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The Influence of Estrogen on Skeletal Muscle

Sex Matters

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Abstract

As women enter menopause, the concentration of estrogen and other female hormones declines. This hormonal decrease has been associated with a number of negative outcomes, including a greater incidence of injury as well as a delay in recovery from these injuries. Over the past two decades, our understanding of the protective effects of estrogen against various types of injury and disease states has grown immensely. In skeletal muscle, studies with animals have demonstrated that sex and estrogen may potentially influence muscle contractile properties and attenuate indices of post-exercise muscle damage, including the release of creatine kinase into the bloodstream and activity of the intramuscular lysosomal acid hydrolase, β-glucuronidase. Furthermore, numerous studies have revealed an estrogen-mediated attenuation of infiltration of inflammatory cells such as neutrophils and macrophages into the skeletal muscles of rats following exercise or injury. Estrogen has also been shown to play a significant role in stimulating muscle repair and regenerative processes, including the activation and proliferation of satellite cells. Although the mechanisms by which estrogen exerts its influence upon indices of skeletal muscle damage, inflammation and repair have not been fully elucidated, it is thought that estrogen may potentially exert its protective effects by: (i) acting as an antioxidant, thus limiting oxidative damage; (ii) acting as a membrane stabilizer by intercalating within membrane phospholipids; and (iii) binding to estrogen receptors, thus governing the regulation of a number of downstream genes and molecular targets. In contrast to animal studies, studies with humans have not

as clearly delineated an effect of estrogen on muscle contractile function or on indices of post-exercise muscle damage and inflammation. These inconsistencies have been attributed to a number of factors, including age and fitness level of subjects, the type and intensity of exercise protocols, and a focus on sex differences that typically involve factors and hormones in addition to estrogen. In recent years, hormone replacement therapy (HRT) or estrogen combined with exercise have been proposed as potentially therapeutic agents for postmenopausal women, as these agents may potentially limit muscle damage and inflammation and stimulate repair in this population. While the benefits and potential health risks of long-term HRT use have been widely debated, controlled studies using short-term HRT or other estrogen agonists may provide future new and valuable insights into understanding the effects of estrogen on skeletal muscle, and greatly benefit the aging female population. Recent studies with older females have begun to demonstrate their benefits.

Over the past 15 years, several reviews have documented the potential for estrogen to mitigate post-injury disruption and inflammatory responses.[1-3] This review, while providing new insights into this discussion, further incorporates recent developments in our understanding of the potential for estrogen to positively affect muscle repair mechanisms and muscle contractility as well as its application to the aging female population. This review incorporates most of the studies related to estrogen and muscle contraction/damage/repair mechanisms that have appeared in the literature since the last major reviews by the authors and others in 2001–3,[2-4] as well as numerous relevant earlier works. An initial PubMed search using the keywords 'estrogen', 'muscle', 'force', 'strength', 'injury' and 'repair' yielded a significant number of papers from 2000 onward, which were selected for relevance for this focused updated overview.

While not intended to be exhaustive, this review does highlight the major areas of advances and controversies within this area of research. For example, while many studies with animals have tended to support the potential of estrogen to mitigate indices of muscle damage and inflammation, until recently the literature has been much less clear with regard to humans. These discrepancies, while widely debated,[5] have generally been attributed to a number of factors, including differences in age, fitness levels and exercise protocols, as well as a focus on sex-based differences rather than estrogen-specific effects. As sex differences are likely complicated by factors other than estrogen alone, the most effective experimental models for teasing out estrogenic effects are also included in this review. A summary of some of the suggested effects of estrogen on muscle function, as well as markers of post-injury damage, inflammation and repair, are included in table I. In addition, emerging insights into potential mechanisms of estrogenic influence on muscle repair, particularly relating to the activation and proliferation of satellite cells, are highlighted. Finally, a discussion of the potential application of hormone replacement therapy (HRT) and/or estrogen as therapeutic agents to the aging female population round out this updated review.

1. The Action of Estrogens: An Overview

The term 'estrogens' describes a group of 18-carbon corticosteroid molecules secreted primarily by the ovaries in females and, to a lesser extent, by the testes in males.[3] Estrogens are primarily involved in the development and maintenance of normal sexual and reproductive function,[79] although they have also been shown to exert a wide range of biological effects in many physiological systems, including the cardiovascular, musculoskeletal, immune and central nervous systems.[80] The most potent and abundant form of estrogen produced in the body is 17β-estradiol, although two other metabolites of estrogen,

Table I. Summary of some potential estrogen effects on skeletal muscle

Indicator	Effect measured	References		
		sex/estrogen/ HRT effects	mixed effects	no sex/estrogen/ HRT effects
Muscle structure and function	Muscle growth, size and mass	Rodents[6] Humans[7-12] Myoblasts[13]		Humans[14-19]
	Twitch characteristics	Rodents[20-22]		
	Tetanic force development, strength, endurance, fatigability or performance	Rodents[23-26] Humans[10,14,27-38]	Rodents[39] Humans[7,11,40]	Rodents[20,41-43] Humans[15-19,30,34,44-54]
Muscle damage indicators	Strength loss	Rodents[26]		Rodents[25,41] Humans[5,44-46,55-57]
	Blood creatine kinase activity	Rodents[58-63] Humans[55,64-66]		Rodents [67] Humans[5,55-57]
	Histology	Rodents[68,69]		Rodents[26] Humans[55]
	Lysosomal enzyme activity	Rodents[69-72]		
Post-damage muscle inflammation	Muscle leukocyte infiltration (neutrophils and macrophages)	Rodents[63,67,68,71-75] Humans[55]		Humans[45]
Post-damage muscle repair	Satellite cells, muscle regeneration	Rodents[70,71,73,76-77] Humans[78]		

HRT = hormone replacement therapy.

estriol and estrone, are also present at lower levels, and exhibit tissue-specific effects.[81]

Over the past two decades, our understanding of the protective roles of estrogen in a number of physiological systems has grown immensely. For example, estrogen has been reported to attenuate inflammation and damage, and enhance repair in skin, neural and hepatic tissues.[82-84] With respect to muscle, estrogen has been shown to exert protective effects on cardiac, smooth and skeletal muscle. For example, the incidence of cardiac disease in pre-menopausal women is lower than age-matched men, and this observation has been largely attributed to the presence of estrogen.[85] As well, several studies have reported an estrogen-mediated reduction in the degree of myocardial injury following ischaemia-reperfusion injury.[86-90] With respect to skeletal muscle, most animal studies have demonstrated that female and estrogen-supplemented rodents exhibit less myofibre injury and inflammation following exercise-induced muscle injury,[2,4] while in humans these effects have not been as clearly delineated.[5] In addition, our laboratory has recently demonstrated that estrogen may also influence post-damage repair processes through activation and proliferation of satellite cells.[70,71,73]

While the protective effects of estrogen on muscle have been well documented, the potential mechanism(s) underlying estrogenic action remain elusive. Three schools of thought are often used to explain the influence of estrogen:

1. Estrogen, due to its 18-carbon phenolic backbone and structural similarity to other potent antioxidants such as vitamin E, is thought to have a high antioxidant capacity and as such may have the ability to scavenge free radicals and stimulate the expression and activities of certain antioxidant enzymes, thus limiting oxidative damage.[91-93]
2. Due to its structural similarity to cholesterol, estrogen may have the ability to intercalate within membrane phospholipids in a similar fashion and exert a membrane-stabilizing effect.[1,94]
3. The discovery of three types of estrogen receptors (ERs) [ERα, ERβ and plasma membrane ER] has led to the discovery that estrogen may govern the regulation of a number of downstream genes and molecular targets.[3,71,95]

While each of these topics is addressed in individual subsections, it should be noted that

one, two or all of these processes is likely active during conditions of muscle injury.

2. Estrogen Influence on Muscle Structure and Function

With respect to muscle size, estrogen has been shown to influence growth of myoblast cells *in vitro*,[6] and is also associated with *in vivo* development of muscle size in female mice.[13] In humans, however, the effects of estrogen on muscle size are not as well understood. For example, while some studies have demonstrated that estrogen or hormone replacement may attenuate or even reverse the age-related decline in lean muscle mass and size observed in postmenopausal women,[7-12] other studies have shown no effects of estradiol on muscle mass, size or cross-sectional area.[14-19]

A number of studies have examined the influence of estrogen on muscle contractile properties. While some reports have clearly demonstrated a positive influence of estrogen or HRT on parameters such as twitch characteristics, tetanic force development and strength, other studies, particularly those involving humans, have been unable to demonstrate any estrogen-specific effects (table I). In animals, estrogen has been shown to affect muscle fatigue as well as twitch characteristics such as peak tension and half-relaxation time.[20-22] Reductions in skeletal muscle contractility and isometric tetanic force production have also been observed in mature, ovariectomized rodents,[23-25] although not all rodent studies have shown a positive estrogenic influence on tetanic force development.[20,41-43] While the underlying mechanisms for these potential force reductions by estrogen are still not known, some evidence is available. For example, Moran et al.[23,26] reported that the decrements in maximal tetanic force observed in ovariectomized rats were reversed with estrogen replacement. Interestingly, these authors also observed that the fraction of strong-binding myosin was greater in estrogen-supplemented animals, and suggested that estrogen may influence muscle contractile properties through direct binding to myosin.[26] Estrogen may also potentially modulate force development through its effects on specific contractile proteins. For example, Kadi et al.[96] found that estrogen administration altered the expression patterns of myosin heavy chain (MHC) proteins in both fast- and slow-twitch muscles of rodents. In addition, Suzuki and Yamamuro[39] reported that the isometric twitch tension of the extensor digitorum longus muscle in rats (which primarily contains fast-twitch fibres) was lower in estrogen-supplemented and ovary-intact rats compared with ovariectomized rats; however, estrogen had no effect on isometric twitch tension in the soleus, which primarily contains slow-twitch fibres. In contrast, McCormick et al.[20] observed no changes in MHC composition with estrogen replacement.

While a number of human studies have examined sex differences in muscle strength and fatigability during exercise, the findings are inconsistent and therefore few conclusions can be drawn. For example, some studies have demonstrated that the skeletal muscles of women have greater muscular endurance (i.e. a longer time to fatigue) compared with men, particularly following intermittent or isometric contraction protocols of low to moderate intensity,[27-31] whereas studies using more intense protocols or dynamic exercise (i.e. mixed concentric and eccentric contractions) have reported no such differences.[30] Studies using eccentric exercise protocols, which induce a greater degree of muscle disruption, also tend to show no differences between the sexes with respect to relative strength loss and skeletal muscle fatigability,[44-46] although differences are often observed with other indices of muscle injury, as seen in section 3.

As studies based on sex differences may be confounded by variables beyond the presence of estrogen, a more valid model may be to examine differences in muscle properties in pre- versus postmenopausal females or between age-matched older females with or without estrogen or hormone replacement. At the time of writing, approximately 25–30 studies and reviews were available in the literature that specifically examined differences in maximal isometric tension development, strength and/or muscle performance between males and females as well as between pre- and

postmenopausal women with or without HRT. While some studies have reported that HRT may have the potential to at least partly overcome the age-related declines in strength and increased levels of post-exercise muscle damage experienced by many women during and following menopause,[10,14,32-36] there is also a large body of evidence suggesting that HRT has little to no influence on muscle strength, performance or force development in humans.[15-19,34,47-53] Other studies have shown mixed results and demonstrated either increases in power or enhanced muscle performance with HRT, with no corresponding changes in maximal isometric tension.[7,11,40] In addition, a few studies have examined changes in muscle strength and force generation during the menstrual cycle. While some reports have noted significant increases in strength and force generation during the follicular and mid-cycle phases of the menstrual cycle (when estrogen levels are at their highest or rising),[37,38] others have reported no changes.[17,54] A very recent study compared 15 postmenopausal monozygotic twin pairs in which one twin had been using HRT for an average of approximately 7 years while the other had no history of HRT use.[11] They concluded that "long term HRT use was associated with better mobility, greater muscle power and favourable body and muscle composition among 54–62 year old women."[11] While another recent study which also compared postmenopausal females with or without HRT use reported that those women using HRT had significantly greater upregulation of proanabolic gene expression both at rest and following eccentric exercise.[97]

Collectively, evidence concerning the effects of estrogen on muscle structure and contractile function has tended to be conflicting and depends upon a number of factors, including the species examined, study type (i.e. cross-sectional vs longitudinal), age of the subjects, types of comparisons made (e.g. pre- vs postmenopausal females, males vs females, or postmenopausal females with or without HRT), size and fibre type composition of the muscles examined, the type, duration and intensity of exercise, and the contractile properties chosen for testing. Thus, caution should be exercised before any definite conclusions regarding the efficacy of estrogen or HRT on muscle function can be drawn. However, some recent well-controlled studies have supported positive effects of HRT on skeletal muscle function and composition in postmenopausal females[11,97] and may now have shifted the balance of evidence toward a positive influence of estrogen and HRT on skeletal muscle.[98]

3. Estrogen and Muscle Damage, Inflammation and Repair

3.1 Indices of Muscle Damage

Unaccustomed exercise, exercise involving lengthening or eccentric contractions or myotrauma often result in muscle membrane disruption and injury to myofibres. This has been documented directly through ultrastructural analysis of muscle tissue and biopsy samples[99-101] and indirectly through indicators such as losses in muscle strength, appearance of myofibre proteins in the blood, and muscle soreness.[102] Following this type of exercise, a well-characterized series of events involving oedema, an infiltration of inflammatory cells (i.e. neutrophils and macrophages), and activation and proliferation of satellite cells takes place to repair and replenish the damaged tissue. Each of these steps is regulated by a myriad of factors released both systemically as well as from the damaged tissue.[103,104]

Although many animal studies have demonstrated that estrogen and sex may significantly attenuate some indices of muscle membrane disruption and injury, including strength losses,[58-61,68-70,72] not all animal studies have found protective effects.[25,26,41,67] One of the most common markers of muscle membrane disruption is the appearance of the muscle protein creatine kinase (CK) in the bloodstream, and levels of this marker are significantly higher in male rats compared with female rats following conditions of muscle injury.[59-61] These differences have been specifically attributed to the presence of estrogen, as both male and ovariectomized

female rats supplemented with estrogen demonstrate reductions in CK activity similar to ovary-intact female rats.[58,60-63] While some human studies have shown similar trends with respect to post-injury indices of damage, strength loss and CK release,[55,64,65] many others have shown no differences between the sexes.[5,44-46,55-57]

Use of blood CK levels as an indicator of post-exercise muscle membrane disruption in human and intact animal studies has been shown to be problematical due to its high variability and to factors related to CK clearance rates from the blood.[105,106] For example, it has been reported that female mice and humans may clear CK from blood faster than male mice, and this could at least in part account for some of the reported sex differences in post-exercise blood CK activities.[105] However, this observation does not negate the possibility that post-exercise CK release from muscle is also attenuated by estrogen through its role as a membrane stabilizer. Indeed, an early study by Amelink et al.[61] used an isolated *in vitro* preparation to electrically stimulate muscles from normal male, female and ovariectomized female rats with or without prior estrogen treatment. They reported a direct inverse relationship between estrogen supplementation and *in vitro* CK *release* from the isolated muscles in all groups of animals. This and other studies by this group suggest that the membrane-stabilizing effects of estrogen may attenuate post-exercise CK release from skeletal muscle and that changes in circulating CK levels can at least indirectly and qualitatively reflect changes in exercise-induced muscle membrane disruption.[59,60]

Less is known about whether estrogen and sex can specifically influence muscle structural damage. One of the first studies to explore this question was performed by Komulainen et al.,[69] who found that the hindlimb muscles of female rats exhibited significantly less myofibre structural damage and swelling compared with male rats up to 96 hours after downhill treadmill running. In addition, the muscles of male rats had significantly greater losses of sub-membrane proteins such as desmin and dystrophin compared with females. Activity of the intramuscular lysosomal enzyme, β-glucuronidase, which is commonly used as an indirect indicator of muscle damage, was higher post-exercise in male versus female hindlimb muscles. Our laboratory has confirmed that this protective effect is due to the presence of estrogen, as we have observed similarly attenuated post-exercise β-glucuronidase activities in red and white hindlimb muscles of ovariectomized female rats supplemented with estrogen.[70,71] Collectively, the above data suggest that early losses in sub-membrane proteins in the muscles of male and estrogen-deficient female rats following exercise-induced muscle damage may originate at the plasma membrane, and estrogen may prevent this disruption through potential membrane-stabilizing properties.[71,94] In this regard, a very recent study that examined a number of muscle and blood markers of exercise-induced muscle damage and inflammation in postmenopausal females concluded that postmenopausal women lacking estrogen replacement via HRT experienced significantly greater muscle damage following eccentric exercise and that there appeared to be a protective effect of HRT against exercise-induced muscle damage.[66]

It is also possible that at least some of the reported estrogen-related differences in indicators of post-exercise muscle damage and inflammation can be attributed to differences in animal size. Ovariectomized female rats without estrogen supplementation are often larger than ovariectomized female rats that are estrogen supplemented,[70,71] possibly contributing to the differences in muscle damage reported in these groups following downhill running. For example, one study reported that large (100–200%) differences in weights of male rats may have been a factor contributing to higher indices of a specific marker of muscle damage following downhill running.[107] However, this study lacked statistical rigor and conceded that the differences between weight groups could also have been due to the large differences in rat ages, with heavier groups representing significantly older animals. Whether these much smaller weight differences in ovariectomized female animals of the same age are a factor in the repeatedly observed differences in indices of post-exercise muscle damage is not

known. However, we have previously reported that both ovary-intact female and heavier estrogen-supplemented male rats exhibited attenuated post-exercise muscle inflammation markers to a greater degree than heavier male rats who lacked estrogen supplementation.[74] As well, one study involving ischaemia reperfusion-induced injury (where rat bodyweight was not a factor) also demonstrated a post-damage attenuation effect of estrogen on muscle leukocyte infiltration.[68]

As estrogen may also increase voluntary physical activity in rats,[108] it is possible that estrogen-supplemented animals may be more 'trained' and hence less susceptible to exercise-induced muscle damage than unsupplemented ovariectomized females. However, most of the studies demonstrating stimulatory effects of estrogen on rodent activity have used voluntary wheel running or open field observation to assess physical activity patterns.[108] It is uncertain whether relatively brief exposures to estrogen for animals confined to small cages with no access to running wheels may result in different levels of 'training effect' between groups. These questions should be further investigated.

It is also possible that estrogen, due to its structural similarity to known antioxidants such as vitamin E, may protect muscle from damage and inflammation through a similar mechanism.[91,92] When cells are exposed to conditions of stress or injury, free radical-induced peroxidation reactions can occur, leading to membrane disruption. While *in vitro* studies have demonstrated that estrogen is able to substantially inhibit membrane lipid peroxidation,[62,92] there is some question as to whether the picomolar concentration of estrogen normally observed in physiological systems is high enough to exert significant antioxidant effects. Some studies have demonstrated *in vivo* antioxidant effects of estrogen following running exercise[109] and muscle injury,[58,68] while others have failed to find estrogen-related changes in post-exercise indices of oxidative stress.[110] Intriguingly, estrogen appears to reduce levels of antioxidants such as vitamin C and glutathione in some muscles and tissues,[110,111] thereby potentially undermining some of its antioxidant effects.

Studies examining the antioxidant effects of estrogen in humans are limited and the findings are equivocal, likely because human studies tend to examine chemical indicators of post-exercise oxidative stress in the blood (rather than muscle biopsies) and focus on sex-based differences rather than estrogen effects *per se*. Nevertheless, Dernbach et al.[112] found that female rowers had lower levels of an oxidative stress marker in the blood compared with males after a strenuous 4-week training programme, while Ayres et al.[113] reported that amenorrhoeic female athletes demonstrated a significantly greater potential for lipid peroxidation after an acute bout of exercise compared with eumenorrhoeic females. More recently, Kerksick et al.[57] found that females at the mid-luteal phase of their cycle had higher serum concentrations of the antioxidant enzyme superoxide dismutase (SOD) compared with males after eccentric exercise. However, Chung et al.[114] failed to observe any differences in post-exercise oxidative stress markers between females who exercised at different phases of the menstrual cycle. As mentioned previously, many factors complicate the interpretation of data generated from human studies, including prior state of fitness, use of indirect rather than direct markers, and the presence of other sex hormones such as progesterone and testosterone. Future studies aimed at comparing these indices between pre- and postmenopausal women will hopefully yield further insights in this area.

In addition to its potential role as an antioxidant, estrogen may also protect muscle from secondary damage through its influence on various regulators of muscle catabolism and apoptosis. Although relatively few studies are available in this area, Willoughby and Wilborn[115] reported that women in the midluteal phase of their cycle had decreased levels of myostatin mRNA, a regulator of skeletal muscle catabolism, 24 hours after a session of eccentric exercise, while men had increased levels. Stupka et al.[55] found that men had a greater number of skeletal muscle cells positive for the apoptotic indicator bcl-2 compared with women 48 hours after eccentric exercise, while Kerksick et al.[57] noted a significant decrease in the bax/bcl-2 ratio (an

indicator of the apoptotic state of the cell) in women versus men after a similar eccentric exercise protocol. Although more research needs to be done in this area, the current findings do support evidence of sex differences in apoptotic mechanisms following damaging exercise.

Estrogen may also protect muscle from structural damage by interacting with heat shock proteins (HSPs), often referred to as molecular chaperones, which play an important role in protein assembly and maintenance of structural integrity following conditions of stress, trauma or injury. While there are many types of HSPs, each with their own expression patterns and regulatory properties, the most widely studied HSP in muscle is HSP70. Although HSP70 is constitutively expressed in skeletal muscle, its induction can be rapidly triggered by various stressors, including exercise-induced muscle damage.[109]

As post-exercise expression of HSP70 in rodent skeletal muscle has been shown to differ between the sexes,[116] it has been suggested that estrogen, through its role as a potential antioxidant and/or membrane stabilizer, may protect muscle from injury and hence diminish HSP70 induction.[109] For example, Paroo et al.[109] reported that both female and estrogen-supplemented ovariectomized rats exhibited a diminished post-exercise HSP70 response. However, very recent collaborative work involving our laboratory further clarified these findings by suggesting that estrogen may in fact protect skeletal muscle from injury by augmenting basal HSP70 concentrations, as only minor further increases in muscle HSP70 expression are observed with exercise.[117] This suggestion is consistent with previous reports, which demonstrated that constitutive myocardial HSP70 expression was enhanced in the presence of estrogen, and that unlike male and ovariectomized female animals lacking estrogen replacement, exercise or training did not greatly increase myocardial HSP70 expression.[85,118] Taken together, the data suggest that some of the protective potential of estrogen on skeletal muscle may be due to its ability to upregulate basal levels of HSP70 expression.

Our laboratory has also provided evidence that estrogen may protect skeletal muscle from post-exercise damage and inflammation-related events through inhibition of calcium-activated proteases (calpains).[4,63] Calpains are a family of intracellular non-lysosomal proteases that, once activated, may further exacerbate muscle damage through their degradation of various muscle structural proteins. During exercise-induced muscle membrane disruption, calcium floods into the cell down its concentration gradient. If intracellular calcium levels are elevated for prolonged periods, activation of calpains may occur.[119,120] Since estrogen can act as a membrane stabilizer,[121] it may act to limit membrane disruption during injury and hence prevent the influx of calcium down its concentration gradient, which would in turn limit calpain activation and hence further structural damage. As well, because muscle proteins degraded by calpains may act as chemoattractants for inflammatory cells such as neutrophils,[122] estrogen may also protect muscle from further damage by inhibiting the recruitment of inflammatory leukocytes such as neutrophils into muscle.[63]

3.2 Inflammation and Leukocyte Infiltration

Leukocytes such as neutrophils and macrophages play an important role in the inflammatory response following muscle injury, and may also play a role in initiating downstream repair processes. Neutrophils are usually the first leukocytes to arrive at the site of injury, typically between 1 and 12 hours post-damage.[63,68,123] Their main function is to remove and degrade damaged tissue by generating hypochlorous acid and superoxide radicals through a series of oxidation reactions mediated by the enzymes myeloperoxidase and nicotinamide adenine dinucleotide oxidase, respectively. They also release a number of chemoattractants that serve to recruit more neutrophils to the site of injury or infection and amplify the response. While neutrophils play a beneficial role in eliminating damaged tissue, they are unable to distinguish between healthy and damaged structures, and as such may exacerbate tissue damage.[3,124]

Macrophages are the other major type of leukocyte to infiltrate muscle tissue following injury.

Two major subpopulations, ED1+ and ED2+, have been shown to play important roles in the inflammatory response and may also be responsible for initiating downstream repair mechanisms. ED1+ macrophages invade tissue first, usually within 12 hours of injury, and are mainly responsible for phagocytic removal of damaged tissue and cytokine release, while ED2+ macrophages invade muscle later (24–48 hours post-injury) and are essential for activating downstream regeneration processes, including activation of satellite cells.[125-127]

Over the past decade, a number of research studies aimed at exploring sex differences and, more specifically, the potential of corticosteroid hormones (particularly estrogen) to influence post-injury damage and repair processes have been performed. The protective effects of estrogen on brain, neural tissues and cardiac muscle have been well characterized,[95,128] and demonstrate the attenuating influence of estrogen on tissue damage and inflammation as well as its accentuating effect on regenerative processes.

Animal studies from our laboratory[63,68,71,74,75] and others[72] have demonstrated that post-injury infiltration of leukocytes into skeletal muscle is influenced by sex and estrogen status. In an initial study, Tiidus and Bombardier[74] reported that compared with male rats, female rats had significantly attenuated neutrophil infiltration into skeletal muscles 24 hours after running exercise. When the male rats were supplemented with estrogen, they exhibited the same blunted response of post-exercise neutrophil infiltration as female rats.[74] While these earlier studies relied mainly on indirect quantification of neutrophils through myeloperoxidase activity, later studies using histochemical identification of neutrophils confirmed these findings.[68,71,73,75] Later studies from our laboratory using ovariectomized female rats, with or without estrogen replacement, confirmed that the attenuation of neutrophil infiltration into skeletal muscle was estrogen dependent.[63,68,71,75] For example, Tiidus et al.[63] found that estrogen-supplemented ovariectomized female rats demonstrated significantly attenuated neutrophil infiltration into red and white skeletal muscles 1 hour post-exercise compared with unsupplemented rats, while Stupka and Tiidus[68] noted similar findings 2 hours following hindlimb ischaemia-reperfusion injury.

Relatively few studies have examined the influence of sex and estrogen on infiltration of macrophages following exercise or injury. St Pierre Schneider et al.[72] reported that macrophage infiltration was delayed in female versus male mice (peaking at 7 and 5 days, respectively) following lengthening exercise. More recently, our laboratory demonstrated that infiltration of ED1+ macrophages into rat skeletal muscles 24 hours after lengthening exercise (i.e. downhill running) was attenuated with estrogen supplementation.[71,75] Interestingly, a recent study examining damage to endothelial tissues reported that while estrogen also diminished post-damage leukocyte infiltration into endothelial cells, the presence of progesterone negated these effects.[129] This suggests that *in vivo*, when both progesterone and estrogen are present, estrogen may not have as great an anti-inflammatory effect on muscle as previously reported in studies using ovariectomized animals with estrogen supplementation alone. However, very recent work from our laboratory has suggested that progesterone does not alter the attenuating ability of estrogen on post-exercise leukocyte infiltration into skeletal muscle and that progesterone independently may have a small but significant ability to diminish post-exercise muscle leukocyte invasion.[75]

Human studies examining the influence of estrogen on post-injury leukocyte infiltration tend to be less consistent and focus primarily on sex differences rather than estrogen replacement. While some studies have reported that females exhibit lower levels of muscle leukocyte infiltration after eccentric (i.e. lengthening) exercise compared with men,[55] others have reported no such differences.[45] These differences may be attributed in part to differences in exercise protocols and/or experimental methods; however, it is also possible that other sex hormones may exert independent effects on post-exercise leukocyte infiltration into skeletal muscle.

The mechanisms by which estrogen may influence post-damage leukocyte infiltration into muscle are not yet known. Systemically, estrogen may prevent leukocyte entry from the bloodstream

into the damaged tissue by limiting the availability of endothelial adhesion molecules.[72] Estrogen has been shown to regulate leukocyte rolling and adhesion into damaged tissue by increasing the activity of endothelial nitric oxide synthase (NOS).[130,131] Nitric oxide (NO) may also play an important role in initiating muscle repair mechanisms, which is addressed in section 3.3.

Similar to muscle damage, estrogen may also exert its protective influence on post-injury inflammatory processes through various estrogen hormone receptor-mediated and non-receptor-mediated mechanisms. While estrogen has been shown to inhibit inflammation and accelerate healing in a number of other tissues, including liver and nervous tissue through both hormone receptor- and non-receptor-mediated processes,[128,132] relatively little information is available on the mechanisms of post-injury estrogenic protection in skeletal muscle.

Recent studies from our laboratory have provided compelling evidence that estrogen protects muscle from muscle injury and leukocyte infiltration primarily through non-receptor-mediated events.[68,70,71] As mentioned previously, estrogen, by intercalating within plasma membranes, can act as both an antioxidant or as a membrane stabilizer,[3,4] which may in turn limit membrane disruption and subsequent inflammation following an injury. Furthermore, we have proposed that the attenuation of neutrophil infiltration post-injury may be mediated through estrogen-mediated stabilization of muscle membranes and inhibition of Ca^{2+}-activated proteases (calpains).[63] This theory was originally based on findings from the laboratory of Belcastro,[119,122] who established a connection between calpain activity and neutrophil invasion of skeletal muscle 1–2 hours after running exercise. As protein fragments generated through the proteolytic actions of calpains may act as chemoattractants for neutrophils following exercise or injury, stabilization of membranes by estrogen may limit post-exercise membrane disruption and influx of Ca^{2+} into the cell, which would in turn inhibit the upregulation of calpain activity post-exercise. In a 2001 study from our laboratory,[63] we provided support for this theory by demonstrating that estrogen supplementation in ovariectomized female rats limited post-exercise neutrophil infiltration into muscle and simultaneously attenuated calpain activity.

As mammalian skeletal muscle contains both α and β ERs,[133-135] it has been hypothesized that estrogen may exert its protective influence through one or more receptor-mediated events. Through estrogen binding, ERs regulate a number of diverse intracellular signalling pathways, including the phosphatidylinositol-3 kinase (PI3K)/protein kinase B (Akt) pathway, which stimulates protein synthesis and growth of skeletal muscle.[95,136] To date, the only study examining the receptor-mediated influence of estrogen on inflammatory cell infiltration into skeletal muscle is from our laboratory. In this recent work,[71] ovariectomized female rats were either exposed to estrogen supplementation, estrogen supplementation plus the ER antagonist ICI 182,780, or a sham procedure. ICI 182,780 is known as a 'pure' antiestrogen because it inhibits ERs with extremely high affinity and specificity and does not possess the partial agonistic properties commonly seen in nonsteroidal antiestrogens such as tamoxifen.[137] After prolonged exposure to the hormone treatments, a subset of animals ran downhill for 90 minutes on a treadmill. Hindlimb skeletal muscles were examined 1 and 3 days post-exercise for markers of damage (β-glucuronidase activity), inflammation (neutrophil and macrophage invasion) and repair (activation and proliferation of satellite cells). While estrogen treatment significantly attenuated post-exercise skeletal muscle β-glucuronidase activity and leukocyte infiltration, the ER antagonist had no influence on either of these indices. Collectively, the findings provide strong evidence that estrogenic influence on muscle injury and leukocyte invasion is primarily regulated through non-receptor-mediated mechanisms, while ERs may play a more vital role in downstream repair processes, as seen below.

3.3 Muscle Repair and Regeneration: The Role of Satellite Cells

Strenuous, unaccustomed exercise or exercise involving eccentric contractions can result in trauma or injury to myofibres. Following this

type of injury, skeletal muscle fibres undergo a period of regeneration to repair and replenish the damaged tissue. Chemotactic signals, including cytokines and growth factors, are generated by the injured tissue, which activates the inflammatory response, and attract leukocytes to the site of injury. This cascade of events also leads to the activation and proliferation of satellite cells, which is a pivotal event in muscle repair and regeneration.[138]

Satellite cells are small, mononucleated cells that reside between the basal lamina and sarcolemma of muscle fibres.[139] While normally quiescent in adult skeletal muscle, in response to myofibre injury[140,141] or overload[142] they re-enter the cell cycle, where they proliferate and differentiate to provide muscle-specific proteins needed for skeletal muscle growth and regeneration.[143] Satellite activation and proliferation are regulated by a myriad of factors released from both the damaged tissue as well as from the leukocytes that are recruited to the site of injury.[104,144] As many of these factors are in turn influenced by circulating levels of estrogen,[68,89,145] we have hypothesized that estrogen may play an important role in satellite cell activation, and hence muscle repair.

While the influence of estrogen on muscle damage and inflammatory processes has been relatively well characterized, much less is known about the potential for estrogen to stimulate muscle regenerative processes such as satellite cell activation and proliferation. McClung et al.[76] reported that regeneration and regrowth of rat skeletal muscle following a period of muscle atrophy induced by hindlimb suspension is dependent upon estrogen status. In addition, sex differences in satellite cell activation and proliferation have been observed in both human and animal studies.[77,78] For example, Roth et al.[78] reported that women exhibited a greater increase in the number of satellite cells in the vastus lateralis muscle than men after 9 weeks of resistance training. In animals, a study by Salimena et al.[77] involving *mdx* mice (which have a dysfunctional sarcolemma and undergo repeated cycles of damage and repair) noted that the skeletal muscles of female mice had less damage and a greater number of myofibres staining positively for satellite cells compared with male mice.

Our laboratory recently performed several studies aimed at examining the influence of estrogen on satellite cell populations following exercise-induced muscle injury.[70,71,73,146] In a preliminary study,[73] we observed that the skeletal muscles of estrogen-supplemented male rats had increased numbers of satellite cells 72 hours after a session of downhill running. We next attempted to determine which stages of the satellite cell cycle were influenced by estrogen by examining histochemical changes in numbers of total (Pax7-positive), activated (MyoD-positive) and proliferating (BrdU-incorporated) satellite cells following a similar exercise protocol. In this follow-up study,[70] ovariectomized female rats were either supplemented with estrogen or given a sham procedure. We observed post-exercise increases in the number of skeletal muscle fibres staining positively for all three satellite cell markers; moreover, the increases in all three of these markers were significantly augmented with estrogen. Taken together, the results suggest that (i) sex-mediated differences in muscle fibre regeneration and satellite cell numbers may be directly attributed to estrogenic influence, and (ii) estrogen may exert its influence on post-exercise muscle satellite cell populations through events upstream of satellite cell activation.

Although the mechanisms by which estrogen may augment post-exercise satellite cell numbers and potentially influence other muscle repair processes are as yet unknown, it is likely that, as with muscle injury, various receptor- and non-receptor-mediated roles for estrogen also exist with muscle repair. Our recent study employing the ER antagonist ICI 182,870 provides compelling evidence that ERs play an important role in influencing muscle repair processes through augmentation of satellite cell activation and proliferation.[71] As shown in figure 1, blocking ERs completely abolished both exercise- and estrogen-mediated increases in all three satellite cell populations.[71] While the finding that the ER antagonist decreased post-exercise satellite cell populations to levels below the sham condition was unexpected, it was not surprising given that

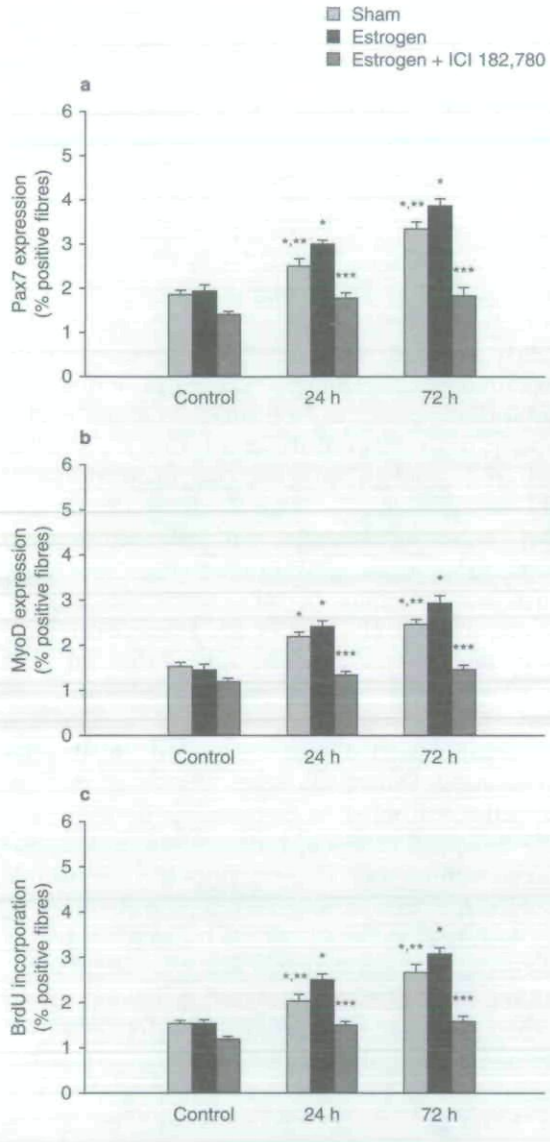

Fig. 1. Effects of estrogen supplementation and ICI 182,780 administration on numbers of fibres positive for (**a**) Pax7 (paired box homeotic gene 7), (**b**) MyoD (myogenic differentiation factor D), and (**c**) BrdU (5-bromo-2'-deoxyuridine) satellite cell markers in rat soleus muscle 24 and 72 h following downhill running. Values are means ± SEM. (reproduced from Enns et al.,[71] with permission of the authors). * $p<0.05$ compared with control group, ** $p<0.05$ compared with treatment-matched estrogen group, *** $p<0.05$ compared with treatment-matched sham and estrogen groups.

ERs are expressed in many different organs of the body[80] and were also likely inhibited by the antagonist. This systemic inhibition of ERs may have also led to additional protection of skeletal muscle from injury by estrogen via other receptor- and non-receptor-mediated mechanisms, as discussed below. We recently repeated this study, this time using an ER-α-specific agonist, which lacked other estrogenic properties and demonstrated that it is specifically through the ER-α that estrogen effect on satellite cells is manifested.[146]

A number of downstream signalling pathways and targets of ER binding exist that could potentially be responsible for the upregulation of post-exercise satellite cell populations observed in the presence of estrogen. For example, the PI3K/Akt pathway has been shown to stimulate growth and protein synthesis through binding of estrogen to ERs.[95,136] In addition, 17β-estradiol is involved in the ER-mediated induction of the immediate early genes *c-fos* and *egr-1* in myoblasts, which promotes cell growth.[6] As these pathways are essential for the growth and repair of myofibres, there appears to be support for a role of estrogen in this process.

NO may also be a potential downstream effector of estrogenic influence during conditions of muscle injury.[130,131] NOS activity and NO levels are enhanced with estrogen in a number of tissues, and through a combination of receptor- and/or non-receptor-mediated events may influence muscle damage and repair.[89,147] In skeletal muscle, inhibition of NOS with the antagonist N-nitro, L-arginine methyl ester prior to muscle injury prevents satellite cell activation.[148] In addition, the release and localization of hepatocyte growth factor, which regulates satellite cell activation,[149] is an NO-dependent process during conditions of muscle injury.[150] The question of whether estrogen influences post-exercise skeletal muscle damage and repair mechanisms through NO-mediated signalling is an intriguing one and merits further study.

It is also possible that estrogen may influence muscle repair through its effects on specific leukocyte populations. A number of studies have postulated that leukocytes, and in particular ED2+ macrophages, may be important promoters of satellite cell activation and proliferation.[72,127,151,152] *In vitro* studies have demonstrated that macrophages added to myoblast

cultures increase satellite cell proliferation and enhance myotube formation.[127,152] In addition, Tidball and Wehling-Henricks[151] reported that depletion of macrophages *in vivo* following a modified loading protocol (hindlimb suspension-induced muscle atrophy followed by reloading) impaired myofibre regeneration and satellite cell activation.

New evidence has revealed that estrogen may also influence macrophage-mediated muscle repair through receptor-mediated mechanisms. ERs have been identified on murine and rat macrophages[153,154] and appear to have potent regulatory effects on macrophage function.[155] For example, a recent study by Calippe et al.[156] demonstrated that chronic administration of estrogen to ovariectomized mice markedly increased the expression of interleukin-6 and NOS, which are known satellite cell activators,[104,148] through ERα-mediated events. These data provide new and compelling evidence that estrogen may accentuate post-exercise muscle satellite cell activation and proliferation even while it attenuates muscle macrophage infiltration.[71]

4. Conclusion

Studies with animals have provided some evidence that estrogen and sex may influence muscle membrane stability and limit exercise-induced muscle damage. Furthermore, estrogen appears to exert significant influence on post-damage leukocyte infiltration into skeletal muscle and may promote downstream repair processes through activation and proliferation of satellite cells as well as through leukocyte-mediated events. Although the mechanisms of estrogenic influence on skeletal muscle during conditions of muscle injury and repair have not been fully characterized, membrane stabilization, antioxidant activities and receptor-mediated processes likely play an important role.

In partial contrast to animal studies, the influence of sex and estrogen on indices of muscle damage and repair has not been as clearly delineated in humans.[56] These inconsistencies have been attributed to a number of factors, including age of subjects, pre-study level of fitness, type and intensity of exercise protocol, and a focus on sex-based differences rather than estrogen-specific effects. However, despite these inconsistencies, a limited body of evidence exists to support the contention that estrogen may influence skeletal muscle contractile properties as well as mitigate post-injury leukocyte infiltration and repair in humans.

5. Implications for Humans and Future Research

The study of estrogenic influence on muscle function, damage, inflammation and repair is particularly relevant to the postmenopausal female population, as females tend to experience greater strength declines, decreased functional capacity, impairments in muscle repair and increased rates of sarcopenia with age than their male counterparts.[7,8] As studies with animals have demonstrated that estrogen reduces muscle atrophy[157,158] and accelerates recovery from experimentally induced atrophic conditions,[76,136] it is reasonable to speculate that estrogen or HRT may have similar beneficial effects on preserving muscle size, strength and injury protection in humans. Indeed, while some studies utilizing different therapeutic strategies such as strength training and/or HRT to limit postmenopausal losses in strength and muscle mass and accelerate post-damage muscle repair with this population have proven encouraging, other studies have shown no effects of estrogen or HRT on muscle size or function in postmenopausal women (table I). Interestingly, strength training has been shown to significantly increase satellite cell numbers in both younger and older individuals, with the biggest increases seen in older women;[78] thus, it is possible that HRT, combined with exercise, may be the most beneficial method for preserving muscle mass and strength in older women.[33,159] Several recent studies that found positive effects of HRT use in postmenopausal women on muscle mass, function, protection from exercise-induced damage and induction of proanabolic environment have further strengthened the case for positive estrogenic effects on skeletal muscle of older

women.[11,97,98] Future studies using postmenopausal women exposed to estrogen replacement, either with or without specific exercise regimens, may help us better determine the potential effects of estrogen and HRT, if any, in this population.

Unfortunately, a significant drawback to HRT in older females, despite its potential to diminish muscle damage and speed repair, is the increased risk of cancer and other diseases associated with prolonged postmenopausal exposure to estrogen replacement.[160,161] Further research involving other pharmacological agents that mimic estrogenic effects and/or activate ERs[162] without inducing carcinogenic or other undesirable effects may be an important new avenue to influence the health and musculoskeletal functional abilities of aging females. Thus, increasing our understanding of the mechanisms by which estrogen may exert its protective effects and designing effective counter-measures to preserve the strength and functional abilities of older adults will greatly benefit this population.

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