0.6 |
| | WBC | ± | ± | ± | | ± | | ± | | ± |
| | Neut | 2.7±0.5 | 6.8±3.0 | 3.1±0.5 | | 3.1±0.8 | | 2.9±0.6 | | 3.2±0.8 |
| | Elast | 102.6±57.9 | 99.7±84.6 | 66.7±28.0 | | 90.8±42.2 | | 101.3±71.3 | | 56.1±31.5 |
| | Elast/cell | 13.0±4.8 | 9.5±6.4 | 19.6±9.5 | | 16.0±11.7 | | 28.7±18.2 | | 18.3±7.6 |
| | LPS Elast | 1196±416 | 2352±1220 | 1141±553 | | 1493±822 | | 1289±440 | | 1342±546 |
| | LPS Elast/cell | 239±65 | 188±47 | 208±111 | | 255±107 | | 249±88 | | 230±76 |

Note: CK is log CK values, Iso is percentage of maximal isometric strength (at 10, 20 and 80 degrees), Sore is perceived soreness, Circ is mid-thigh circumference, elast is elastase concentration, elast/cell is elastase expressed per neutrophil, LPS elast is stimulated elastase concentration and LPS elast/cell is stimulated elastase concentration expressed per cell.

Study 3

Measures of exercise-induced muscle damage between males and females following two bouts of eccentrically biased exercise. Data are mean ± SD

| | | | Base | day 1 | day 2 | day 3 | day 4 |
|---|---|---|---|---|---|---|---|
| Females | sore | bout 1 | 0±0 | 6.3±1.7 | 5.2±1.4 | 3.4±1.7 | 2.2±1.8 |
| | | bout 2 | 0±0 | 3.4±0.8 | 3±1.0 | 1.6±1.1 | 0.4±0.5 |
| | CK | bout 1 | 100±0 | 1660±1144 | 1030±860 | 855±697 | 951±1013 |
| | | bout 2 | 100±0 | 685±791 | 317±315 | 203.8±169 | 164.3±83 |
| | iso 20 | bout 1 | 100±0 | 70.1±15.3 | 82.8±19.8 | 91.6±14.6 | 97±13.8 |
| | | bout 2 | 100±0 | 92.9±17.1 | 87.9±7.3 | 94.4±21.7 | 103.1±24.4 |
| | iso 40 | bout 1 | 100±0 | 76±15.9 | 87.7±13.0 | 91.7±15.3 | 95.4±13.6 |
| | | bout 2 | 100±0 | 95.9±10.0 | 98.2±7.9 | 97.2±13.2 | 103.9±13.8 |
| | iso 80 | bout 1 | 100±0 | 78.9±13.5 | 85.7±10.8 | 88.2±10.7 | 92.7±13.5 |
| | | bout 2 | 100±0 | 92.9±9.0 | 99.3±10.1 | 103.5±9.0 | 106.6±9.3 |
| | wkdone | bout 1 | 100±0 | 79.5±10.7 | 83.7±10.4 | 90.9±15.6 | 82.2±31.3 |
| | | bout 2 | 100±0 | 88.7±8.0 | 99.3±9.4 | 101.5±9.8 | 105.9±9.8 |
| | p torq | bout 1 | 100±0 | 82.2±13.3 | 85.9±13.2 | 88.3±9.2 | 91.5±12.1 |
| | | bout 2 | 100±0 | 91.8±11.6 | 97.8±5.8 | 100.3±8.4 | 104.7±8.7 |
| | m p for | bout 1 | 100±0 | 87.8±16.7 | 91.3±16.9 | 94.9±19.9 | 95.6±20.1 |
| | | bout 2 | 100±0 | 92.5±7.4 | 100.9±7.7 | 102.3±7.8 | 106.7±12.3 |
| | fatigue | bout 1 | 63.4±12.6 | 70.8±11.9 | 71.4±10.7 | 66.3±10.4 | 66.2±7.8 |
| | | bout 2 | 63.5±12 | 65.9±10.1 | 67.2±10.5 | 65.9±8.3 | 65.3±7.9 |
| | | | | | | | |
| | | | base | day 1 | day 2 | day 3 | day 4 |
| Males | sore | bout 1 | 0±0 | 4.9±1.7 | 5.2±1.6 | 2.9±1.7 | 1.1±1.0 |
| | | bout 2 | 0±0 | 2.6±1.3 | 2.1±1.5 | 1.1±1.0 | 0.2±0.3 |
| | CK | bout 1 | 100±0 | 786±698 | 994.7±1499 | 685.6±653 | 654.2±675 |
| | | bout 2 | 100±0 | 364.7±348 | 229.3±163 | 148.8±80.1 | 123.2±60.1 |
| | iso 20 | bout 1 | 100±0 | 67.3±31.7 | 91.2±17.0 | 94.1±16.0 | 100.5±22.6 |
| | | bout 2 | 100±0 | 91.4±8.9 | 94.6±13.3 | 94.9±12.5 | 97.5±14.9 |
| | iso 40 | bout 1 | 100±0 | 81.4±9.2 | 90.3±16.7 | 94.8±11.5 | 100.2±18.3 |
| | | bout 2 | 100±0 | 91.7±10.0 | 97.4±12.0 | 99.1±12.2 | 100.8±7.0 |
| | iso 80 | bout 1 | 100±0 | 87.7±10.9 | 89.4±12.6 | 94.8±10.8 | 100.8±11.7 |
| | | bout 2 | 100±0 | 91.9±11.7 | 99.2±7.5 | 101.7±12.3 | 103.7±8.9 |
| | wkdone | bout 1 | 100±0 | 82.1±7.2 | 83.8±11.5 | 86.3±14.1 | 89.9±12.4 |
| | | bout 2 | 100±0 | 90.7±11.2 | 92.4±9.4 | 91.3±28.6 | 100.2±17.8 |
| | p torq | bout 1 | 100±0 | 88.1±7.6 | 90.4±11.7 | 91.1±11.7 | 93.8±13.8 |
| | | bout 2 | 100±0 | 96.8±8.8 | 96.5±8.6 | 103.7±8.0 | 102.9±12.1 |
| | m p for | bout 1 | 100±0 | 90.1±14.9 | 94±22.1 | 96.5±19.4 | 101.8±22.7 |
| | | bout 2 | 100±0 | 96.6±11.2 | 95.3±10.4 | 101.5±7.7 | 100.5±11.5 |
| | fatigue | bout 1 | 60.6±14 | 57.4±16.7 | 62.2±11.1 | 67.6±8.6 | 73.1±9.0 |
| | | bout 2 | 62.5±13.6 | 66.6±6.0 | 67.2±7.6 | 66.5±7.0 | 66.9±9.2 |

Note: CK is relative change in creatine kinase (100% is baseline), Iso is percentage of maximal isometric strength (at 20, 40 and 80 degrees), Sore is perceived soreness, wkdone is total work done during 30 maximal isokinetic contraction expressed relative to baseline, p torq is peak torque expressed relative to baseline during the 30 isokinetic contractions, m p forc is mean peak force expressed relative to baseline during 30 maximal isokinetic contractions and fatigue is a fatigue index during 30 maximal isokinetic contractions, where 100% is no fatigue