REVIEW ARTICLE

Sports Med 2002; 32 (2): 103-123
0112-1642/02/0002-0103/$25.00/0

Exercise-Induced Muscle Damage and the Potential Protective Role of Estrogen

Becky Kendall and *Roger Eston*

School of Sport, Health and Exercise Sciences, , University of Wales, Bangor, UK

Contents

Abstract . . . 103
1. Model of Muscle Damage . . . 105
   1.1 Free Radicals and Muscle Damage . . . 105
   1.2 Initial Stimulus of Damage . . . 106
   1.3 Autogenic Processes . . . 107
      1.3.1 Role of Calcium . . . 107
      1.3.2 Calpain . . . 108
   1.4 Inflammatory and Immune Response to Muscle Damage . . . 108
      1.4.1 Cytokines . . . 108
   1.5 Leucocytes . . . 108
   1.6 Regeneration . . . 109
2. Estrogen and Muscle Damage . . . 109
   2.1 Estrogen as an Antioxidant . . . 111
   2.2 Estrogen and Membrane Stabilisation . . . 111
   2.3 Estrogen and Gene Regulation . . . 111
3. Estrogen and Muscle Damage Research . . . 112
   3.1 Effect of Estrogen on Creatine Kinase Activity . . . 112
   3.2 Histopathological Studies . . . 113
   3.3 The Effect of Estrogen on Indirect and Functional Measures of Muscle Damage . . . 113
   3.4 Estrogen, Time Course of Muscle Damage and Immune Response . . . 114
   3.5 Estrogen and the General Inflammatory Response . . . 114
   3.6 Estrogen and Specific Events Associated with Inflammation . . . 115
      3.6.1 Calcium . . . 115
      3.6.2 Adhesion Molecules . . . 116
      3.6.3 Cytokines . . . 116
4. Estrogen and Pain Perception . . . 118
5. Implications for Further Research . . . 119
6. Conclusion . . . 119

Abstract

Exercise-induced muscle damage is a well documented phenomenon that often follows unaccustomed and sustained metabolically demanding activities. This is a well researched, but poorly understood area, including the actual mechanisms involved in the muscle damage and repair cycle. An integrated model of muscle damage has been proposed by Armstrongand is generally accepted.

A more recent aspect of exercise-induced muscle damage to be investigated is the potential of estrogen to have a protective effect against skeletal muscle damage. Estrogen has been demonstrated to have a potent antioxidant capacity that plays a protective role in cardiac muscle, but whether this antioxidant capacity has the ability to protect skeletal muscle is not fully understood.

In both human and rat studies, females have been shown to have lower creatine kinase (CK) activity following both eccentric and sustained exercise compared with males. As CK is often used as an indirect marker of muscle damage, it has been suggested that female muscle may sustain less damage. However, these findings may be more indicative of the membrane stabilising effect of estrogen as some studies have shown no histological differences in male and female muscle following a damaging protocol.

More recently, investigations into the potential effect of estrogen on muscle damage have explored the possible role that estrogen may play in the inflammatory response following muscle damage. In light of these studies, it may be suggested that if estrogen inhibits the vital inflammatory response process associated with the muscle damage and repair cycle, it has a negative role in restoring normal muscle function after muscle damage has occurred.

This review is presented in two sections: firstly, the processes involved in the muscle damage and repair cycle are reviewed; and secondly, the possible effects that estrogen has upon these processes and muscle damage in general is discussed. The muscle damage and repair cycle is presented within a model, with particular emphasis on areas that are important to understanding the potential effect that estrogen has upon these processes.

It is well documented that strenuous and repeated eccentric contractions are associated with exercise-induced muscle damage and delayed onset muscle soreness.[1-3] 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.[4] For sedentary individuals, a single episode of exercise involving eccentric muscular contractions may produce significant muscle soreness and damage.[4]

The ability of muscle to resist force is ~30% greater during a maximal voluntary eccentric contraction than its ability to exert force during a concentric contraction.[5] Although current research is inconclusive, several studies[5-7] 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. It is therefore considered to be an important inclusion in strength training programmes.

During this type of contraction the length of the muscle is increasing whilst the muscle itself attempts to contract. Compared with concentric contractions, the mechanical strain per muscle fibre is higher, as fewer fibres are recruited.[8,9] The 'loading profile' places a high stress on the tissues involved and is most likely a primary factor of muscle damage.[9] During shortening contractions, work is done by the muscle, but during eccentric contraction, work is done on the muscle by the external lengthening forces.[3] 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.[10] 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.[10]

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.[11] 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.[11]

In general, this review considers the muscle damage that follows eccentric activity. However, as discussed later, sustained metabolically demanding activities can also be the initiating stimulus resulting in very similar muscle damage. This is discussed in more detail within the model of muscle damage subsection.

The symptoms of exercise-induced muscle damage include: soreness;[3,12,13] increase in volume of limb with injured muscle;[12] increase in circumference of limb with injured muscle;[13-15] decrease in resting arm angle;[12-15] decrease in range of motion of the affected limb;[3,14,16] decrease in muscular strength;[3,12,17] leakage of myofibre proteins into the blood, the most commonly measured being creatine kinase (CK);[3,18-20] swelling and structural damage.[3]

The most frequently reported symptom of muscle damage is delayed-onset muscle soreness. The soreness associated with this type of activity appears between 8 and 24 hours following the damaging exercise, and peaks between 24 and 48 hours later, but can remain for up to 7 days.[13,21,22]

The sensation of pain in skeletal muscle is transmitted by myelinated group III and unmyelinated group IV afferent fibres.[2,8] The myelinated group III fibres are believed to transmit sharp pain, where as the unmyelinated transmit dull aching pain,[2,23] which is more commonly associated with delayed-onset muscle soreness and muscle damage. It is believed that the pain afferents are sensitised by chemicals released during the muscle damage and repair cycle,[2,8] which include prostaglandin, bradykinin, serotonin, histamine and potassium.[2,8]

1. Model of Muscle Damage

An integrated model of muscle damage has been proposed by Armstrong,[24] which defines four stages: (i) initial events; (ii) autogenic processes; (iii) phagocytic stage; and (iv) 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 estrogen in preventing the potential destructive activities of these reactive species.

1.1 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,[25] across all stages of the theoretical model.

Free radicals are molecules or molecule fragments containing an unpaired electron in their outer valence shell.[26] The unpaired electron is usually extremely exchangeable, which is the chemical and physical reason for the reactivity of radical species.[27] They have a potent oxidising effect, which is the basis for their destructive effect against lipids, proteins, nucleic acid and the extracellular matrix.[28]

McArdle and Jackson[25] explained that free radicals can be generated through the mitochondrial electron transport system,[29] membrane

bound oxidases[30] and infiltrating phagocytic cells.[31] It is known that inflammatory events involve the generation of free radicals via reduced nicotinamide-adenine dinucleotide phosphate (NADPH) and myeloperoxidase.[32,33] More recent evidence suggests that superoxide radicals are also of importance in neutrophil attraction and neutrophil adherence to the endothelium.[32]

Free radicals can cause damage by lipid peroxidation of unsaturated fatty acids in the muscle membrane.[34] They can also cause oxidative damage to DNA and proteins.[25,35] It is suggested that as well as playing a role in direct tissue damage, the generation of ROS may also amplify the general inflammatory response of the body and promote further cell injury, for example, through up-regulation of pro-inflammatory cytokines.[36]

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.[33] There is abundant evidence for the involvement of neutrophil-generated ROS in the inflammatory response of tissues to various types of injury,[33] and growing evidence of their involvement in post-exercise muscle inflammatory response and damage.[4,37] However, the results from one recent study[38] 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.

Since eccentric exercise requires less oxygen consumption than equivalent concentric exercise,[7] 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,[33] 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 in this review.

1.2 Initial Stimulus of Damage

The initial events are thought to occur either via mechanical stress or metabolic stress.[4,24,39] In terms of mechanical stress, high tension or a tension imbalance[11,40,41] (associated with eccentric contractions) can disrupt the sarcolemma (resulting in calcium entry), the sarcoplasmic reticulum (resulting in impaired calcium sequestration) and myofibrillar structures.[24] Metabolic events include high temperature, lowered pH, insufficient mitochondrial respiration and oxygen free radical production.[24] A number of paradigms, including rhythmic exercise and repeated eccentric muscle activity have related ROS production to muscle inflammation and injury.[36]

Oxygen free radicals are produced in tissue that is highly metabolically active. These substances can cause irreversible damage to many cellular constituents.[39] Oxidation of the sulfhydryl groups of the ATPase pump by free radicals is highly correlated with a reduction in the rate of Ca^{2+} uptake by the sarcoplasmic reticulum,[39] 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.[39]

The increase in hydrogen ions (decrease in pH) that 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.[39]

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.[39] 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+}.[39]

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,[4] that is, whether it is eccentric in nature and involving a relatively low metabolic demand, or a sustained highly metabolic activity.

1.3 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.[24]

1.3.1 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.[24] 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.[25,42] Studies that have inhibited the flux of Ca^{2+} across the sarcoplasmic reticulum, following an exercise protocol in rats, have demonstrated a decrease in damage.[39,43] 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 the sarcolemma through production of leukotrienes and prostaglandins through free oxygen radical formation and/or through release of detergent-like lysophospholipids.[24] 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.[26] 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.[39,44]

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.[39] 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.[39]

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.[25] A calpain hypothesis was proposed by Belcastro et al.,[45] 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.[46] This in turn has been related to the reduction of maximal isometric titanic force associated with eccentric exercise.[46]

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.[47] A rise in free cytosolic calcium may also be related, independently, to the activation of the respiratory burst in phagocytic cells.[4] This phenomena suggests links between the mechanisms involved in the early stages of exercise-induced

tissue damage and the activation of cells involved in nonspecific immune responses.[4]

1.3.2 Calpain

While loss of Ca^{2+} homeostasis has been suggested as a primary factor in producing muscle damage, Belcastro et al.[45] proposed a calpain hypothesis of exercise-induced muscle damage. It is believed that Ca^{2+} stimulates proteases, such as calpain, which directly act upon proteins within the muscle. Belcastro et al.[45] reported that this nonlysosomal 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.[45] 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,[45] thus aiding the inflammatory response and repair.

1.4 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.[48] 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.[4]

Inflammation is characterised by the movement of fluid, plasma proteins and leucocytes into the tissue in response to injuries, infections or antigens.[49] Signalling occurs between the injured muscle cells and the mononucleated cells that subsequently appear at the site of injury.[47] 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.[47] Infiltration of these cells into the muscle is orchestrated by specific cytokines.[4]

1.4.1 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.[4,49] Host defence cytokines are produced by circulating and tissue resident leucocytes as well as other cells.[50] It is believed that a small group of cytokines, including interleukin (IL)-1, interferon, IL-2, IL-6 and tumour necrosis factor-α (TNFα), are the principle mediators of inflammation.[51] IL-1 is expected to have broad and important influences in muscle inflammation, as well as possible roles in stimulating protease synthesis.[47] TNFα and IL-1 have overlapping mechanisms within the body and have been shown to increase leucocyte adhesion, priming leucocyte function and causing macrophage activation.[49] In addition, IL-1 is believed to induce the expression of many other cytokines including IL-2, IL-3, IL-6, TNFα and interferons.[47] 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.[47] To stimulate the activity of antigen specific host defences, these cytokines regulate the growth, differentiation and functional activities of T and B lymphocytes.[4]

1.5 Leucocytes

Leucocytes, 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:[44,47] (i) attack and breakdown of debris (neutrophils and macrophages); (ii) removal of cellular debris (macrophages); and (iii) regeneration of cells (macrophages).

Leucocytes are attracted to injured muscle cells, via various chemotactic factors, possibly including resident leucocytes,[47] calpain or peptide fragments[45] and cytokines.[4,28] To enter the inflamed tissue, leucocytes bind to specific adhesion molecules of endothelial cells that line blood vessel walls.[52]

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 ROS via a respiratory burst, which is catalysed by the enzyme NADPH oxidase, located in the plasma membrane.[4]

It has been suggested that the neutrophil is programmed for overkill not caution.[53] It has little intrinsic ability to distinguish between foreign and host antigens, thus destroying healthy as well as damaged cell and debris.[4] Macrophages, like neutrophils are capable of producing oxygen free radicals.[49] 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 release.[48]

Following degradation processes, some macrophages may play a role in muscle repair.[52] Two populations have been observed within animal muscle,[44,47] ED1+ cells act as phagocytes and ED2+ cells regulate the consequent repair process.[44]

1.6 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.[23] Indeed, it is strongly suggested that macrophage infiltration is an important part of the regeneration phase particularly in terms of satellite cell proliferation.[54-56]

A brief review of the muscle damage and repair cycle has been presented. This has, in 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 figure 1.

A more recent area of interest with regard to muscle damage is the potential protective effect of estrogen. In the following sections is an explanation of the possible mechanisms for the protective role of estrogen in the muscle damage and repair cycle.

2. Estrogen and Muscle Damage

Estrogen 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 compared with age-matched males is believed to be partially attributable to the protective effect of the female sex hormone estrogen.[57-63]

Estrogens 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.[64] The term estrogen refers to three structurally similar steroid hormones, estradiol-17β (E2), estrone (E1) and estriol (E3). Of these, E2 is the primary estrogen in humans and the one with the greatest estrogenic properties,[63,65] and as such is studied in the majority of investigations.[64] Estrogen 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 estrogen 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 estrogen may have an effect in reducing skeletal muscle

damage (if indeed it does) is very difficult to determine. Nevertheless, the review considers the possible effect of estrogen across the muscle damage and repair cycle, including initial events, secondary damage, inflammatory processes and regeneration. The review is presented within subsections

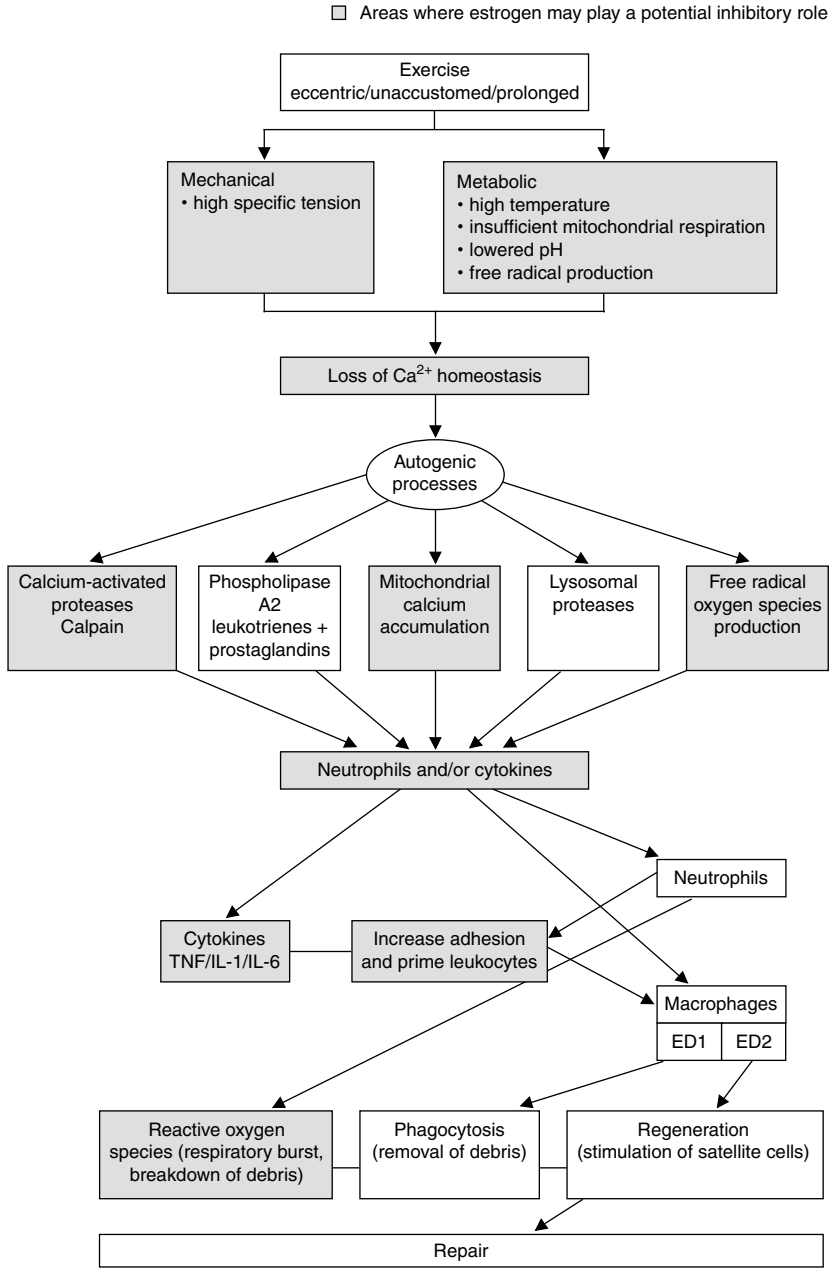

Fig. 1. Illustration of a simple model of the muscle damage and repair cycle. TNF/IL-1 = tumour necrosis factor/interleukin-1.

but the interaction between processes and thus subsections should not be forgotten.

2.1 Estrogen 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.[27] A common denominator for these compounds is that their molecular structure is based on a carbon-ring structure, which originates from phenol species.[27] Phenol species have one or more hydroxyl groups, which have a unique ability to reduce electrons.[27]

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[66] and ultimately affects membrane stability.[63] It has been demonstrated *in vitro* and *in vivo* in both rat and human investigations, that estrogen (at physiological and supraphysiological concentrations) possesses a potent antioxidant characteristic,[63,66-75] although the mechanisms by which estrogen acts as an antioxidant have not been fully determined. Estrogens do possess a hydroxyl group on their A (phenolic) ring, in the same configuration and position as tocopherol (vitamin E) [known to possess a strong antioxidant capacity][65,73,76] and similar to thyroxine,[68] which also possesses potent antioxidant activity. Estrogen may donate hydrogen atoms from the phenolic hydroxyl group, thus terminating peroxidation chain reactions, in a way similar to tocopherol.[63,65,68,73,76]

An increase in oxygen radical production results in a decrease in tocopherol concentrations as a result of the above reaction.[65] However, this has only been shown to occur in studies with male and sexually immature female rats[77,78] in which estrogen levels are obviously low. Sexually mature female rats (high estrogen) are not affected in the same manner, that is, tocopherol levels are maintained.[79] These results suggest that estrogen may offer an additional line of defence against oxygen free radicals and may render skeletal muscle less susceptible to exercise-induced oxidative damage.[65]

2.2 Estrogen and Membrane Stabilisation

Due to its figuration and antioxidant capacity, estrogen is believed to have membrane stabilising characteristics. It has been suggested that estrogen may protect membranes from peroxidative damage by decreasing membrane fluidity and increasing membrane stability in ways similar to cholesterol.[80,81] Estrogen is a fat-soluble hormone and this type of stabilisation involves an interaction between membrane phospholipids and estrogen in ways similar to the stabilising mechanisms of tocopherol and cholesterol.[63,76,80] As steroid hormones are lipophilic,[82] 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.[66] Wiseman and Quinn[81] demonstrated a positive association between decreased membrane fluidity and antioxidant ability. They stated that this ability, by estrogen in particular, to decrease membrane fluidity is a mechanism of their antioxidant action, which results in stabilisation of the membrane against peroxidation.

2.3 Estrogen and Gene Regulation

Pro-inflammatory cytokines, for example IL-6 and TNFα have been shown to increase during the muscle damage and repair cycle.[32,83-88] Nuclear factor kappa B is known to govern gene expression involving various cytokines and cell adhesion molecules[89-92] and it has been shown that tocopherol inhibits the activation of this factor.[92] Yoshikawa and Yoshida[92] demonstrated that tocopherol can prevent leucocyte-endothelial cell adhesion by inhibiting signal transduction. They concluded that tocopherol may have a protective effect against the progression of inflammation. Research suggests that it could be the antioxidant properties of tocopherol which leads to this gene regulatory effect.[92-94] Caulin-Glaser et al.[95] explained that es-

trogen has been shown to have important gene regulatory effects,[96-98] which again could be explained through its strong antioxidant capacity. Therefore, estrogen 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 estrogen may have a capacity to reduce muscle damage, based on the above three interacting processes. This possible protective role of estrogen and the subsequent effect upon the muscle damage and repair cycle is presented in figure 2. There follows a review of the research in this area and a discussion of how the interacting processes outlined above may account for recent observations in the literature.

3. Estrogen and Muscle Damage Research

3.1 Effect of Estrogen on Creatine Kinase Activity

CK is a commonly measured marker of muscle damage, although its interindividual variability has been criticised.[99] Despite this, a large difference in CK activity has been demonstrated between males and females, which has generally been attributed to the effect of estrogen.

Women have been shown to have lower CK activity at rest[100] and show less CK efflux after bicycle exercise[101] or long distance running.[102,103] In addition, Bär et al.[104] investigated the effects of 2 hours of running in rats on CK activity. They observed that ovariectomised females (source of

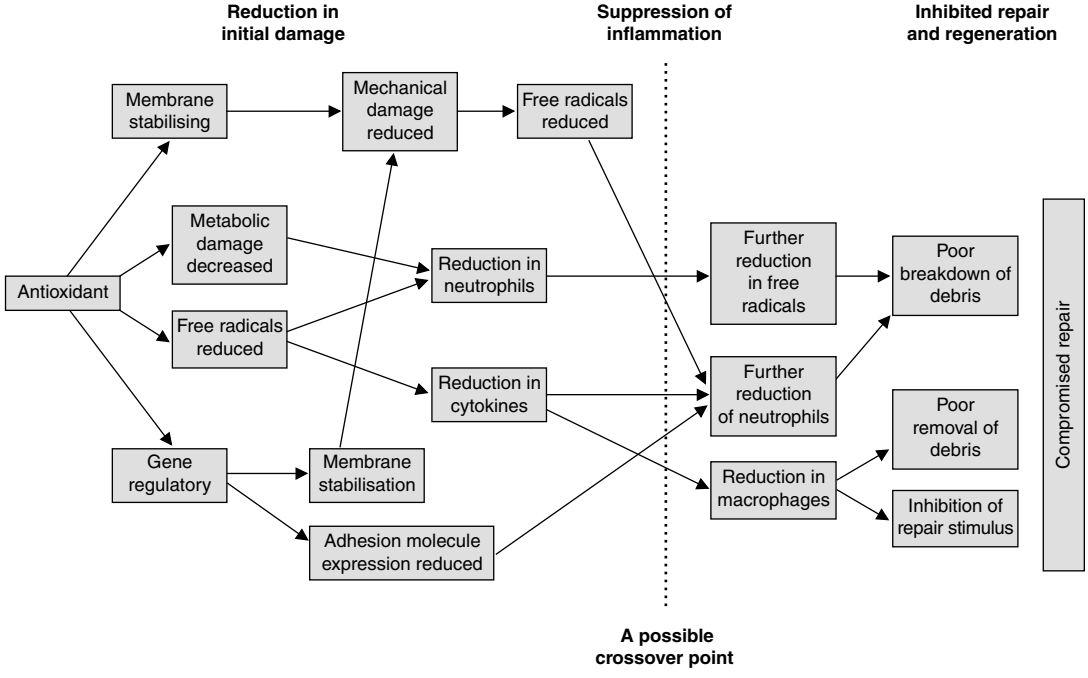

Fig. 2. Summary of how the interacting properties of estrogen potentially play a role in the muscle damage and repair cycle. To date, it is not known whether estrogen-mediated inhibition of inflammatory processes would compromise repair. The potential effect of estrogen during the early stages of the 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 estrogen may potentially have a negative effect.

estrogen removed) showed similar levels to male rats and that feeding of exogenous estrogen to this group and to male rats reduced the efflux of CK. Following a similar exercise protocol, Amelink and Bär[105] 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 suggested that estrogen 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,[96-100] the actual protection in terms of the aetiology of muscle damage is undetermined. These findings have led to more recent and in-depth investigations into muscle damage and estrogen.

3.2 Histopathological Studies

Van der Meulen et al.[106] and Dumke[107] measured histological damage and enzyme release following a treadmill protocol in rats. Van der Meulen et al.[106] 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[107] 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 estrogen offers no real protection against damage, but simply helps maintain a more stable membrane. These procedures assumed a similar time course for males and females and only measured 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.[106] and Dumke[107] are those by Reijneveld et al..[108] A marked attenuation of the CK response, as found in many of the studies in this area,[101,104,105] was concurrent with a decrease in the amount of histological damage. Clearly this area requires further investigation.

Warren et al.[99] 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 indicate changes in function should be incorporated to assess and follow muscle damage.

3.3 The Effect of Estrogen on Indirect and Functional Measures of Muscle Damage

Buckley-Bleiler et al.[109] investigated the response of women at different ages to exercise-induced muscle damage. Comparisons were made between pre-pubescent, eumenhorrheic and post-menopausal females. They found no significant differences between these groups in measures of CK, muscle soreness and maximal isometric strength. However, these 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 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.[110] investigated the effects of regularly ingesting estrogen, in the form of oral contraceptives (at least 30mg ethinylestradiol for more than 6 months), on post-exercise muscle

damage of the 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 nonusers. The authors concluded that estrogen 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, oral contraceptive 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. This has been replicated in our laboratory, where following eccentric exercise of the elbow flexors, the only marker of muscle damage to show a significant difference between oral contraceptive users and nonusers was perceived soreness on activation.[111] The effect of estrogen on pain will be discussed in section 4.

Rinard et al.[112] 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 estrogen has no protective effect on muscle damage. However, it should be noted that participants were selected based on their soreness response, thus making generalisations from the study very difficult.

3.4 Estrogen, Time Course of Muscle Damage and 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.[113] This is an area that has recently received greater attention, and has enhanced our understanding of the gender differences in muscle damage and repair.

3.5 Estrogen and the General Inflammatory Response

Komulainen et al.[114] investigated the time course of structural changes within the muscle following downhill running. The results demonstrated that the general histopathological changes in both genders 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[113] hypothesised that as estrogen 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 estrogen 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. These investigators measured post-exercise tissue myeloperoxidase activity in male and female rats which were treated (40 μg/kg bodyweight) and untreated with estrogen. Their results suggested that estrogen might significantly affect post-exercise leucocyte 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,[45,113,115] to name but a few, with interactions amongst these given factors. This and the possible effect that estrogen has on some of these factors will be discussed in section 3.6.

Stupka et al.[116] reported that the possible difference in the extent of muscle damage between males and females is caused by differences in the inflammatory response and not by differences in sarcomere damage. They demonstrated that following an eccentric protocol, women showed less muscle inflammation compared with 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 experiencing a similar amount of initial damage.

St Pierre Schneider et al.[52] investigated the time course and concentration of leucocyte invasion in injured soleus muscles of male and female mice, to determine if any gender differences existed. Leucocyte invasion began 1 day after injury in both genders and diminished on day 5 in males, but remained evident after day 7 in females. During the period of maximal leucocyte invasion at 1 day after injury, muscle sections from males contained more fibres invaded by acid phosphatase-positive cells than muscle sections from females. They suggested that estrogen prevented elevated macrophage concentrations in blood vessels by limiting the availability of endothelial cell adhesion molecules. This suggests that estrogen could reduce macrophage or other leucocyte emigration into inflamed tissue, because fewer endothelial cell adhesion molecules result in an inability of leucocytes 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.,[117] who observed a greater presence of neutrophils in muscles of women 4 hours after exercise compared with men. They concluded that this was probably caused by 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 estrogen may be the most likely factor affecting the infiltration of the inflammatory cells into the muscle.

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

3.6 Estrogen and Specific Events Associated with Inflammation

3.6.1 Calcium

The 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.[118] investigated the effect that estrogen had on smooth muscle cells. They observed that estrogen 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 that can result in more severe muscle damage. If the same is true for skeletal muscle, this could be an important process by which estrogen may affect the inflammatory response to exercise-induced muscle damage.

Jovanovic et al.[119] 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 estrogen in cardiac muscle may also be found within skeletal muscle, preventing, or certainly dampening, the secondary muscle damage pro-

cesses. However, this requires empirical verification with skeletal muscle cells.

A recent investigation by Tiidus et al.[120] seems to suggest that estrogen has the potential to reduce Ca^{2+} overload within skeletal muscle following an exercise protocol. In the calpain hypothesis proposed by Belcastro et al.,[45] a very important role is suggested for this Ca^{2+} sensitive protease, including chemotaxis of leucocytes (see section 1.3.2). The investigation by Tiidus et al.[120] demonstrated that following an exercise protocol, ovariectomised rats treated with estrogen, not only showed significant attenuation of 1-hour post-exercise neutrophil number and myeloperoxidase activity, but also reduced calpain-like activity, compared with placebo-treated ovariectomised rats. They suggested that estrogen 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 1-hour post-exercise observations only, the findings certainly suggest an important role for estrogen within this specific (Ca^{2+} and calpain) process of the muscle damage and repair cycle.

3.6.2 Adhesion Molecules

To enter the inflamed tissue, leucocytes bind to specific adhesion molecules of endothelial cells that line blood vessel walls.[52] St Pierre Schneider et al.[52] attributed the reduced leucocyte infiltration in injured soleus muscle to the inhibitory effect of estrogen on adhesion molecules.

Previous research has shown that E2 can inhibit cytokine-mediated endothelial cell adhesion molecule activation, thereby reducing leucocyte chemotaxis and adhesion.[95] However, Cid et al.[121] reported that estradiol treatment of cultured human umbilical vein endothelial cells stimulated up to a 2-fold increase in TNFα-induced adhesion of leucocytes.

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)[122-125] and this has been suggested to be caused by estrogen. This could in some way explain the findings of Cid et al.,[121] who explained that their results demonstrate that estradiol has important regulatory functions in promoting leucocyte-endothelial cell interactions. It is unclear why Caulin-Glaser et al.[95] and St Pierre Schneider et al.[52] observed a different relationship. To elucidate further on the potential effects of estrogen 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.[126] The possible effect that estrogen has upon cytokines is discussed in section 3.6.3. Another important consideration in relation to the influence of estrogen, is the role that oxidative stress may play in regulating adhesion molecule gene expression. Marui et al.[126] 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 estrogen has been demonstrated to have a strong antioxidant capacity, it is feasible that it could also reduce expression of these molecules through a similar pathway.

3.6.3 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.[127] explained that the effect of estrogen on autoimmunity and inflammation may involve changes in secretion of inflammatory mediators, for example, cytokines. They showed that treatment of mice with pharmacological doses (0.2 to 2 μg/kg) of estriol resulted in a significant increase in serum TNFα levels and a rapid elevation in serum IL-6 levels following challenge.

Both Schwarz et al.[128] and Angstwurm et al.[129] investigated the influence of menstrual cycle status upon cytokine levels. Angstwurm et al.[129] 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- to 4.4-fold drop in plasma IL-6. Schwarz et al.[128] demonstrated that in male participants no differences existed in cytokine response between initial samples and those taken 1 to 3 weeks later. In pre-menopausal females, release of TNFα and IL-6 was significantly decreased in the luteal phase compared with 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 with the follicular phase. Thus, it would appear that estrogen has an inhibitory effect on cytokine activation. However, Schwarz et al.[128] also demonstrated 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 studies demonstrate the complicated relationship between estrogen and cytokines. During the follicular phase (when estrogen is at its lowest) cytokine release was enhanced compared with the luteal phase. However, during the luteal phase, cytokine release has a positive relationship with estrogen concentration.

Pottratz et al.[130] reported an inhibitory effect of E2, in *in vitro* and *in vivo* studies. These investigators observed that ovariectomised mice demonstrated an up-regulation of granulocytes and macrophages and that such changes could be prevented by a neutralising antibody against IL-6, as well as estrogen replacement. They suggested that the inhibition of IL-6 production by E2 was via a receptor-mediated action.

Chao et al.[122] observed that estrogen can, in some cases, inhibit 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.

The complexities of this area are clearly demonstrated in this review. Estrogen is known to have a potent antioxidant capacity and a membrane stabilising ability. As free radicals are implicated in the development of damage (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.[33] This, in turn, can prime leucocytes enhancing superoxide production. Thus, a reduction in initial free radical production, possibly through estrogen, could feasibly reduce inflammation. In addition to this, estrogen 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 estrogen could play a role within the muscle damage and repair cycle, as presented in figure 2.

Thus, 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: (i) aids repair by reducing further secondary damage; or (ii) is negative in terms of the repair and the return of normal function to the muscle. Tidball[47] 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 nonsteroidal anti-inflammatory drugs and muscle regeneration have been inconclusive,

with reports of both compromised[131] and uninhibited repair.[132] Understanding the role of estrogen in terms of repair would be useful to both recreational and elite sports participants.

4. Estrogen and Pain Perception

In addition to the potential mediating effect of estrogen on the muscle damage and repair cycle, it may also affect the perception of pain associated with muscle damage. Individuals with higher levels of estrogen (oral contraceptive users) have shown similar responses in markers of muscle damage to those with lower levels (non-oral contraceptive users) following a bout of damage-inducing exercise, but have reported significantly less soreness.[110,111] 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.[133] indicated that the ovarian steroid hormones, estrogens and progestogens, 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 perception.

Riley et al.[134] 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 ischaemic 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 estrogen and progesterone are at their lowest, pain thresholds are at their highest. Potentially, this suggests that estrogen and/or progesterone increase sensitivity to pain.

In contrast, Martinez-Gomez et al.[135] investigated tail flick latency across the cycle in rats. Shorter latencies were recorded in phases with lower estrogen. Ovariectomy (removal of estrogen source) abolished these fluctuations. Administration with estrogen, but not progesterone increased response time, which suggests that estrogen increases pain thresholds. Similarly, Rao et al.[136] 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 oophorectomised women (who were not on hormone replacement therapy), 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 midcycle, when estrogen concentrations are highest. While findings are inconclusive, there is certainly the possibility that estrogen 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 enkephalins, endorphins and dynorphins, which have the capability to modify pain transmission and inhibit prostaglandin synthesis.[137] 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.[138] These receptors include mu (μ), kappa (κ), and delta (δ) receptors. It has been suggested that both estrogen and progesterone could potentially play a role within this natural pain relief system. Antinociception of pregnancy and parturition has been observed in rats[139-141] and in women.[142,143] It has been suggested that the elevated levels of the hormones estrogen 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.[144-147]

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.[144,146] Conversely, other research has rejected such a conclusion.[148]

Estrogen receptors have also been suggested to play a role in this natural analgesia. Estrogen receptors are present on small-diameter dorsal root ganglion neurons.[149] Estrogen has been shown to regulate the expression of messenger RNA encoding receptors, which modulate nociception.[149] Additionally, it has been shown that enkephalin synthesising neurons display intracellular estrogen receptors,[150] which have been demonstrated to increase the gene expression of an enkephalin precursor.[150,151]

Thus, although equivocal, the research demonstrates the potential for estrogen to mediate pain perception. Studies investigating exercise-induced muscle damage and estrogen levels therefore need to consider the possibility that estrogen levels may also mediate the perception of pain.

5. Implications for Further Research

The implications for further research are clearly dependent on verification of the role of estrogen in muscle damage. This therefore requires further investigation. At what levels does estrogen have an effect during the damage and repair cycle? Are these effects in fact negative because of inhibition of inflammation, a necessary process for clearance of debris, or is the initial damage reduced sufficiently so that an inhibited inflammatory response is non-consequential?

If estrogen 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 estrogen found across the menstrual cycle may need to be considered within a training environment and schedule, as should the perception of pain as a gauge of damage.

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

There are also health considerations for postmenopausal females. Older males have the advantage of testosterone, known for its anabolic properties, to help maintain, in some way, their muscle mass. Postmenopausal females could therefore benefit from estrogen replacement in terms of protecting their skeletal muscle from degeneration. Phillips et al.[152] have shown that there is no significant difference in the specific force of muscles between young males and premenopausal 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 of estrogen is believed to account for the increases in falling incidents in postmenopausal women.[152] Naessen et al.[153] also found that estrogen replacement therapy has a positive effect in terms of postural balance in postmenopausal females. However, direct contradictions to these findings have also been reported.[154-157]

6. Conclusion

Research in terms of the protective effect of estrogen against skeletal muscle damage is sparse, especially in terms of secondary damage and inflammatory processes. Assumptions and theories on the possible protective role of estrogen on skeletal muscle can be made based upon the known properties of estrogen 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.

Acknowledgements

The authors have no conflicts of interest.

References

1. Newham DJ, Mills KR, Quigley BM, et al. Pain and fatigue after concentric and eccentric muscle contractions. Clin Sci (Lond) 1983; 64: 55-62
2. Armstrong RB. Mechanisms of exercise-induced delayed onset muscle soreness: a brief review. Med Sci SportsExerc 1984; 16: 529-38
3. Clarkson PM, Newham DJ. Associations between muscle soreness, damage and fatigue. In: Gandevia SC, Enoka RM, McComas AJ, et al., editors. Fatigue: neural and Muscular Mechanisms. New York (NY): Plenum, 1994: 457-69
4. Pyne DB. Regulation of neutrophil function during exercise. Sports Med 1994; 17: 245-58
5. Wilmore JH, Costill DL. Physiology of sport and exercise. Champagne (IL): Human Kinetics, 1994
6. Hortobagyi T, Hill JP, Houmard JA, et al. Adaptive responses to muscle lengthening and shortening in humans. J Appl Physiol 1996; 80: 765-72
7. Lastayo PC, Reich TE, Urquhart M, et al. Chronic eccentric exercise improvements in muscle strength can occur with little demand for oxygen. Am J Physiol 1999; 276: R611-5
8. Bär PR, Reijneveld JC, Wokke JHJ, et al. Muscle damage induced by exercise: nature, prevention, and repair. In: Salmon S, editor. Muscle damage. New York (NY) : Oxford Medical Publications, 1997: 1-27
9. Enoka RM. Eccentric contractions require unique activation strategies by the nervous system. J Appl Physiol 1996; 81: 2339-46
10. McComas AJ. Skeletal muscle: form and function. Champagne (IL): Human Kinetics, 1996
11. Friden J, Leiber RL. Structural and mechanical basis of exercise-induced muscle injury. Med Sci Sports Exerc 1992; 24: 521-30
12. Talag T. Residual muscle soreness as influenced by concentric, eccentric and static contractions. Res Q 1973; 44: 458-69
13. Howell JN, Chlebourn G, Conatser R. Muscle stiffness, strength loss, swelling and soreness following exercise-induced injury in humans. J Physiol 1993; 464: 183-96
14. Cleak MJ, Eston R. Delayed onset muscle soreness: mechanisms and management. J Sports Sci 1992; 10: 325-41
15. Eston R, Peters D. Effects of cold water immersion on the symptoms of exercise-induced muscle damage. J Sports Sci 1999; 17: 231-8
16. Yackzan L, Adams C, Francis KT. The effects of ice massage on delayed onset muscle soreness. Am J Sports Med 1984; 12: 159-65
17. Eston RG, Finney S, Baker S, et al. Muscle tenderness and peak torque changes after downhill running following a prior bout of isokinetic eccentric exercise. J Sports Sci 1996; 14: 291-9
18. Jones DA, Newham DJ, Round JM, et al. Experimental human muscle damage: morphological changes in relation to other indices of damage. J Physiol 1986; 375: 435-48
19. Clarkson PM, Nosaka K, Braun B. Muscle function after exercise-induced muscle damage and rapid adaptation. Med Sci Sports Exerc 1992; 24: 512-20
20. Child RB, Saxton JM, Donnelly AE. Comparison of eccentric knee extensor muscle actions at two muscle lengths on indices of damage and angle-specific force production in humans. J Sports Sci 1998; 16: 301-8
21. Cleak MJ, Eston R. Muscle soreness, swelling, stiffness and strength loss after intense eccentric exercise. Br J Sports Med 1992; 26: 267-72
22. Nosaka K, Clarkson PM. Muscle damage following repeated bouts of high force eccentric exercise. Med Sci Sports Exerc 1995; 27: 1263-9
23. Jones DA, Round JM. Skeletal muscle in health and disease. Manchester: Manchester University Press, 1990
24. Armstrong RB. Initial events in exercise-induced muscular injury. Med Sci Sports Exerc 1990; 22: 429-35
25. McArdle A, Jackson MJ. Intracellular mechanisms involved in skeletal muscle damage. In: Salmon S, editor. Muscle damage. New York (NY): Oxford Medical Publications, 1997: 90-106
26. Jenkins RR. Free radical chemistry: relationship to exercise. Sports Med 1988; 5: 156-20
27. Karlsson J. Antioxidants and exercise. Champagne (IL): Human Kinetics, 1997
28. Niess AM, Dickhuth H, Northoff H, et al. Free radicals and oxidative stress in exercise-immunological aspects. Exerc Immunol Rev 1999; 5: 22-56
29. Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide. Biochem J 1973; 128: 617-30
30. Crane FL, Sun IL, Clark MG, et al. Transplasma-membrane redox systems in growth and development. Biochim Biophys Acta 1985; 811: 233-64
31. Font B, Vial C, Goldschmidt D. Metabolic levels and enzyme release in the ischaemic myocardium. J Mol Med 1977; 2: 291-7
32. Hellsten Y, Frandsen V, Orthenblad N, et al. Xanthine oxidase in human skeletal muscle following eccentric exercise: a role in inflammation. J Physiol 1997; 498: 239-48
33. Tiidus PM. Radical species in inflammation and overtraining. Can J Physiol Pharmacol 1998; 76: 533-8
34. Davies KJA, Quintanilha AT, Brooks GA, et al. Free radicals and tissue damage produced by exercise. Biochim Biophys Res Com 1982; 107: 1198-205
35. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Claredon Press, 1985
36. Best TM, Fiebig R, Corr DT, et al. Free radical activity, antioxidant enzyme, and glutathione changes with muscle stretch injury in rabbits. J Appl Physiol 1999; 87: 74-82
37. Eston R, Jackson M, Pears J. Association between the production of thiobarbituric reactive substances (malondialdehyde) and markers of muscle damage induced by uphill and downhill running [abstract]. J Sports Sci 1996; 14: 80
38. Lowe DA, Warren GL, Ingalls CP, et al. Muscle function and protein metabolism after initiation of eccentric contraction-induced injury. J Appl Physiol 1995; 79: 1260-70
39. Byrd SK. Alterations in the sarcoplasmic reticulum, a possible link to exercise-induced muscle damage. Med Sci Sports Exerc 1992; 24: 531-6
40. Armstrong RB, Warren GL, Warren JA. Mechanisms of exercise-induced muscle fibre injury. Sports Med 1991; 12: 184-207
41. Lieber R. L. Friden J. Muscle damage is not a function of muscle force but active muscle strain. J Appl Physiol 1993: 526
42. Duncan CJ. Role of intracellular calcium in promoting muscle damage: a strategy for controlling the dystrophic condition. Experientia 1978; 34: 1531-5
43. Amelink GJ, van der Kallen CJM, Wokke JHJ, et al. Dantrolene sodium diminishes exercise-induced muscle damage in he rat. Eur J Pharmacol 1990; 179: 187-92
44. Clarkson PM, Sayers SP. Etiology of exercise-induced muscle damage. Can J Appl Physiol 1999; 24: 234-48

45. Belcastro AN, Shewchuk LD, Raj DA. Exercise-induced muscle injury; a calpain hypothesis. Mol Cell Biochem 1998; 179: 135-45
46. Ingalls CP, Warren GL, Williams JH, et al. E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions. J Appl Physiol 1998; 85: 58-67
47. Tidball JG. Inflammatory cell response to acute muscle injury. Med Sci Sports Exerc 1995; 27: 1022-32
48. Evans WJ, Cannon JG. The metabolic effects of exercise-induced muscle damage. Exerc Sport Sci Rev 1991; 19: 99-125
49. MacIntyre DL, Reid WD, McKenzie DC. The inflammatory response to muscle injury and its clinical implications. Sports Med 1995; 20: 24-40
50. Bagby GJ, Larry CD, Shepherd RE. Exercise and cytokines: spontaneous and elicited responses. In: Hoffman-Goetz L, editor. Exercise and immune function. Boca Raton (FL): CRC Press, 1996: 55-79
51. Imura H, Fukata J, Mori T. Cytokines and endocrine function: an interaction between the immune and neuroendocrine systems. Clin Endocrinol 1996; 35: 107-15
52. St Pierre Schneider B, Correia LA, Cannon JG. Sex differences in leukocyte invasion in injured murine skeletal muscle. Res Nurs Health 1996; 22: 243-51
53. McCord J. Superoxide radical: controversies, contradictions, and paradoxes. Proc Soc Exp Biol Med 1995; 209: 112-7
54. Cantini M, Carraro U. Macrophage-released factor stimulates selectively myogenic cells in primary muscle culture. J Neuropathol Exp Neurol 1995; 54: 121-8
55. Merly F, Lescaudron L, Rouaud T, et al. Macrophages enhance muscle satellite cell proliferation and delay their differentiation. Muscle Nerve 1999; 22: 724-32
56. Lescaudron L, Peltekian E, Fontaine-Perus J, et al. Blood borne macrophages are essential for the triggering of muscle regeneration following muscle transplant. Neuromuscul Disord 1999; 9: 72-80
57. Stumpf W, Sar M, Aumuller G. The heart: a target organ for estradiol. Science 1977; 196: 319-20
58. Bush TL, Barret-Conner E, Cowan LD. Cardiovascular mortality and non-contractile estrogen use in women; results from the lipid research clinics program follow up study. Circulation 1987; 75: 1002-9
59. Gruchow HW, Anderson AJ, Barbarak JJ. Postmenopausal use of estrogen and occlusion of coronary arteries. Am Heart J 1988; 115: 954-63
60. Barret-Connor E, Bush T. Estrogen and coronary heart disease in women. JAMA 1991; 265: 1861-5
61. Chisholm G. Antioxidants and atherosclerosis: a current assessment. Clin Cardiol 1991; 12: 125-30
62. Stampfer MJ, Colditz GA, Willet WC. Postmenopausal estrogen therapy and cardiovascular disease. N Engl J Med 1991; 325: 756-62
63. Tiidus PM. Can estrogens diminish exercise induced muscle damage? Can J Appl Physiol 1995; 20: 26-38
64. Bunt J. Metabolic actions of estradiol: significance for acute and chronic exercise response. Med Sci Sports Exerc 1990; 22: 286-90
65. Tiidus PM. Nutritional implications of gender differences in metabolism: estrogen and oxygen radicals: oxidative damage, inflammation and muscle function. In: Tarnopolsky M, editor. Gender differences in metabolism: practical and nutritional implications. Boca Raton (FL): CRC Press, 1999: 265-81
66. Wiseman H, O'Reilly J. Oestrogen as antioxidant cardioprotectants. Biochem Soc Trans 1997; 25: 54-9
67. Yagi K, Komura S. Inhibitory effect of female hormones on lipid peroxidation. Biochem Int 1986; 13: 1051-5
68. Sugioka K, Shimosegawa Y, Nakano M. Estrogens as natural antioxidants of membrane phospholipid peroxidation. FEBS Lett 1987; 210: 37-9
69. Huber L, Scheffler E, Poll T, et al. 17β-estradiol inhibits LDL oxidation and cholesteryl ester formation in cultured macrophages. Free Radic Res Commun 1990; 8: 167-75
70. Mooradian AD. Antioxidant properties of steroids. Biochem Mol Biol 1993; 45: 509-11
71. Subbiah M, Kessel B, Agrawal M, et al. Antioxidant potential of specific estrogens on lipid peroxidation. J Clin Endocrinol Metabol 1993; 77: 1095-7
72. Ayres S, Tang M, Subbiah MTR. Estradiol 17β as an antioxidant: some distinct features when compared with common fat-soluble antioxidants. J Lab Clin Med 1996; 128 (4): 367-75
73. Ayres S, Abplanalp W, Liu JH, et al. Mechanisms involved in the protective effect of estradiol-17β on lipid peroxidation and DNA damage. Am J Physiol 1998; 274: E1002-8
74. Ruehlmann DO, Mann GE. Actions of oestrogen on vascular endothelial and smooth muscle cells. Biochem Soc Trans 1997; 25: 40-5
75. Bär PR, Amelink GJ. Protection against muscle damage exerted by oestrogen; hormonal or antioxidant action? Biochem Soc Trans 1997; 25: 50-4
76. Persky AM, Green PS, Stubley L, et al. Protective effect of estrogen against oxidative damage to heart and skeletal muscle in vivo and in vitro. Proc Soc Exp Biol Med 2000; 223: 59-66
77. Bowles D, Torgan C, Ebner S, et al. Effects of acute submaximal exercise on skeletal muscle vitamin E. Free Radic Res Commun 1991; 14: 138-43
78. Sen CK, Atalay M, Agren J, et al. Fish oil and vitamin E supplementation in oxidative stress at rest and after physical exercise. J Appl Physiol 1997: 95
79. Tiidus PM, Houston ME. Vitamin E status does not affect the responses to exercise training and acute exercise in female rats. J Nutr 1993: 840
80. Wiseman H, Quinn P, Halliwell B. 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 Lett 1993; 330: 53-6
81. Wiseman H, Quinn P. 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 Radic Res 1994; 21: 187-94
82. Whiting KP, Restall CJ, Brain PF. Steroid hormone-induced effects on membrane fluidity and their potential roles in non-genomic mechanisms. Life Sci 2000; 67: 743-57
83. Esperson GT, Elbeak A, Ernst E, et al. Effects of physical exercise on cytokines and lymphocyte subpopulations in human peripheral blood. APMIS 1990; 98: 395-400
84. Fielding RA, Manfredi TJ, Ding W, et al. Acute phase response in exercise III: neutrophil and IL-1β accumulation in skeletal muscle. Am J Physiol 1993; 265: R166-72
85. Bruunsgaard H, Galbo H, Halkjaer-Kristensen J, et al. Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. J Physiol 1997; 499: 833-41
86. Pedersen BK, Bruunsgaard H, Klokker M, et al. Exercise-induced immunomodulation: possible roles of neuroendocrine and metabolic factors. Int J Sports Med 1997; 18: S2-7

87. Suzuki K, Totsuka M, Nakaji MY, et al. Endurance exercise causes interaction among stress hormones, cytokine, neutrophil dynamics and muscle damage. J Appl Physiol 1999; 87: 1360-7
88. Smith LL, Anwar A, Fragen M, et al. Cytokines and cell adhesion molecules associated with high intensity eccentric exercise. Eur J Appl Physiol 2000; 82: 61-7
89. Montgomery KF, Osborn L, Hession C, et al. Activation of endothelial-leukocyte adhesion molecule 1 (ELAM-1) gene transcription. Proc Natl Acad Sci U S A 1991; 88: 6523-7
90. Degitz K, Lian-Jie L, Caughman SW. Cloning and characterization of the 5′transcriptional regulatory region of the human intercellular adhesion molecule 1 gene. J Biol Chem 1991; 266: 14024-30
91. Iadermarco M, McQuillan J, Rosen G, et al. Characterization of the promoter for vascular cell adhesion molecule-1 (VCAM-1). J Biol Chem 1992; 267: 16323-9
92. Yoshikawa T, Yoshida N. Vitamin E and leukocyte endothelial cell interactions. Antioxid Redox Signal 2000; 2: 821-5
93. Sen CK. Antioxidant and redox regulation of cellular signalling: introduction. Med Sci Sports Exercise 2001; 33: 368-70
94. Sen CK, Roy S. Antioxidant regulation of cell adhesion. Med Sci Sports Exerc 2001; 33: 377-81
95. Caulin-Glaser T, Watson CA, Pardi R, et al. Effects of 17β-estradiol on cytokine-induced endothelial cell adhesion molecule expression. J Clin Invest 1996; 98: 36-42
96. Beato M. Gene regulation by steroid hormones. Cell 1989; 56: 335-44
97. Gaub MP, Bellard M, Scheuer I, et al. Activation of the ovalbumin gene by the estrogen receptor involves the fos-jun complex. Cell 1990; 63: 1267-76
98. Shyamala G, Guiot MC. Activation of Kβ-specific proteins by estradiol. Proc Natl Acad Sci U S A 1992; 89: 10628-32
99. Warren GL, Lowe DA, Armstrong RB. Measurement tools used in the study of eccentric contraction-induced injury. Sports Med 1999; 27: 43-59
100. Meltzer HY. Factors affecting serum creatine phosphokinase levels in the general population: the role of race, activity and sex. Clin Chim Acta 1971; 33: 165-72
101. Shumate JB, Brooke MH, Carroll JE, et al. Increased serum creatine kinase after exercise: a sex-linked phenomenon. Neurology 1979; 29: 902-4
102. Berg A, Keul J. Physiological and metabolic responses of female athletes during laboratory and field exercise. Med Sport 1981; 14: 77-96
103. Rogers MA, Stull GA, Apple FS. Creatine kinase isoenzyme activities in men and women following a marathon race. Med Sci Sports Exerc 1985; 17: 679-82
104. Bär PR, Amelink GJ, Oldenberg B, et al. Prevention of exercise-induced muscle membrane damage by oestradiol. Life Sci 1988; 42: 2677-81
105. Amelink GJ, Bär PR. Exercise-induced muscle protein leakage in the rat: effects of hormonal manipulation. J Neurol Sci 1986; 76: 61-8
106. Van der Meulen JH, Kuipers H, Drukker J. Relationship between exercise-induced muscle damage and enzyme release in rats. J Appl Physiol 1991; 71: 999-1004
107. Dumke CL. Protective mechanism of estradiol on eccentrically induced muscle damage [thesis]. Eugen (OR): University of Oregon, 1996
108. Reijneveld JC, Ferrington DA, Amelink GJ, et al. Estradiol treatment reduces structural muscle damage after exercise in male rats [abstract]. Med Sci Sports Exerc 1994; 25 Suppl.: S72
109. Buckley-Bleiler R, Maughan RJ, Clarkson PM, et al. Serum creatine kinase activity after isometric exercise in pre-menopausal and post-menopausal women. Exp Aging Res 1989; 15: 195-8
110. Thompson HS, Hyatt JP, De Souza MJ, et al. The effects of oral contraceptives on delayed onset muscle soreness following exercise. Contraception 1997; 56: 59-65
111. Kendall BK, Eston RG. The effect of menstrual cycle status and oral contraceptive use on exercise-induced muscle damage [abstract]. J Sports Sci. In Press
112. Rinard J, Clarkson PM, Smith L, et al. Response of males and females to high-force eccentric exercise. J Sports Sci 2000; 18: 229-36
113. Tiidus PM, Bombardier F. Oestrogen attenuates post exercise myeloperoxide activity in skeletal muscle of male rats. Acta Physiol Scand 1999; 166: 85-90
114. Komulainen J, Koskinen SO, Kalliokoski R, et al. Gender differences in skeletal muscle fibre damage after eccentrically biased downhill running in rats. Acta Physiol Scand 1999; 165: 57-63
115. Cannon JG, Meydani SM, Fielding R, et al. Acute phase response in exercise II: associations between vitamin E, cytokines, and muscle proteolysis. Am J Physiol 1991; 260: R1235-40
116. Stupka N, Lowther S, Chorneyka K, et al. Gender differences in muscle inflammation after eccentric exercise. J Appl Physiol 2000; 89: 2325-32
117. MacIntyre DL, Reid WD, Lyster DM, et al. Different effects of strenuous eccentric exercise on the accumulation of neutrophils in muscle in women and men. Eur J Appl Physiol 2000; 81: 57-3
118. Prakash YS, Togaibayeva AA, Kannan MS, et al. Estrogen increases Ca2+ efflux from female porcine coronary arterial smooth muscle. Am J Physiol 1999; 276: H926-34
119. Jovanovic S, Jovanovic A, Shen WK, et al. Low concentrations of 17β-estradiol protect single cardiac cells against metabolic stress-induced Ca2+ loading. J Am Coll Cardiol 2000; 36: 948-52
120. Tiidus PM, Holden D, Bombardier E, et al. Estrogen effect on post-exercise skeletal muscle neutrophil infiltration and calpain activity. Can J Physiol Pharmacol 2001; 79: 400-6
121. Cid MC, Kleinman HK, Grant DS, et al. 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. J Clin Invest 1994; 93: 17-25
122. Chao TZ, Van Alten PJ, Greager JA, et al. Steroid sex hormones regulate the release of tumor necrosis factor by macrophages. Cell Immunol 1995; 160: 43-9
123. Rider V, Foster RT, Evans M, et al. Gender differences in autoimmune diseases: estrogen increases calcineurin expression in systemic lupus erythematosus. Clin Immunol Immunopathol 1998; 89: 171-80
124. Ahmed SA, Hissong BD, Verthelyi D, et al. Gender and risk of autoimmune diseases: possible role of estrogenic compounds. Environ Health Perspect 1999; 107: 681-6
125. Tornwall J, Carey AB, Fox RI, et al. Estrogen in autoimmunity; expression of estrogen receptors in thymic and autoimmune T cells. J Gend Specif Med 1999; 2: 33-40
126. Marui N, Offerman M, Swerlick R, et al. Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. J Clin Invest 1993; 92: 1866-74

127. Zuckerman SH, Ahmari SE, Bryan-Poole N, et al. Estriol: a potent regulator of TNF and IL-6 expression in a murine model of endotoxemia. Inflammation 1996; 20: 581-97
128. Schwarz E, Schafer C, Bode JC, et al. Influence of the menstrual cycle on the LPS-induced cytokine response of monocytes. Cytokine 2000; 12: 413-6
129. Angstwurm MWA, Gärtner R, Ziegler-Heitbrock HWL. Cyclic plasma IL-6 levels during normal menstrual cycle. Cytokine 1997; 9: 370-4
130. Pottratz ST, Bellido T, Macharla H, et al. 17β-estradiol inhibits expression of human interleukin-6 promoter-reporter constructs by a receptor-dependent mechanism. J Clin Invest 1994; 93: 944-50
131. Mishra DK, Friden J, Schmitz MC, et al. . Anti-inflammatory medication after muscle injury. A treatment resulting in short-term improvement but subsequent loss of muscle function. J Bone Joint Surg Am 1997; 79: 1270-1
132. Thorsson O, Rantanen J, Hurme T, et al. Effects of nonsteroidal anti-inflammatory medications on satellite cell proliferation during muscle regeneration. Am J Sports Med 1998; 26: 172-6
133. Bethea CL, Pecins-Thompson M, Schutzer WE, et al. Ovarian steroids and serotonin neural function. Mol Neurobiol 1998; 18: 87-123
134. Riley JL, Robinson ME, Wise EA, et al. A meta-analytic review of pain perception across the menstrual cycle. Pain 1999; 81: 225-35
135. Martinez-Gomez M, Cruz Y, Salas M, et al. Assessing pain thresholds in the rat: changes with estrus and time of day. Physiol Behav 1994; 55: 651-7
136. Rao SS, Ranganekar AG, Saifi AQ. Pain thresholds in relation to sex hormones. Indian J Physiol Pharmacol 1987; 31: 250-4
137. Thomas VN. Pain: its nature and management. London: Bailliere Tindall, 1997
138. Macintyre PE, Ready LB. Acute pain management: a practical guide. London: WB Saunders Co. LTD, 1996
139. Gintzler AR. Endorphin-mediated increase in pain threshold during pregnancy. Science 1980; 210: 193-5
140. Kristal MB, Thompson AC, Abbott P, et al. Amniotic-fluid ingestion by parturient rats enhances pregnancy-mediated analgesia. Life Sci 1990; 46: 693-8
141. Iwasaki H, Collins JG, Saito Y, et al. Naloxene-sensitive pregnancy-induced changes in behavioural responses to colorectal distension: pregnancy-induced analgesia to visceral stimulation. Anesthesiology 1991; 74: 927-33
142. Cogan R, Spinnato JA. Pain and discomfort thresholds in late pregnancy. Pain 1986; 27: 63-8
143. Whipple B, Josimovich JB, Komisaruk BR. Sensory threshold during the antepartum, intrapartum, and postpartum periods. Int J Nurs Stud 1990; 27: 213-21
144. Gintzler AR, Bohan MC. Pain thresholds are elevated during pseudopregnancy. Brain Res 1990; 507: 312-6
145. Dawson-Basoa ME, Gintzler AR. Estrogen and progesterone activate spinal kappa-opiate receptor analgesic mechanisms. Pain 1996; 64: 607-15
146. Dawson-Basoa ME, Gintzler AR. Gestational and ovarian sex steroids antinociception: synergy between spinal κ and δ opioid systems. Brain Res 1998; 794: 61-7
147. Liu N, Gintzler AA. Prolonged ovarian sex steroid treatment of male rats produces antinociception: identification of sex-based divergent analgesic mechanisms. Pain 2000; 85: 273-81
148. Gordon FT, Solimon MRI. The effects of estradiol and progesterone on pain sensitivity and brain opioid receptors in ovariectomized rats. Horm Behav 1996; 30: 244-50
149. Dina OA, Aley KO, Isenberg W, et al. Sex hormones regulate the contribution of PKC and PKA signalling in inflammatory pain in the rat. Eur J Neuro Sci 2001; 13: 2227-33
150. Amandusson A, Hallbeck M, Hallbeck AL, et al. Estrogen-induced alterations of spinal cord enkaphalin gene expression. Pain 1999; 83: 243-8
151. Amandusson A, Blomqvist A. Estrogen receptors can regulate pain sensitivity. Lakartidningen 2001; 15: 1774-8
152. Phillips SK, Rook KM, Siddle NC, et al. Muscle weakness in women occurs at an earlier age than in men, but strength is preserved by hormone replacement therapy. Clin Sci 1993; 84: 95-8
153. Naessen T, Linmark B, Larsen HC. Better postural balance in elderly women receiving estrogens. Am J Obstet Gynecol 1997; 177: 412-6
154. Seeley DG, Cauley JA, Grady D, et al. Is postmenopausal estrogen therapy associated with neuromuscular function or falling in elderly women?. Study of osteoporotic fractures research group. Arch Int Med 1995; 155: 293-9
155. Taaffe DR, Luz Villa M, Delay R, et al. Maximal muscle strength of elderly women is not influenced by oestrogen status. Age Ageing 1995; 24: 329-33
156. Armstrong AL, Oborne J, Coupland CA, et al. Effects of hormone replacement therapy on muscle performance and balance in post-menopausal women. Clin Sci 1996; 91: 685-90
157. Brooke-Wavell K, Prelevic GM, Bakridan C, et al. Effects of physical activity and menopausal hormone replacement therapy on postural stability in postmenopausal women: a cross sectional study. Maturitas 2001; 37 (3): 167-72

Correspondence and offprints: *Becky Kendall*, School of Sport, Health and Exercise Sciences, University of Wales, Bangor, LL57 2PX, UK.
E-mail: pep604@bangor.ac.uk