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Estrogen, muscle damage, and the repeated bout effect

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LIST OF ABBREVIATIONS

ATP	Adenosine tri phosphate
CG	Control group
CK	Creatine kinase
DH1	First downhill run
DH2	Second downhill run
DOMS	Delay onset muscle soreness
ELISA	Enzyme-linked immunosorbent assay
ER	Estrogen receptor
HE	High estrogen group
HC	Hormonal contraceptive
HR	Heart rate
LE	Low estrogen group
LDH	Lactate dehydrogenase
MPO	Myeloperoxidase
Pre	Before exercise
RBE	Repeated bout effect
RER	Respiratory exchange ratio
ROS	Reactive oxygen species

ABSTRACT

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PURPOSE: The aim of this study was to investigate estrogen's effect on markers of muscle damage and the repeated bout effect in women following multiple downhill runs. METHODS: Thirteen moderately trained females (VO_2 max: 36-47 ml/kg/min), 18-35 years old, and who used hormonal contraception participated in the study. They completed two 40 min downhill runs (-10% grade) at 65-70% VO_2 max during either the third week of hormone use (low estrogen group (LE), n=7) or the placebo week (high estrogen group (HE), n=6). Trials were separated by four weeks. Creatine kinase (CK) activity, pressure tolerance (front thigh, shin, and calf), and circumferences (thigh and calf) were measured before (pre) and at 24 hr, 48 hr, and 72 hr after exercise. Muscle soreness of the front thigh, shin and calf was reported immediately (0 hr) and at 24 hr, 48 hr, and 72 hr after exercise. Knee extension and ankle dorsiflexion peak torque were measured pre and 72 hr postexercise. RESULTS: A run effect was found in front thigh ($P = 0.009$) and calf ($P = 0.02$) pressure tolerance such that DH1 was lower than DH2. Soreness of the front thigh was lower following DH2 compared to DH1 ($P = 0.05$). A group x time interaction was observed for shin soreness such that soreness in LE was higher at 24 hr and 48 hr compared to 0 hr ($P < 0.001$; $P < 0.001$, respectively) and 72 hr

($P < 0.01$; $P = 0.04$, respectively). Following DH1, CK activity was higher at 24 hr ($P < 0.001$), 48 hr ($P = 0.04$), and 72 hr ($P = 0.03$) compared to pre-exercise. CK activity was lower at 24 hr following DH2 ($P < 0.001$) compared to 24 hr following DH1. No significant changes were noted for shin pressure tolerance, calf soreness, circumference, or peak torque measurements. CONCLUSION: Women with lower estrogen levels may show a greater response in markers of muscle damage following the initial bout of exercise as evidenced by increased soreness in the shin. The RBE was observed as evidenced by a less robust response to pressure tolerance, soreness and CK activity following DH2, with no group differences in the RBE response. Higher estrogen levels at the time of exercise may mitigate markers of muscle damage following an initial bout of eccentric exercise but does not seem to influence the repeated bout effect.

CHAPTER 1. INTRODUCTION

1.1 Introduction

Eccentric exercise is characterized by a lengthening of the muscle while it produces force in an attempt to shorten. This type of work done on the muscle by forced external lengthening often leads to muscle damage if the muscle is unaccustomed to the exercise (Armstrong 1984, Byrnes et al. 1985, Friden et al. 1981). Indeed, eccentric exercise induces more muscle damage compared to concentric exercise (Armstrong 1984) possibly due to metabolic and/or mechanical stress (Ebbeling and Clarkson 1989, Armstrong 1990). There are five general characteristics of muscle damage: muscle fiber disarrangement, decreased muscle performance, muscle protein release into the blood, delayed onset muscle soreness (DOMS), and an acute-phase immune response (Stupka et al. 2000).

Muscle damage results in muscle fiber disarrangement such as z-disc disruptions (Friden et al. 1981, Armstrong 1984) which are visible in muscle biopsy samples. A limitation of biopsies, however, is that it is possible to miss damaged fibers when the biopsy is taken, or induce more damage to the surrounding muscle which may compromise ensuing biopsies (Malm 2001).

Muscle force production decreases immediately after muscle-damaging eccentric exercise to about 45-55% of the pre-exercise performance (Chen 2006, Sewright et al.

2008). Recovery may take as little as three days to more than ten days (Kerksick et al. 2008, Eston et al. 2000) depending on exercise intensities and protocols (Kerksick et al. 2008, Eston et al. 2000, Nosaka et al. 2002).

Due to disruption of the muscle membrane, proteins [e.g. creatine kinase (CK)] are able to leak out into the blood. Elevated plasma (Chen 2006, Van Der Meulen et al. 1991) or serum (Sewright et al. 2008, Thompson et al. 1997) CK activity is commonly used as an indicator of damage (Chen 2006). It should be noted, however, that the increase in enzyme concentration is not necessarily proportional to the amount of muscle damage (Van Der Meulen et al. 1991). Values also show high intersubject variability (Chen 2006).

Delayed onset muscle soreness (DOMS) is soreness that does not appear immediately post exercise, but rather a day or two later. While the direct cause of DOMS is unknown, it is associated with swelling and decreased muscle performance (Chleboun 1998). DOMS usually peaks one to three days after exercise and may persist for up to seven days (Friden et al. 1981, Armstrong 1984).

Following muscle damaging exercise, leukocytes (neutrophils and macrophages) migrate to the area to remove dead and damaged cells and to promote the repair process. This is known as an acute-phase immune response (Field et al. 1991, Tidball 2005). While a necessary part of the repair process, it can also lead to more damage in the surrounding tissue through the release of reactive oxygen species (ROS) by these cells (Tidball 2005).

There is evidence, albeit somewhat inconsistent, of sex differences in the muscle damage response to eccentric exercise. Differences in muscle damage between male and

female rats have been found in multiple studies (Bar et al. 1988, Amelink et al. 1990, Van Der Meulen et al. 1991, Komulainen et al. 1999). For example, markers of muscle damage (β-glucuronidase, CK) were lower in female compared to male rats following a downhill running protocol (Bar et al. 1988, Komulainen et al. 1999). Additionally, swelling and loss of structural proteins from the muscle cell appear to be greater and appear sooner in male rats and is hypothesized to be due to greater sarcolemma disruption (Komulainen et al. 1999). In comparison to women, men show greater muscle soreness, strength loss, and muscle protein leakage (Kerksick et al. 2008), but these findings are not consistently reported (Sewright et al. 2008, Stupka et al. 2000).

Estrogen is being investigated as a possible explanation for the observed gender differences in muscle damage. Estrogen is a group of steroid hormones that contains eighteen carbon atoms and is derived from cholesterol (Johnson 2007, Tortora and Derrickson 2009). There are three forms of estrogen: 17-β estradiol, estrone, and estriol. Estrogen is produced mainly by the ovaries with some production from the adrenal glands. While often referred to as the female sex hormone, estrogen is also produced in males but in significantly lower concentrations (Johnson 2007).

Estrogen concentrations vary throughout a woman's menstrual cycle. Estrogen levels are low during the early follicular stage when menstruation is occurring. During the follicular stage, the follicle (which contains the developing oocyte) begins to grow and eventually starts to produce estrogen. This results in a continual increase in estrogen levels in the body, with an additional surge of estrogen just prior to ovulation. Following ovulation is the luteal stage. Estrogen levels in the luteal phase decrease after the surge but remain higher than the follicular phase. At the end of the luteal phase estrogen levels

decline and menstruation occurs (Johnson 2007, Tortora and Derrickson 2009). The average cycle (starting with the first day of menses and ending the day prior to menstruation) is approximately 28 days, with most cycles occurring between 25 and 31 days (Chiazze et al. 1968).

Estrogen's role in mediating muscle damage is not well understood but it may protect the muscle through its ability to act as an antioxidant, membrane stabilizer, or gene regulator (Kendall and Eston 2002). Evidence for estrogen decreasing muscle damage has been demonstrated in rats. When male rats and ovariectomized female rats were given estrogen supplementation, markers of muscle damage were lower and followed the pattern of female intact rats (Bar et al. 1988, Tiidus et al. 2001). In humans, Carter et al. (2001) found a decrease in plasma CK activity with no difference in muscle soreness when estrogen levels were different between two female groups (Carter et al. 2001). Thompson et al. (1997) found the opposite, i.e. a difference in muscle soreness, but no significant change in CK. Both studies included hormonal contraceptive (HC) users versus non HC users. It should be noted that the effect of exogenous versus endogenous estrogen on muscle damage has not been thoroughly studied (Savage and Clarkson 2002). While both Thompson et al. (1997) and Carter et al. (2001) used eccentric exercise to induce muscle damage in each study, different protocols (aerobic versus anaerobic) were used which may have contributed to the contradictory findings.

An area of research that has not been explored is the effect estrogen levels have on the repeated bout effect (RBE). After completion of an initial bout of eccentric exercise, markers of muscle damage are not as apparent following a second exercise bout of similar intensity. Decreases in factors such as muscle proteins in the blood, muscle

soreness, and muscle fiber disruption following a second bout of eccentric exercise characterize the RBE (Newham et al. 1987, Byrnes et al. 1985). The cause of this attenuation of markers of muscle damage is unknown. It has been proposed that cellular changes, neuronal changes, and/or mechanical changes are occurring (McHugh 2003). Regarding sex differences, the pattern of RBE in women is similar to what has been described in men (Stupka et al. 2001, Fernandez-Gonzalo et al. 2011).

1.2 Statement of the problem

The lack of clarity concerning estrogen and its role in protecting against muscle damage is primarily due to the lack of research in this area and the variability in estrogen among women. Not every woman has a 28 day menstrual cycle and hormone concentrations will vary not only throughout the cycle but also among women (Treloar et al. 1967, Chiazze et al. 1968). HC use among women could also affect markers of muscle damage. There is some evidence that HC may have a beneficial effect on muscle soreness (Kendall and Eston 2002) and strength recovery (Savage and Clarkson 2002) after exercise.

Generally, two types of eccentric exercise are used to induce muscle damage: downhill running and isolated muscle contractions. While isolated muscle contractions focus on a single type of movement to localize damage to a specific area, downhill running affects several major muscle groups. Different methods of exercise used in the studies by Carter et al. (2001) and Thompson et al. (1997) (downhill running and bench stepping, respectively) may be a factor in the contradicting muscle soreness and CK results reported by these researchers.

Currently there is no literature that specifically examines the question of estrogen and its influence on the RBE. Muscle damage induced during the first bout of exercise may be the trigger that leads to adaptations to protect the muscle from future damage. If estrogen can attenuate muscle damage resulting from the first exercise bout, there is some uncertainty as to if and how that may affect the RBE.

1.3 Aims and Hypotheses

Aim 1: To determine if estrogen levels at the time of exercise influence the ensuing muscle soreness and CK response.

Ho: Higher estrogen levels at the time of exercise will not attenuate the ensuing muscle soreness and CK response to a bout of eccentric exercise compared to a lower estrogen level.

Ha: Higher estrogen levels at the time of exercise will attenuate muscle soreness and CK.

Aim 2: To determine if estrogen levels at the time of exercise will change markers of the repeated bout effect.

Ho: There will be no difference in the attenuation of CK and soreness between high and low estrogen groups following a second bout of eccentric exercise.

Ha: There will be a smaller attenuation of CK and soreness following the second bout in the high estrogen group compared to the low estrogen group

1.4 Limitations

There are several limitations to this study. Muscle biopsies were not performed to confirm the extent of muscle damage. Our subject pool included only HC users, so responses in women who do not use HC are unknown. Only moderately trained women were included in this study, therefore, extrapolation of the results to other populations may be limited.

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

Estrogen is a group of hormones commonly referred to as the female sex hormone, although men also have estrogen albeit in much lower concentrations (Johnson 2007). While estrogen is known to play a role in development and reproduction, its significance in protecting against muscle damage is less defined. Animal studies have demonstrated that high estrogen levels decrease markers of muscle damage (Bar et al. 1988). Similar results have been reported in humans but they have not been consistent across studies (Kerksick et al. 2008, Stupka et al. 2000). There is a lack of research on the role of estrogen in the repeated bout effect (RBE) in women. RBE is characterized by a decrease in muscle damage and soreness but any interaction between RBE and estrogen is unknown.

2.2 Eccentric Exercise

Eccentric contractions occur when the muscle lengthens while producing force, i.e. resistance or force is applied to a muscle that is increasing in length while exerting tension or contracting against the applied resistance. Such contractions cause more

damage to skeletal muscle tissues compared to concentric (muscle shortening) contractions (Armstrong 1984, Chleboun 1998). Mechanical and metabolic stressors produced by eccentric contractions may trigger the initiation of exercise induced muscle damage. Mechanical stress (damage to the sarcolemma, sarcoplasmic reticulum, and muscle fiber structures) and metabolic stress (high temperature, decreased ATP levels, ROS, and lower pH) can lead to a disruption in calcium homeostasis in muscle cells (Armstrong 1990). Damage to the sarcomeres and/or sarcolemma results in muscle soreness and/or pain, leakage of muscle proteins into the blood, and decreased strength in the days following a bout of eccentric exercise (Clarkson and Trembley 1988).

2.3 Creatine Kinase

The disruption of muscle fiber structures leads to the leakage of muscle cell contents, some of which are used as indirect markers that muscle damage has occurred. Examples are myoglobin (Armstrong 1984), intracellular calcium concentration (Sonobe et al. 2010), and IL-6 (Chaffin et al. 2011), with the most commonly measured being CK activity (Thompson et al. 1997, Carter et al. 2001, Stupka et al. 2000). CK is found in muscle and in some organs (Amelink et al. 1988) and is released into the blood when muscle tissue is damaged (Chen 2006). CK is an enzyme that catalyzes the formation of phosphocreatine and adenosine diphosphate from creatine and adenosine triphosphate (ATP) in the cells. CK also catalyzes the reverse reaction to create ATP (Wallimann et al. 1992). While not a direct marker, an increase in plasma CK activity after exercise is believed to demonstrate that muscle damage has occurred (Byrnes et al. 1985).

Large variability exists in the CK response to exercise among individuals. Chen (2006) found that males who were divided into groups based on plasma CK response (low, medium and high responders) following an initial bout of eccentric exercise showed a similar response in plasma CK levels a year later. This indicates that people are naturally high or low CK responders and may explain some of the variability in plasma CK values.

In addition to being low or high responders, sex may also influence CK values. Rat studies show that intact females tend to have lower levels of CK activity at rest compared to their male (Bar et al. 1988) and ovariectomized counterparts (Bar et al. 1988, Tiidus 2001 et al.). However, when resting values of plasma CK were similar, Amelink et al. (1988) found a greater increase in male rats compared to females following two hours of running. In humans, Stupka et al. (2000) showed in an underpowered study that the data was trending towards a significant gender difference in CK values following exercise ($p=0.14$) with women tending to have lower levels of CK . When comparing men and women after downhill running, Eston et al. (2000) did not find sex differences in CK levels.

The benefits of using CK as a marker of muscle damage is that it is easy to measure and there is a vast collection of literature for comparison. The drawbacks to using CK are that there is large intersubject variability (Chen 2006) and a correlation has not been established between the extent of muscle damage and plasma CK activity (Van Der Meulen et al. 1991).

2.4 Delayed-onset muscle soreness

As previously mentioned, unaccustomed eccentric exercise produces soreness in the muscles which generally peaks 24-72 hours after exercise (Friden et al. 1981, Newham et al. 1987, Smith et al. 1994) and dissipates approximately 4-7 days after exercise (Hough 1902, Armstrong 1984). This is commonly referred to as delayed onset muscle soreness or DOMS. Although DOMS has been found in the distal part of the muscle after exercise, it is generally located throughout the whole muscle when maximal exercise is performed. The localization of soreness in the distal end could be due to the damage being focused in that area or pain receptors being located in the distal end of the muscle near the tendons (Armstrong 1984). The cause of DOMS is unknown but is associated with swelling (Chleboun 1998), decreased muscle performance (Smith et al. 1994, Chleboun 1998), and muscle damage in the exercised muscle (Armstrong 1984).

Swelling is often determined by comparing the circumference of the limb before and after exercise. While measuring changes in circumference does not indicate where the swelling is occurring (e.g. in the muscles or surrounding tissue), it indicates that volume is increasing in that area (Nosaka et al. 2002, Chleboun 1998). Chleboun (1998) found that muscle circumference was the largest four days after performing isolated eccentric elbow flexor. The increase in volume was as much as 26%, and returned to pre-exercise values approximately nine days after exercise.

Decreased muscle performance is often associated with muscle soreness. The decreased ability to produce force could be caused by an unwillingness to fully contract the muscle due to increased pain or because the contractile units are too damaged to produce the same level of force (Armstrong 1984, Hough 1902). Regaining full pre-

exercise force production usually takes about two weeks (Newham et al. 1987). Chelboun et al. (1998) found that muscle performance decreased by as much as 47% of voluntary maximal contraction following eccentric exercises of the elbow flexors. The greatest decrease in performance was observed one day following exercise and was yet to be fully recovered ten days after exercise.

To determine muscle damage, indirect measures such as protein (e.g. CK activity) in the blood and direct measures such as muscle biopsies are used. Muscle biopsies give a direct view of muscle tissue; however, muscle biopsies only show a small segment of muscle tissue, and the section observed may or may not be taken from tissue that is damaged. The process of taking the biopsy can also damage the surrounding tissue and may confound results if another sample is taken from the compromised surrounding area (Malm 2001).

2.4.1 Possible Mechanisms

The dull pain associated with DOMS may be due to nerves being stimulated by a chemical signal (e.g., from molecules such as bradykinins), mechanical pressure from swelling, or a thermal change caused by increased temperature associated with inflammation. Armstrong (1984) proposed a model for DOMS starting with disruption of the muscle fibers due to the mechanical stress of the exercise, particularly eccentric exercise. Damage to the muscle fibers results in increased calcium in the cells. The calcium imbalance eventually leads to the destruction of the cell, ultimately releasing muscle proteins into the interstitial fluid (Kendall and Eston2002).

After muscle-damaging exercise and the ensuing leakage of cellular contents such as calcium, an inflammatory response is triggered to repair the damaged tissue. The inflammatory response may be activated by calpain (calcium-activated) which attracts neutrophils to the area through chemo-attractants (Belcastro et al. 1998). Neutrophils and macrophages migrate to the site to clean-up the muscle tissue. This inflammatory process itself can lead to more muscle damage. For example, neutrophils release ROS or factors that increase the levels of ROS while cleaning up debris from the original damage which can lead to more tissue damage (Kendall and Eston 2002). While it is not believed that neutrophils contribute directly to muscle repair, it could be that removal of damaged tissue by neutrophils can help the regeneration process continue. Other phagocytic cells (i.e., macrophages) also migrate to the area to clean up debris and could also be involved with the repair process itself (Tidball 2005, Tiidus et al. 2001). Therefore, while a decrease in the number of neutrophils and macrophages that invade the area may reduce the amount of additional damage that occurs, it may also hinder the regeneration process.

2.5 Repeated Bout Effect

One bout of eccentric exercise produces a protective effect whereby a second bout of the same exercise results in less muscle damage and soreness. This phenomenon is termed the repeated bout effect (RBE), and is characterized by less muscle disruption, lower CK levels (Byrnes et al. 1985), decreased soreness, and an attenuated leukocyte response. These adaptations are seen only if the repeated bout is performed within a certain time frame after the first exercise bout. Smith et al. (1994) found that if the second bout of exercise occurs 48 hours after the first, CK and DOMS responses were similar to

an initial single bout of exercise (Smith et al. 1994). Byrnes et al. (1985) found that CK, soreness, and myoglobin levels were lower following a second bout of downhill running when it was performed three and six weeks after the first bout but no difference when the bout was repeated at nine weeks. However, Nosaka et al. (2001) found indicators of the RBE six months following the initial bout of exercise.

2.5.1 Possible mechanisms

There are many theories as to why there is less damage to myofibrils during a second bout of an eccentric exercise. McHugh (2003) attributed this protective adaptation to neuronal, mechanical, and cellular changes. The neuronal theory is based on more muscle fibers or a change in the type of muscle fibers being recruited. Due to straining the muscle tissue during the initial bout of eccentric exercise, more motor units may be activated during the second bout to disperse the stress over a greater area (Nosaka and Clarkson 1995, McHugh 2003). A second possibility could be that while mostly fast twitch fibers are recruited in the first bout, there may be a switch to slow-twitch fiber recruitment during the following bout (Warren et al. 2000, McHugh 2003).

The theory of mechanical changes focuses on alterations in the cytoskeleton of the muscle such as connective tissue and desmin. Restructuring after damage from the first bout may lead to protection from the second bout. Lapier et al. (1995) found that rats with higher connective tissue mass in the hindlimb were less susceptible to contraction-induced injury. However, an increase in connective tissue could also lead to an increase in passive muscle stiffness resulting in less protection from an ensuing bout of exercise. McHugh et al. (1999) found that subjects with higher passive muscle stiffness prior to

exercise showed increased muscle soreness and decreased strength following isokinetic eccentric contractions of the hamstring.

According to the cellular theory, the cell membrane strengthens in response to damage to prevent future disruptions (Clarkson and Tremblay 1988). Other possibilities are that the sarcomeres that were damaged were weak sarcomeres which are then replaced by stronger ones, or that the muscle fiber lengthens by adding more sarcomeres (McHugh et al. 1999, Newham 1988). Lastly, there may be changes to excitation contraction coupling after the initial bout resulting in less attenuation of strength after a repeated bout (Warren et al. 2000).

2.6 Estrogen

Estrogen is a group of 18-carbon hormones. The most common forms are 17β-estradiol, estrone, and estriol. Estrogens are produced in the ovaries as well as in the adrenal cortex. 17β-estradiol is the most abundant form in the premenopausal female after puberty (Johnson 2007). Estrogen has many roles in the body such as regulation of the menstrual cycle and development of secondary sex characteristics (Tortora and Derrickson 2009), and is currently being examined for its possible protective role against muscle damage and muscle soreness.

Evidence from rat studies suggests that high estrogen levels offer protection against skeletal muscle damage. Bar et al. (1988) found that ovariectomized female rats had a significant increase in CK activity after exercise while control female rats did not. The study also showed that when male rats and female ovariectomized rats were treated with estrogen for three weeks prior to exercise, the CK response was lower compared to

their non-treated counterparts. Tiidus et al. (2001) found that ovariectomized rats supplemented with estrogen had decreased levels of CK, myeloperoxidase (MPO) which is generally expressed in neutrophils, and calpain-like activity one hour after exercise compared to their non-supplemented counterparts.

When the sarcolemma is damaged following exercise, homeostasis of molecules and proteins can be disrupted in the muscle fiber. Sonobe et al. (2010) measured intracellular calcium levels in male, female, and ovariectomized female rats in response to eccentric contractions. They found a sex difference in the amount of calcium in the intracellular space following eccentric contractions with higher levels found in the male rats. They also found more hypercontracted muscle fibers in the male group than either female group. While no significant difference was found between intact female and ovariectomized rats, the ovariectomized females tended to have higher values of calcium and hypercontracted fibers than the non-ovariectomized female group.

2.6.1 Possible Mechanisms

The role of estrogen in reducing muscle damage is categorized into three main modes of action, i.e., acting as an antioxidant, a membrane stabilizer, or a regulator of gene expression (Kendall and Eston 2002). It has been proposed that estrogen with its phenol ring may act as an antioxidant. The compounds resulting from estrogen breakdown may also have antioxidant capabilities similar to vitamin E (Sugioka et al. 1987). As an antioxidant, estrogen could bind to ROS to decrease the amount of damage done to muscle cells. While ROS are important for certain gene regulators (e.g., TNF-alpha), too much ROS can result in cell damage (Sen 1995). Sugioka et al. (1987) found

that estrogens were able to decrease the amount of lipid peroxidation in vitro. Out of the three estrogens, estradiol appeared to have the greatest antioxidant properties.

As a fat-soluble hormone, estrogen may interact with the cell membrane to decrease membrane fluidity. In a study by Tiidus et al. (2001) ovariectomized rats were subjected to a running protocol that induced oxidative stress. Rats supplemented with estrogen showed decreased levels of CK and neutrophils compared to the non-supplemented group. MPO and calpain-like activity were also lower in the estrogen group providing evidence that there is a membrane stabilization component.

A third possible mechanism in support of estrogen's involvement in mediating muscle damage is gene regulation. As previously mentioned, ROS is important for certain gene regulation, and estrogen's potential as an antioxidant to bind ROS could impair gene regulation. The administration of estrogen one day prior to exercise in male rats did not result in any protective effects (no significant difference in CK); however, when estrogen was administered for three weeks prior to exercise there was a protective effect (Bar et al. 1988). Due to the apparent delay in the response to estrogen administration before protection against muscle damage occurs, some gene regulation may need to take place prior to exercise.

2.6.2 Estrogen Receptors

Gene regulation via estrogen has been a focus of recent research. Estrogen acts as a ligand binding to a receptor which undergoes a conformational change to alter gene transcription. It was discovered that there were two variations of the receptor that estrogen binds, and they were coined estrogen receptor-α (ERα) and estrogen receptor-β

(ERβ). What the receptors activate individually as well as how they interact with each other is still being studied. Recently, it has been shown that both ERα and ERβ are located on most of the nuclei in human skeletal muscle fibers in (Wiik et al. 2009). The role of estrogen receptors in skeletal muscle is still being elucidated. Recent studies indicate that the inflammatory and regeneration response following muscle damage may be influenced by estrogen receptor mediated pathways (Velders et al. 2012, Enns et al. 2008). For example, proinflammatory markers were elevated in ovariectomized mice following muscle damage, whereas mice treated with ER ligands showed attenuated responses. Three days after damage, mice treated with the ERβ agonist but not the ERα agonist ligand showed increases in markers of satellite cell activation compared to ovariectomized mice. ERβ knockout mice showed a decreased satellite cell activation response following muscle damage compared to both ERα knockout mice and wild type, providing evidence that estrogen may act through ERβ to alter the inflammatory response and muscle satellite cell activation (Velders et al. 2012).

2.6.3 Findings in Females

Data regarding estrogen's protective effects are less conclusive in humans than in animal studies. Whereas there was a decreased CK response when male rats and ovariectomized female rats were given estrogen supplementation (Bar et al. 1988), studies using human subjects are more complicated due to large subject variability that must be taken into account. Moreover, the influence of exogenous vs endogenous estrogen on the responses to eccentric exercise in women remains unclear. Thompson et al. (1997) examined the effects of HC use on DOMS after a 50 min bench stepping

eccentric exercise. Subjects taking HC had lower 17β-estradiol levels compared to the control group (CG), and the CG showed more soreness in the quadriceps compared to HC users. Soreness appeared to peak for both groups at 48 hours after exercise. While not statistically significant, it appeared CG had lower CK activity than the HC users. The highest CK values for CG occurred on day three whereas HC users' highest level was at day five. Higher estrogen levels do not appear to protect the muscle from increased soreness, but CK trends may be impacted by the higher levels. Carter et al. (2001) examined estrogen's influence on markers of muscle damage following downhill running. The group with higher estrogen levels (HC users) showed significantly lower CK activity 72 hours after eccentric exercise with no difference in muscle soreness. Estrogen is also used for hormone therapy in postmenopausal women. Dieli-Conwright et al. (2009) found that postmenopausal women who were taking hormone therapy showed attenuated muscle protein activity (CK and LDH) after maximal eccentric knee extension exercises in contrast to a low estrogen CG which showed an increase in muscle protein activity.

Stupka et al. (2000) examined differences in inflammation between males and females in response to eccentric contractions in an isolated leg muscle. The women (HC users) had significantly higher estrogen levels compared to the men. Contrary to previous reports (Bar et al. 1988), they found no statistical difference in CK between the men and women, perhaps due to the study being underpowered. It was found, however, that even though the level of muscle damage displayed in muscle biopsies was the same, markers of leukocytes (bcl-2-positive inflammatory cells ($p<0.05$) and leukocyte common antigen ($p=0.052$)) were higher in men compared to women. This provides evidence that there may be a difference in the immune response to muscle damage between men and women,

i.e., fewer neutrophils or macrophages may be recruited in women than men in response to muscle damage.

There are limitations to the evidence for estrogen's role in protecting against muscle damage in humans such as HC use and differences in testing techniques. Another area that lacks clarity is the role of estrogen in the RBE. If estrogen attenuates muscle damage from the first bout of eccentric exercise it is unclear if markers of the RBE will be altered.

2.7 Summary

Unaccustomed eccentric exercise results in muscle damage, muscle soreness, decreased muscular strength, inflammation, and an acute immune response. Estrogen has been shown in animal studies to attenuate these responses while results of human studies have been mixed. The RBE also attenuates these markers, but it is unknown how these two factors (estrogen and RBE) interact.

CHAPTER 3. MATERIALS AND METHODS

3.1 Participants

Participants for this study were 13 moderately active premenopausal women between 18 and 35 years of age. They completed questionnaires detailing menstrual cycle history and daily activity level (Paffenbarger 1997) to assess inclusion/exclusion qualifications. Only women who had regular menstrual cycles (no missed periods for the last 6 months), using HC, were moderately active [VO_2 max between 36 to 47 ml/kg/min (Heyward 2006) and not engaged in intense exercise for more than one hour a day three times a week] were included. Participants were excluded if they were irregularly menstruating, ovariectomized, smokers, taking cardiac or anti-inflammatory medications, had any musculoskeletal limitations, or had ovarian or breast cancer.

All procedures were approved by the Institutional Review Board (Protocol #1308013928). Participants provided written consent prior to participation in the study.

3.2 VO_2 max Testing Protocol

A progressive intensity, continuous VO_2 max test protocol on a treadmill was used to determine if women met the abovementioned criterion for being moderately active. Women walked on the treadmill at a pace of 3.5 mph at 0% incline for three minutes to warm-up. The test began at a speed of 4 mph with 0% incline. Treadmill speed

increased every two minutes by 1 mph until reaching 7 mph, after which the treadmill grade increased by 2% every two minutes. Criteria for determining if a subject reached her VO_2 max included meeting two of the following criteria: RER ≥1.15, heart rate at or near the age predicted maximum, voluntary termination, and < 150 ml/min increase in VO_2 with an increase in workload.

3.3 Experimental Design

After qualifying for the study, participants were divided into two groups: a low estrogen group (LE; n=7) and a high estrogen group (HE; n=6). Women during their third week of hormone use were classified as LE (due to endogenous estrogen concentration being suppressed during this part of the menstrual cycle) and HC users during their placebo week were classified as HE. They subsequently completed two 40-min downhill running trials on a treadmill separated by approximately one month. Exercise intensity was 65-70% VO_2 max at a -10% grade.

3.4 Experimental Trials

Women were asked to fast overnight and refrain from strenuous activities for two days prior to the downhill runs. They were given a three minute warm-up consisting of walking on the treadmill at a speed of 3.5 mph. Speed was then incrementally increased to a target value (based on the VO_2 max test) that elicited 65-70% of the VO_2 max. The speed for the second downhill was identical to that of the first run. The two trials were approximately 4 weeks apart so that participants completed both trials during the same

phase of their menstrual cycle. Measurements recorded during the treadmill runs included rating of perceived exertion (RPE), HR, and VO_2.

3.5 Measurements

Muscle soreness, leg pressure tolerance, leg circumference, peak muscle torque, 17 β-estradiol and creatine kinase activity measurements were recorded at various time points before and after the trials as shown in Table 1.

Table 1. Timeline of measurements.

	Pre-exercise	**0hrs**	**24hrs**	**48hrs**	**72hrs**
Soreness		X	X	X	X
Circumference	X		X	X	X
Pressure Tolerance	X		X	X	X
Estradiol	X				
CK	X		X	X	X
Peak Torque	X				X

Duplicate circumference measurements were taken on the right leg at mid-thigh and calf prior to each trial and at 24, 48, and 72hrs post exercise using a tape measure. If the two readings were more than 5mm apart then a third measurement was performed. For thigh circumference, women placed their knee on a bench with the leg at a 90 degree angle. The measurement was taken midway between the inguinal crease and the proximal border of the patella. For the calf circumference, participants stood with both feet on the

ground and approximately 20 cm apart. The measurement was taken at the point of maximal circumference between the knee and the ankle. The distance from the medial malleolus to the area of measurement on the calf was recorded for repeated measurements.

Muscle soreness of the front thigh, calf, and shin was assessed using a subjective rating scale ranging from 0-6 (Rodenburg et al. 1993) immediately after exercise and at 24, 48, and 72hrs post exercise. An algometer (JTECH Medical Commander Algometer, Midvale, UT) was used to determine pressure tolerance prior to the trials and at 24, 48, and 72hrs post exercise. Pressure was applied on the right front thigh, shin (1cm lateral to the tibia) and calf at the location of the circumference measurements at a rate of 2 lb/sec using a 1 cm^2 probe. Subjects were asked to say "yes" when they felt pain. Subjects were supine for front thigh and shin measurements and prone for calf measurements. Each area was measured once in rotation before a repeated measurement was taken.

Peak isometric torque production of the right leg during leg extension and ankle dorsiflexion was measured using a Humac Cybex (Henley Healthcare, Sugar Land, TX) machine. Participants performed three sets of five repetitions (30 sec rest between sets) during which they were asked to exert as much force as possible against the resistance. Leg extension testing was performed at a speed of 60deg/sec with the subject in a sitting position. Ankle dorsiflexion was performed at a speed of 30deg/sec with the subject in a supine position with her upper leg perpendicular and lower leg about parallel to the floor. Measurements were performed pre-exercise and at 72hrs post exercise.

3.6 Blood Sampling and Preparation

A 4 ml blood sample was drawn into serum separator tubes from an antecubital vein immediately before the first (DH1) and second (DH2) downhill runs, and 2 ml blood samples were taken for the post exercise samples. Blood was allowed to clot for 30 min following five inversions of the tubes then centrifuged for 10 min at 1000-1300g. Serum was collected and stored at -80 °C until analyzed for CK and 17β-estradiol.

3.7 Analyses

3.7.1 CK activity

Blood samples were analyzed for CK activity using an enzymatic assay according to the manufacturer's protocol (Pointe Scientific, Inc. Lincoln Park, MI). 50µl of serum was pipetted into a cuvette containing 1ml of pre-warmed reagent. The cuvette was inverted three times and measured two minutes later using a spectrophotometer (SpectraMax M2e, Molecular Devices, LLC Sunnyvale, CA) at 37°C. The average change in absorption per minute (ΔAbs/min) was record and used to calculate CK activity (U/L) by using the equation (ΔAbs/min x 1.05)/(0.00622 x 0.05).

3.7.2 Estrogen (17 β-estradiol)

Estrogen concentrations, specifically 17 β-estradiol, were measured using an enzyme-linked immunosorbent assay (ELISA). Following the manufacturer's protocol (ALPCO, Salem, NH) serum samples were analyzed in duplicate. A 4-parameter curve was calculated (SoftMax Pro 5.4 Molecular Devices, LLC. Sunnyvale, CA) and used to determine the samples' concentrations in pg/ml.

3.8 Statistical Analysis

Results are shown as the mean ±SE. Subject characteristics were analyzed using t-tests. All other data were analyzed using a group (HE and LE) x run (DH1 and DH2) x time (pre, and 0, 24, 48, and 72hrs postexercise) mixed model ANOVA. A Tukey post-hoc analysis was performed when applicable. Significance was accepted at $\alpha \leq 0.05$.

CHAPTER 4. RESULTS

Subject characteristics are summarized in Table 2. There was no significant difference between the groups for VO_2 max, height, body mass, or age. A dependent t-test showed that estradiol did not differ between DH1 and DH2, so the values were averaged across the runs. Although not statistically significant, estradiol concentration in HE tended to be higher than in LE (P=0.07).

Table 2. Subject characteristics.

	Estradiol (pg/ml)	**VO_2 Max (ml/kg/min)**	**Height (cm)**	**Mass (kg)**	**Age (years)**
High Group	75.1 ± 11.8 (34-107)	40.4 ± 1.7 (37-46)	165.8 ± 1.7 (160-170)	67.0 ± 3.1 (59-78)	23.7 ± 1.9 (20-29)
Low group	45.2 ± 9.7 (13-94)	41.2 ± 1.4 (37-46)	166.3 ± 1.8 (165-170)	65.2 ± 6.7 (50-102)	22.0 ± 0.9 (18-25)

Values are X±SEM with ranges underneath. In the high group n=6 and in the low group n=7.

4.1 Pressure Tolerance

Pressure tolerance for the front thigh is shown in Figure 1. Although there was no significant run x time x group interaction, there was a run effect. Pressure tolerance following DH1 (9.2 ± 0.8 lb/cm^2) was lower than DH2 (10.1 ± 0.8 lb/cm^2) (P = 0.009)

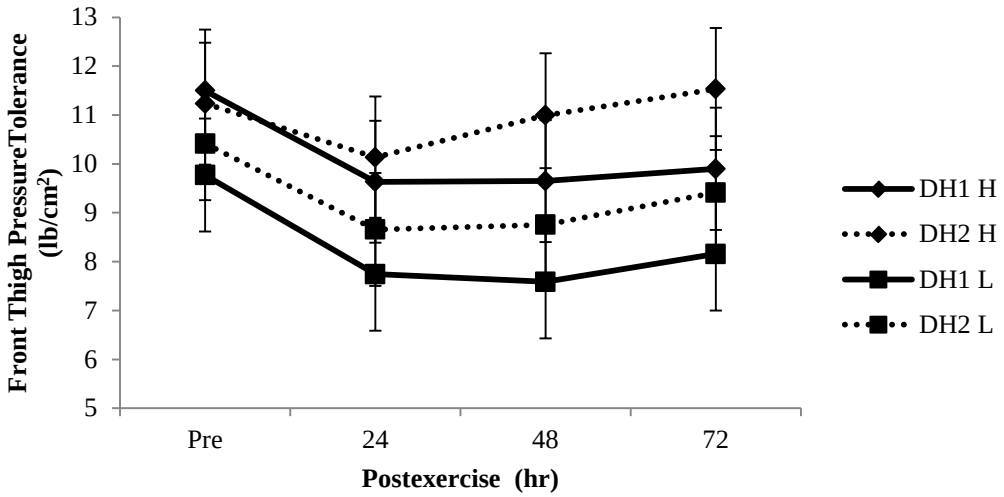

Figure 1. Pressure tolerance of the front thigh for the first (DH1) and second (DH2) downhill runs. Values are X±SEM. H=high estrogen group; L=low estrogen group. Pressure tolerance was lower for DH1 than DH2 (P < 0.01).

Pressure tolerance for the shin is shown in Figure 2. There were no significant main effects or interactions.

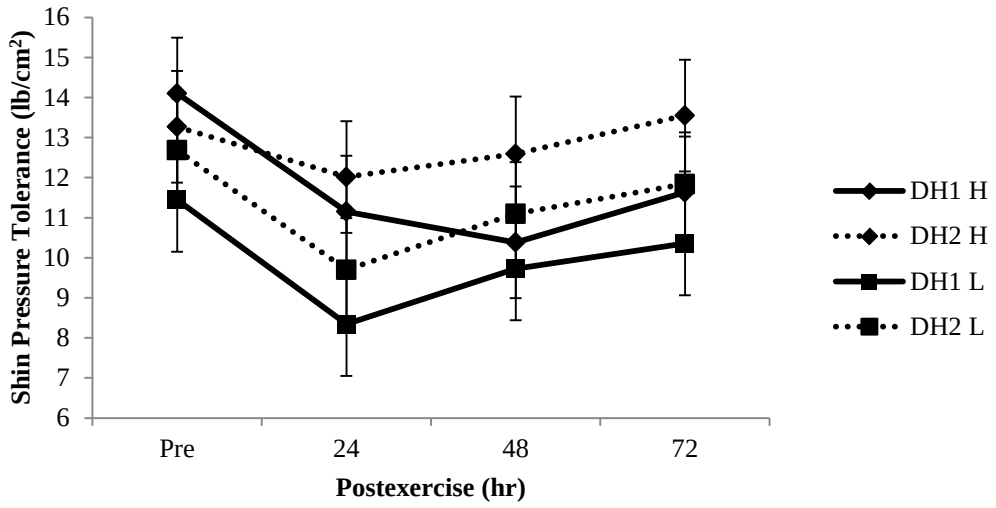

Figure 2. Pressure tolerance of the shin for the first (DH1) and second (DH2) downhill runs. Values are X±SEM. H=high estrogen group; L=low estrogen group.

Pressure tolerance for the calf is shown in Figure 3. A run effect was observed such that pressure tolerance was lower following DH1 (9.1 ± 0.7 lb/cm^2) than DH2 (10.0 ± 0.7 lb/cm^2) (P = 0.02).

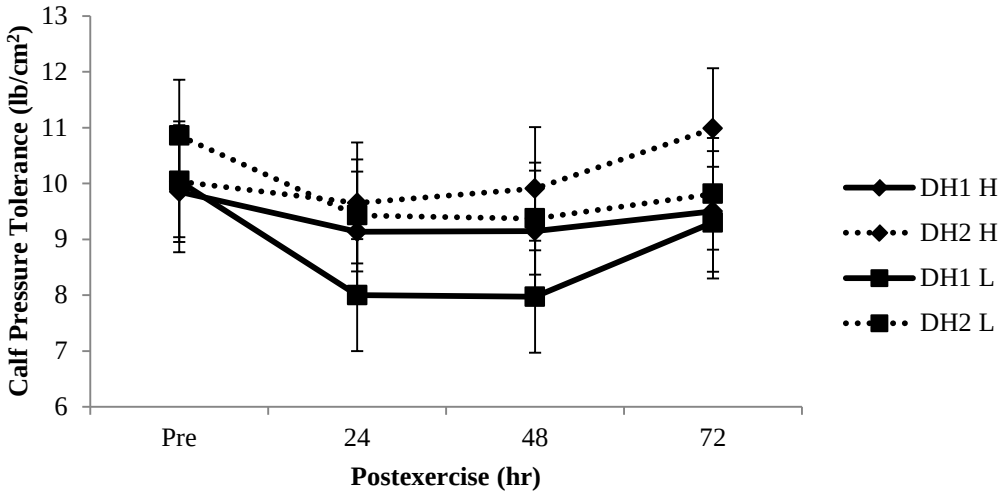

Figure 3. Pressure tolerance of the calf for the first (DH1) and second (DH2) downhill runs. Values are X±SEM. H=high estrogen group; L=low estrogen group. Pressure tolerance was lower for DH1 than DH2 (P = 0.02).

4.2 Perceived Soreness

Muscle soreness of the front thigh is shown in figure 4. A significant run effect was observed. Front thigh muscle soreness was lower after DH2 (2.5 ± 0.2) than DH1 (3.2 ± 0.2) (P = 0.05).

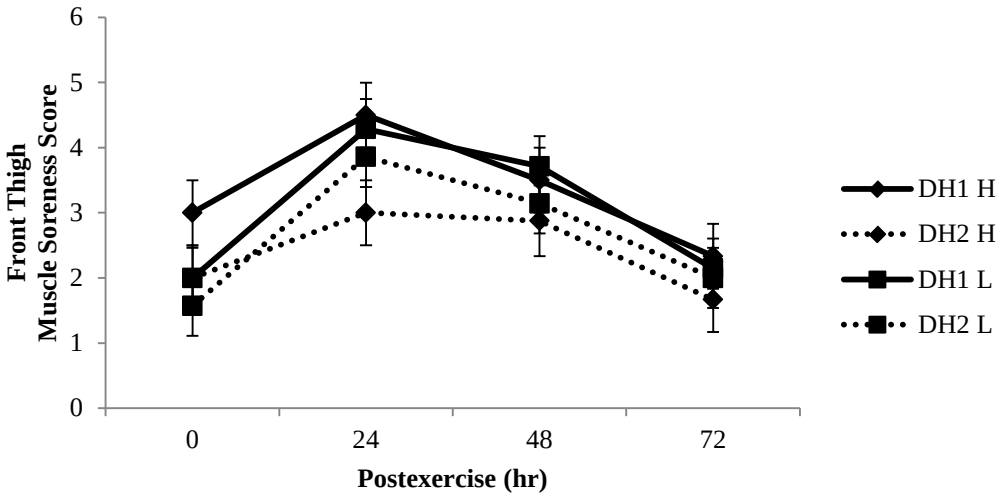

Figure 4. Perceived soreness of the front thigh for the first (DH1) and second (DH2) downhill runs. Values are X±SEM. H=high estrogen group; L=low estrogen group. Soreness was higher for DH1 than DH2 (P = 0.05).

Muscle soreness of the shin is shown in figure 5. There was a significant group x time interaction (P = 0.05). LE had increased shin soreness at 24 (3.0 ±0.4) (P < 0.001) and 48 hr (2.6 ± 0.4) (P < 0.001) compared to 0 hr (1.0 ± 0.4) and 72 hr (1.5 ± 0.4) (24 hr: P < 0.01; 48 hr: P = 0.04).

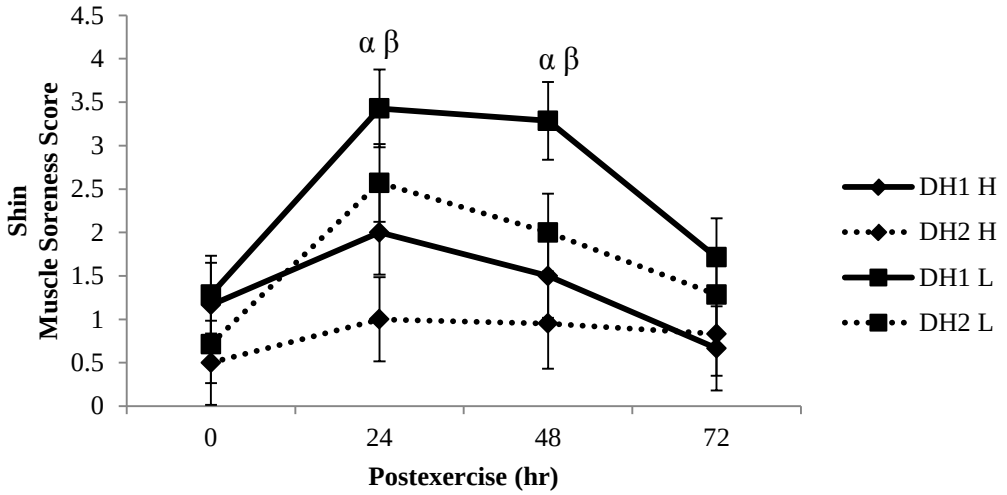

Figure 5. Perceived soreness of the shin for the first (DH1) and second (DH2) downhill runs. Values are X±SEM. H=High estrogen Group; L=low estrogen group. α Higher than 0 hr in L. β Higher than 72 hr in L.

Muscle soreness of the calf is shown in Figure 6. There were no significant interactions or main effects.

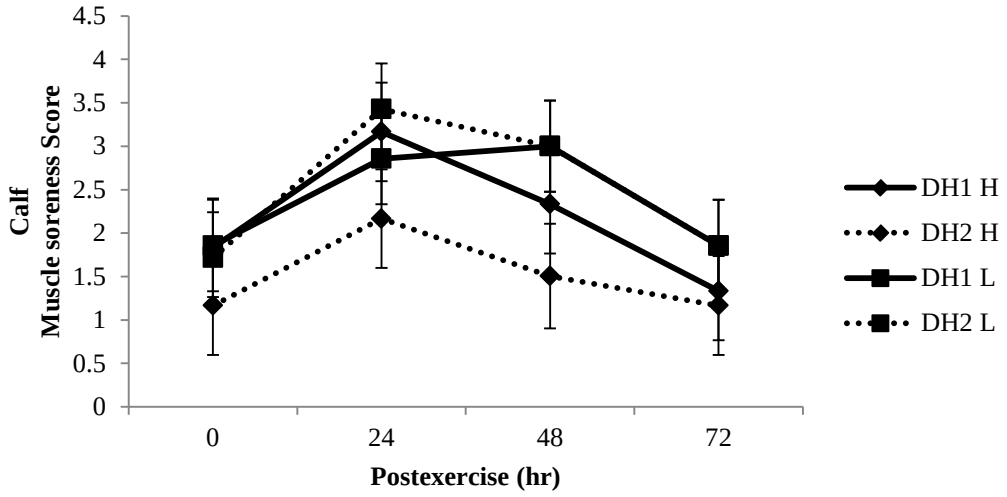

Figure 6. Perceived soreness of the calf for the first (DH1) and second (DH2) downhill runs. Values are X±SEM. H=high estrogen group; L=low estrogen group.

4.3 CK Activity

CK activity is shown in Figure 7. A significant run x time interaction was observed. Compared to pre (76.1 ± 44.8 U/L), CK activity following DH1 was higher at 24 hr (433.5 ± 44.8 U/L) ($P < 0.001$), 48 hr (224.4 ± 44.8 U/L) ($P = 0.04$), and 72 hr (230.8 ± 44.8 U/L) ($P = 0.03$), with 24 hr also higher than 48 hr ($P < 0.001$) and 72 hr ($P < 0.01$). CK activity at 24 hr following DH2 (202.4 ± 44.8 U/L) was lower than at 24 hr following DH1 ($P < 0.001$).

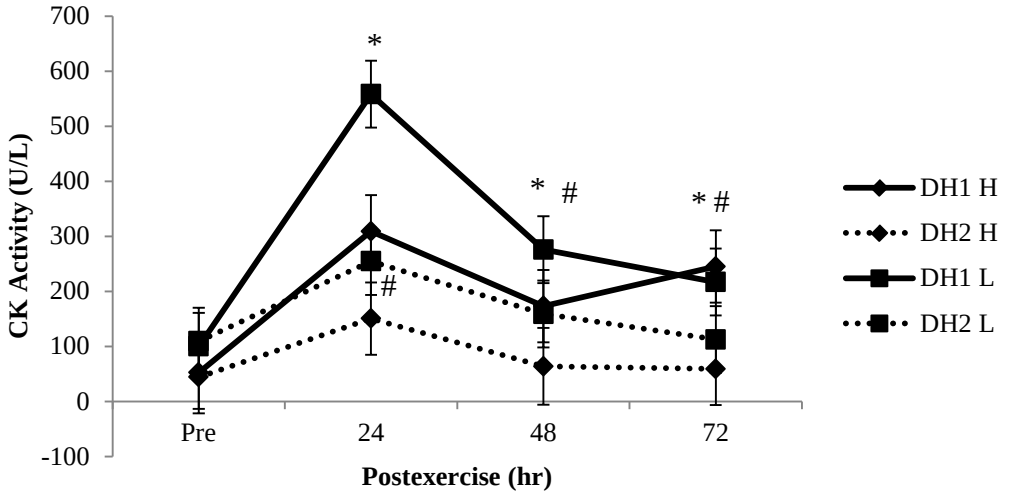

Figure 7. Creatine Kinase(CK) activity for the first (DH1) and second (DH2) downhill runs. Values are X±SEM. H=high estrogen group; L=low estrogen group. * Higher than 0 hr in DH1. # Lower than 24 hr DH1.

4.4 Muscle Circumference

Circumference measurements of the thigh and calf are shown in Figure 8 and 9, respectively. No significant changes were noted for thigh or calf circumference.

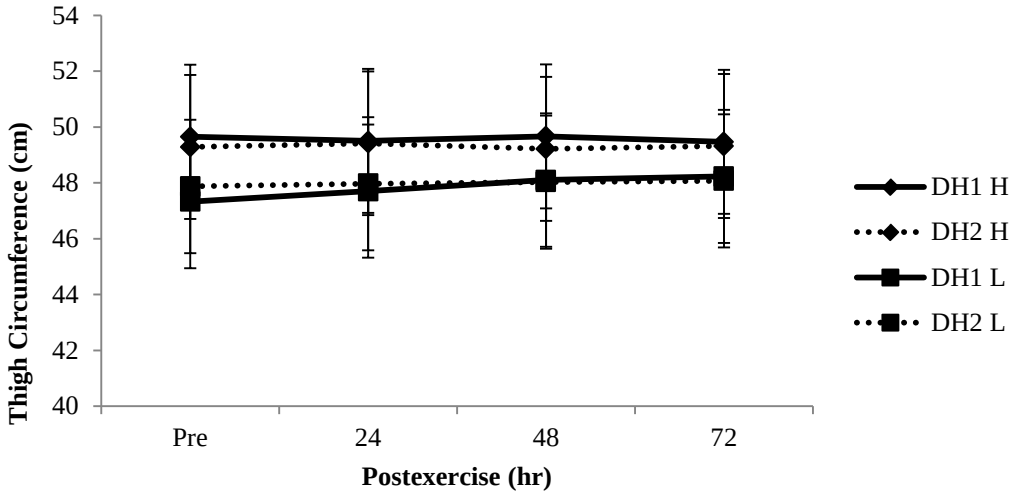

Figure 8. Circumference of the thigh for the first (DH1) and second (DH2) downhill runs. Values are X±SEM. H=high estrogen group; L=low estrogen group.

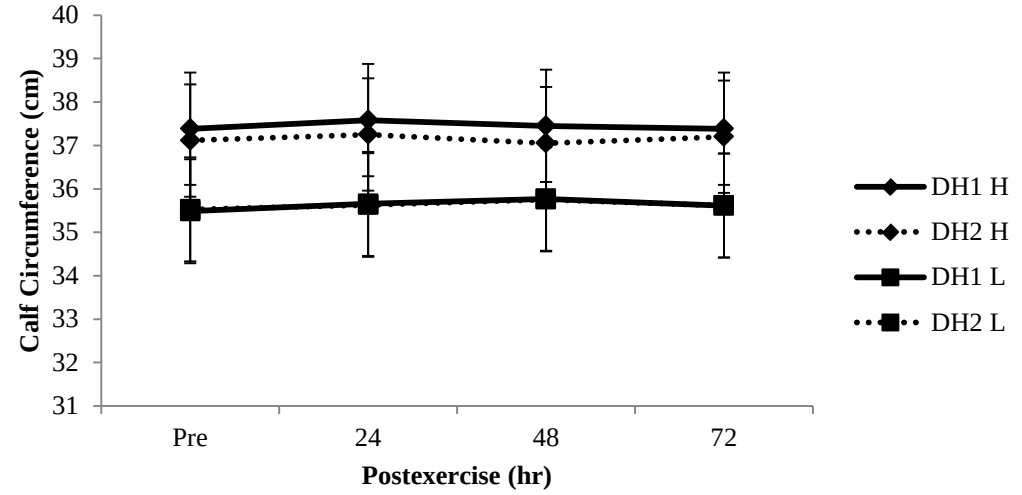

Figure 9. Circumference of the calf for the first (DH1) and second (DH2) downhill runs. Values are X±SEM. H=high estrogen group; L=low estrogen group.

4.5 Peak Torque

Peak torque for knee extension and ankle dorsiflexion is shown in Figures 10 and 11, respectively. No significant changes, main effects, or interactions were observed.

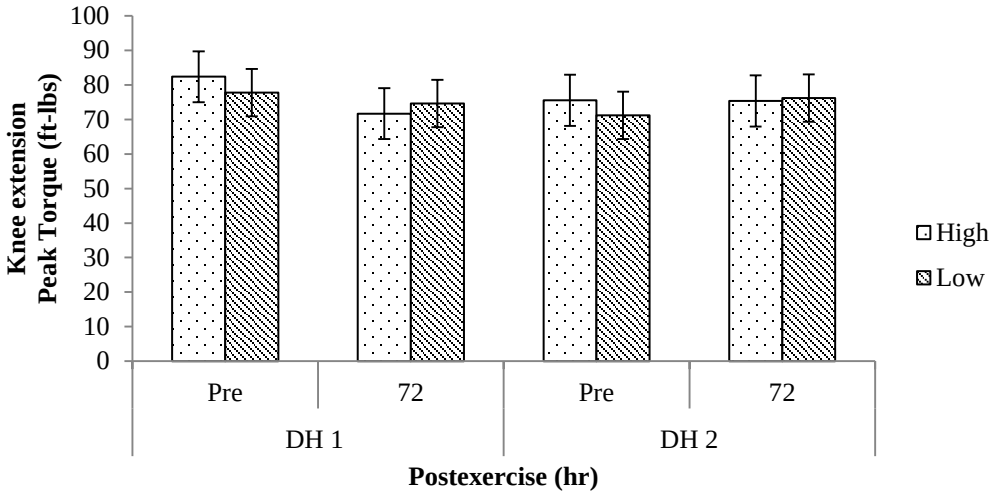

Figure 10. Peak torque for knee extension for the first (DH1) and second (DH2) downhill runs. Values are X±SEM. High=high estrogen group; Low=low estrogen group.

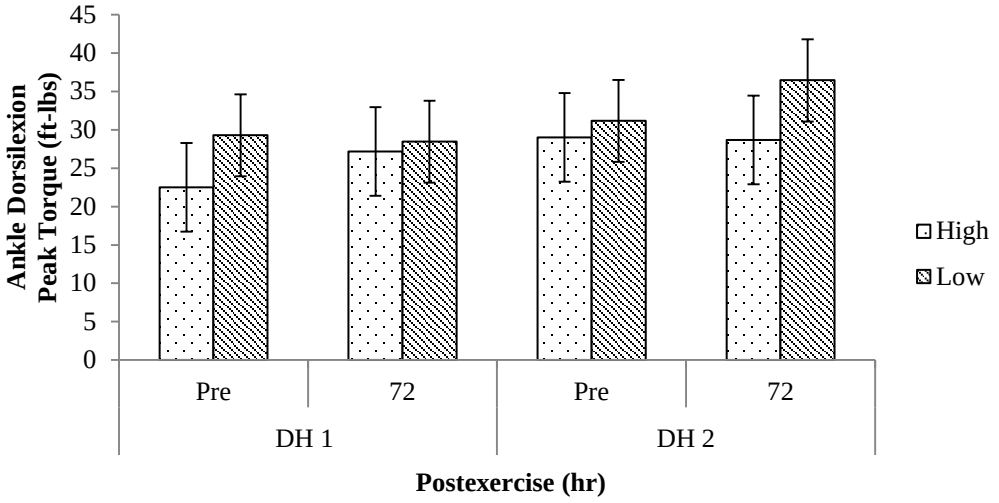

Figure 11. Peak torque for ankle dorsiflexion for the first (DH1) and second (DH2) downhill runs. Values are X±SEM. High=high estrogen group; Low=low estrogen group.

CHAPTER 5. DISCUSSION

Increased tenderness (Eston et al. 2000) and muscle soreness (He et al. 2015), which are reflective of exercise-induced muscle damage, generally occur following the first exposure to an eccentrically-biased exercise such as downhill running. In the present study, increased muscle soreness and CK activity were observed following the initial bout of exercise. Hence, the exercise protocol was successful in inducing markers of muscle damage in our women participants.

Because it has been suggested that estrogen may influence muscle damage and recovery through its properties as a membrane stabilizer, antioxidant, or gene regulator (Kendall and Eston 2002), the first aim of this study was to determine if estrogen levels at the time of exercise influenced the ensuing muscle soreness and CK response following unaccustomed eccentric exercise. In the present study, women with lower estrogen showed greater perceived muscle soreness in the shin than women with higher estrogen, providing partial support for the hypothesis. Thus, the higher concentration of estrogen in HE may have attenuated the muscle soreness response following the first exercise bout. Estrogen, as a fat-soluble hormone, may interact with the cell membrane to stabilize the membrane by decreasing fluidity and lipid peroxidation and therefore reduce damage (Kendall and Eston 2002).

Increased plasma/serum CK activity is observed following muscle-disrupting activities (He et al. 2015, Eston et al. 2000). In the present study CK activity increased following the initial bout of exercise, but no differences were detected between the groups. These results are not consistent with previous animal (Bar et al. 1988, Tiidus et al. 2001) and human (Carter et al. 2001) studies where attenuated CK responses in higher estrogen groups were reported. Due to CK activity's variability between subjects, the sample size of this study may not have been large enough to detect a significant difference as was the case for Stupka et al. (2000).

Decreased muscle performance is an indirect marker of muscle damage (Radaelli et al. 2014, Jakeman and Eston 2013). No changes in peak torque for knee extension or ankle dorsiflexion were observed in the present study. Although decreased force production is known to persist for as long as three days following downhill running (Eston et al. 2000), it is possible that if our women experienced any changes in peak torque, recovery may had occurred such that changes in peak torque were not detected at 72 hr postexercise when these measurements were made. Because all of our participants were female, and most likely had higher estrogen concentrations than males, it may be that recovery occurred sooner than participants in the study by Eston et al (2000) who were predominately men.

An inflammatory response occurs following muscle damage, and often results in swelling of the affected area. This acute-phase immune response is initiated in order to clean up debris, but the process itself may cause more damage through the release of ROS. Estrogen's antioxidant properties may help neutralize ROS, thus reducing secondary damage (Sugioka et al. 1987) and perhaps the ensuing swelling.

Increases in circumference have been shown following various anaerobic isolated muscle eccentric exercises (Radaelli et al. 2014, Nosaka et al. 2002, Savage and Clarkson 2002), but not after aerobic bench stepping exercises (Thompson et al. 1997). It is possible that swelling from muscle damage following aerobic exercise (in contrast to isolated muscle group exercise) may be dispersed over a larger area such that circumference measurements are not adequately sensitive to detect significant changes and may be why no significant change in circumference measurements was noted in the present study.

The RBE is characterized by attenuated markers of muscle damage following a second bout of the same exercise (Byrnes et al. 1985). As evidenced by Chen (2006), muscle damage following the initial bout must be substantial enough to initiate an adaptive response so that the RBE becomes evident. While, mechanisms of the RBE are not thoroughly understood, it has been theorized that changes in muscle fiber recruitment (number or type), muscle cytoskeleton, and/or the muscle cell membrane may be involved in the RBE (McHugh 2003). Estrogen receptor mediated pathways may influence the inflammatory and regeneration response (Velders et al. 2012, Enns et al. 2008). It is possible that higher estrogen levels may enhance the recovery process through increased activity of certain pathways, such as satellite cell activation (Velders et al. 2012). The second aim of this study was to determine if estrogen levels at the time of exercise would alter markers of the RBE. In general, pressure tolerance, muscle soreness, and CK activity were attenuated following the second downhill run, indicating that the RBE was evident in our women participants. Contrary to the hypothesis, however there was no difference in the RBE response

between the groups. To our knowledge, this is the first study to examine the effect of estrogen on the RBE in women, thus estrogen's role in the recovery process following muscle damage in women needs further investigation.

There are several limitations of this study that could have impacted the outcomes. The difference in estrogen concentration between the two groups of women in this study was not as large as in several previous reports (Thompson et al. 1997, Carter et al. 2001). Therefore, a different sampling technique that would have resulted in a larger difference in estrogen between our groups may have increased our ability to detect changes in some of the markers of muscle damage. Armstrong (1990) noted that soreness tended to localize in the distal end of the muscle. Whether this is due to most of the damage occurring in this area or due to pain receptors being localized there, examining pressure tolerance and circumference in the distal end of the muscle may have led to a more robust response. Finally, increasing our sample size would have provided more statistical power, enhancing our ability to detect an estrogen effect if there indeed is one.

In conclusion, it was hypothesized that markers of muscle damage in women with higher estrogen concentrations would be attenuated following both an initial exercise bout and a second bout compared to women with lower estrogen. Results provide evidence that higher estrogen levels at the time of exercise may mitigate markers of muscle damage following an initial bout of exercise, but higher estrogen does not seem to impact markers of the RBE in moderately-trained women.

5.1 Recommendations for Future Research

It is unknown what impact there would be on markers of the RBE if the first bout of muscle damaging exercise is performed when estrogen concentration is high and the second bout is performed when it is low and vice versa. Future studies alternating estrogen levels between bouts of exercise would increase our understanding on the relationship of estrogen levels and the RBE.

Many of the studies examining estrogen's role in muscle protection have compared HC users to nonusers (Thompson et al. 1997, Cater et al. 2001, Savage and Clarkson 2002). In order to remove the factor of exogenous estrogen and progesterone, future research using only non-HC users should be explored in order to determine if the results would support the findings of this study.

While the exact mechanism by which estrogen protects the muscle from damage is unknown, previous research in rats (Bar et al. 1988) showed that some time was needed for estrogen to have a protective effect, suggesting gene regulation may first need to occur. Research exploring the role of estrogen receptors on the muscle cell nucleus indicated that estrogen receptor-β may play a significant role in protection from muscle damage (Velders et al. 2012). Future research focusing on this receptor and its subsequent downstream signaling may elucidate the mechanisms involved in estrogen's protective role against muscle damage.

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APPENDICES

Appendix A Menstrual, and Physical Activity History

Medical History Survey

Name: _____ Local phone no: _____
Local address: _____
Birth date: _____ Age: _____ Sex: _____
Weight: _____ Height: _____

1. Have you ever been diagnosed as having: (check all that apply)

	Never	In past	Presently
Heart Disease	_____	_____	_____
Rheumatic Fever	_____	_____	_____
High blood Pressure	_____	_____	_____
Other vascular disorders	_____	_____	_____
Diabetes	_____	_____	_____
Kidney Disease	_____	_____	_____
Asthma	_____	_____	_____
Allergies	_____	_____	_____
Chronic bronchitis	_____	_____	_____
Other respiratory illness	_____	_____	_____
High serum lipids	_____	_____	_____
Osteoporosis	_____	_____	_____
Other bone disease	_____	_____	_____
Neuromuscular-skeletal disease	_____	_____	_____

2. Please indicate any surgery that you have undergone and the approximate date(s):

3. Please indicate recent illnesses or major injuries you have had approximate dates:

4. Please list any and all medications that you are presently taking:

Medication	Dosage	Dosage per day
_____	_____	_____
_____	_____	_____

5. Do you smoke? _____ Packs per day?_____

6. Describe your present training program (the activity, amount/day, days/week, and length of time you have been training at this level)

Activity	Minutes/Day	Day/week	Weeks of training
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Menstrual Cycle History Questionnaire

Name: _____
Date of Birth: _____

Menstrual History:

1. In recent months, what is the usual length of time from the beginning of your period to the beginning of your next period?

< 21 days _____
21 – 23 days _____
24 – 26 days _____
27 – 29 days _____
30 – 32 days_____
33 – 35 days_____
> 35 days _____

2. A. Disregarding the first 18 months immediately following your first period have you ever skipped a menstrual period? (If answer is "no" go to question #3)

Yes ____
No _____

B. If answer is yes, approximately how old were you when missing a menstrual cycle?_____

C. When was the last time a menstrual cycle was missed? (How many years/months ago?)_____

D. How long did this irregularity continue? _____

3. How many periods have you had in the past year?

3 or less ____
4 – 7 ____
8 – 12 ____

4. How many periods have you had in the last 6 months?

1 or none ____
2 – 4 _____
5 – 6 _____

5. A. Have you ever used hormonal contraceptives?

Yes ___
No ___

B. If yes, when was the last time you used them?

Presently ____
Within the last 6 months____
More than 6 months ago____

6. At what time during each month does you menstrual cycle typically begin?

Approximately the ____ day of the month

7. What to you weigh at present? _____ pounds

8. What did you weigh 6 months ago? _____ 1 year ago? _____

Paffenbarger Physical Activity Questionnaire

1. How many city blocks or their equivalent do you normally walk each day?
_____ Blocks/day (let 12 blocks=1mile)

2. What is your usual pace of walking? (please check one)
a.____ Casual or strolling (less than 2 mph) b. ____Average or normal (2 to 3 mph)
c. ____Fairly brisk (3 to 4 mph) d. ____Brisk or striding (4 mph or faster)

3. How many flights of stairs do you climb up each day? _____ Flights/day (let 1 flight=10 steps)

4. List any sports or recreation you have actively participated in during the past year. Please remember seasonal sports or events.

Sport, Recreation, other Physical Activity	Number of times/year	Average Time/Episode		Years Participation
		Hours	Minutes	
a.				
b.				
c.				
d.				
e.				
f.				

5. Which of these statements best expresses your view? (Please check one.)
a. ___I take enough exercise to keep healthy
b. ___ I ought to take more exercise
c. ___Don't know

6. At least once a week, do you engage in regular activity akin to brisk walking, jogging, bicycling, swimming, etc. long enough to work up a sweat, get your heat thumping, or get out of breath?

___No Why not?_____
___Yes How many times per week?___
Activity:_____

7. When you are exercising in your usual fashion, how would you rate your level of exertion (degree of effort)? (please circle one number.)

0	0.5	1	2	3	4	5	6	7	8	9	10
Normal	Very very week (just noticeable)	Very weak	Weak	Moderate	Some what strong	Strong (heavy)		Very strong		Very very strong (almost Maximal)	Maximal

8. On a usual weekday and a weekend day, how much time do your spend on the following activity? Total for each day should add up to 24 hours.

	Usually Weekday Hours/Day	Usual Weekend Day Hours/Day
a. Vigorous activity (digging in the garden, strenuous sports, jogging, aerobic dancing, sustained swimming, brisk walking, heavy carpentry, bicycling on hills,etc.)		
b. Moderate activity (housework, light sports, regular walking, golf, yard work, lawn mowing, painting, repairing, light carpentry, ballroom dancing, bicycling on level ground, etc.)		
c. Light activity (office work, driving car, strolling, personal care, standing with little motion, etc.)		
d. Sitting activity (eating, reading, desk work, watching TV, listening to radio, etc.)		
e. Sleeping or reclining		

Appendix B Delayed Onset of Muscle Soreness Questionnaire

Delayed onset of muscle soreness questionnaire

Subject: Test:

Rate of Scale (1-6)

0=complete absence of soreness

2=right pain felt only on palpation

4=moderate pain, some stiffness and / or weakness, especially during movement

6=severe pain that limits the range of motion

Immediately after exercise

Buttocks	(0	1	2	3	4	5	6)
Front of thigh	(0	1	2	3	4	5	6)
Back of thigh	(0	1	2	3	4	5	6)
Shin	(0	1	2	3	4	5	6)
Calf	(0	1	2	3	4	5	6)

24 hours after exercise

Buttocks	(0	1	2	3	4	5	6)
Front of thigh	(0	1	2	3	4	5	6)
Back of thigh	(0	1	2	3	4	5	6)
Shin	(0	1	2	3	4	5	6)
Calf	(0	1	2	3	4	5	6)

48hours after exercise

Buttocks	(0	1	2	3	4	5	6)
Front of thigh	(0	1	2	3	4	5	6)
Back of thigh	(0	1	2	3	4	5	6)
Shin	(0	1	2	3	4	5	6)
Calf	(0	1	2	3	4	5	6)

72 hours after exercise

Buttocks	(0	1	2	3	4	5	6)
Front of thigh	(0	1	2	3	4	5	6)
Back of thigh	(0	1	2	3	4	5	6)
Shin	(0	1	2	3	4	5	6)
Calf	(0	1	2	3	4	5	6)

Appendix C Informed Consent

RESEARCH PARTICIPANT CONSENT FORM
Estrogen and Muscle Soreness
Dr. Darlene Sedlock
Purdue University
Department of Health & Kinesiology

Purpose of Research

After you exercise in a way you are not accustomed to, your muscles often become sore a day or two later and then get better within the next few days. The soreness is a result of the body's response to microdamage in the muscles from the exercise. If you then do that same exercise again, after this second bout you won't be nearly as sore if at all. Research has shown that women tend to experience less muscle soreness than men after the first bout of an unaccustomed exercise. This is thought to be due to the different levels of estrogen between men and women. However, estrogen's effect on muscle soreness following a second bout of exercise is unknown. Thus, we are doing this study to see if estrogen levels at the time of doing some unaccustomed exercise (in this case the exercise is running downhill) will reduce muscle soreness and markers of muscle damage following both bouts of exercise. You are being asked to participate because you are a regular exerciser who may occasionally experience muscle soreness.

Specific Procedures

You will first be asked to complete some preliminary requirements to see if you qualify for the study. These requirements include completing some questionnaires related to your menstrual, medical, and health histories, and physical activity participation. We will measure your height, weight, resting heart rate and resting blood pressure, and assess your aerobic fitness with an exercise test (VO2max). For the exercise test, you will be wearing a mouthpiece attached to a breathing valve which lets you breathe in room air while your exhaled air is directed to a machine that performs some measurements on the air. We will also monitor your heart rate by having you put a strap around your chest. The exercise will be performed on a treadmill. Initial speed will be 3.5 mph. Speed will increase by 1 mph every three minutes up to 6.5 mph, after which the grade will increase by 2% every two minutes without a change of speed. You will be asked to give a maximum effort on this test. If you do not qualify for the study based on these preliminary tests, you will be involuntarily withdrawn from the study.

If you qualify for the study based on the questionnaires and exercise test, you will be asked to perform a 40 minute downhill (-10%) run on the treadmill at a specific time during your menstrual cycle. Approximately one month later (at the same time during

your menstrual cycle) you will do the same run. For these exercise trials you will have to fast overnight and refrain from intense exercise for 2 days prior to the runs. The trials will be in the morning.

When you report to the lab, your height and weight will be measured. This will be followed by a 20 min rest after which we will take some blood (~ 1 tablespoon) from an arm vein, measure the circumference of your thigh and calf, test your leg strength, and check for any leg muscle soreness by using an instrument that applies pressure to your leg muscles (similar to poking a finger in the muscle). Then we will attach the same heart rate chest strap and device for the mouthpiece as we did during the VO2max test and begin the exercise: after a 3 minute warm-up you will run downhill for 40 min. In the morning during the next three days (at 24, 48, and 72 hr after the exercise) you will come to the lab and rate any muscle soreness using a 0-6 rating scale, have another blood sample taken (~ 1 teaspoon) and have your leg circumference and muscle soreness (pressure) measurements taken again. During the 72 hr postexercise visit we will also measure your leg strength once more.

Approximately four weeks after the first downhill run you will perform the second downhill run. The exercise, blood sampling procedures, muscle soreness ratings, leg circumference, leg strength, and leg soreness (pressure) measurements for this run will be done in the same way as for the previous run.

Duration of Participation

The preliminary requirements will take a total of approximately one hour, i.e, about 15 min for questionnaires and about 45 min for the exercise test. Each exercise session will take approximately 1.25 - 1.5 hours. The blood draws and other measurements at 24, 48 and 72 hr after exercise will take ~15 min each. Muscle soreness ratings will take a few minutes each. Therefore, the total time commitment will be about 5.5 hours over the approximately 9 week study duration.

Risks

Risks associated with this study are mainly associated with the exercises and with blood sampling. Because you will be engaging in a VO_2max test (aerobic fitness) as well as 2 treadmill runs you will be stressing your cardiovascular system throughout the exercise periods. Thus, there exists the possibility of risks and discomfort during the exercise tests such as: abnormal blood pressure, fainting, irregular, fast or slow heart rhythm, and in rare instances heart attack, stroke or death. However, you must meet specific criteria to participate in the study, i.e., you are already a regular exerciser. In addition you will complete a health history questionnaire prior to the study to ensure that you don't have any risk factors associated with cardiovascular disease. There is also a slight risk of losing your balance when running downhill. To minimize this, your exercise will be monitored and you will be given verbal instructions to move forward, backward, or to the center of the treadmill if necessary. Someone involved with the study will also be by the treadmill while you are running. You will probably experience some degree of muscle soreness following the downhill run. Additionally, it is possible that there also may be some swelling in the leg muscles. Every effort will be made to

promote your safety by carefully monitoring the equipment, your measurements, and you during all tests. Test technicians are trained in exercise testing, are CPR and AED certified, and knowledgeable in obtaining Emergency Medical Services. Emergency equipment is in close proximity to the laboratory.

You may experience some discomfort (pain) when having your blood drawn. There is also a risk of fainting, and a remote risk of infection and/or bruising when sampling blood. These risks will be minimized by having your blood drawn by an individual trained in these procedures.

Finally, there is a risk of breach of confidentiality. This risk will be minimized by using procedures outlined below in the confidentiality section.

Benefits

There are no anticipated direct benefits to you by participating in the study. However, you will receive information about your fitness level, estrogen levels and cardiovascular system responses to exercise. Additionally, as a regular exerciser who might occasionally experience exercise-induced muscle soreness, you may benefit from information derived from this study.

Please feel free to consult with your physician for interpretation of any information you receive from participating in this study.

Compensation

You will receive $50 for participation in this study upon completion of the requirements. There is no compensation for partial completion of the requirements.

Injury or Illness

Purdue University will not provide medical treatment or financial compensation if you are injured or become ill as a result of participating in this research project. This does not waive any of your legal rights nor release any claim you might have based on negligence.

Confidentiality

All of your data will be coded and not associated with your name. Data will be kept until three years after the last presentation or publication of the results, and will be located in a file cabinet in a locked room in Lambert Bldg that is available only to staff associated with the study. The code linking your name with your data will be destroyed at the same time as the other data. Electronic records will be kept on a password-protected drive and are accessible only by staff associated with the study. You may have access to your data upon request. Blood samples will be kept for a maximum of two years from the completion of the study.

The project's research records may be reviewed by departments at Purdue University responsible for regulatory and research oversight.

Voluntary Nature of Participation

You do not have to participate in this research project. If you agree to participate you can withdraw your participation at any time without penalty.

Contact Information

If you have any questions about this research project, you can contact Dr. Darlene Sedlock (765-494-3184, sedlock@purdue.edu) or Jennifer Hockemeyer (260-385-4428, kruseja@purdue.edu). If you have concerns about the treatment of research participants, you can contact the Institutional Review Board at Purdue University, Ernest C. Young Hall, Room 1032, 155 S. Grant St., West Lafayette, IN 47907-2114. The phone number for the Board is (765) 494-5942. The email address is irb@purdue.edu.

Documentation of Informed Consent:

I have had the opportunity to read this consent form and have the research study explained. I have had the opportunity to ask questions about the research project and my questions have been answered. I am prepared to participate in the research project described above. I will receive a copy of this consent form after I sign it.

_____ _____

Participant's Signature Date

_____

Participant's Name

_____ _____

Researcher's Signature Date

Appendix D Raw Data

Raw data set 1: Estradiol (pg/ml)

High Estrogen Group (H)

Sub ID	D1PRE	D2PRE
Sub 1	59.68	45.15
Sub 6	195.75	15.54
Sub 9	45.05	22.97
Sub 10	49.23	99.74
Sub 18	112.56	102.43
Sub 22	73.02	80.23

Low Estrogen Group (L)

Sub ID	D1PRE	D2PRE
Sub 3	21.27	41.95
Sub 5	49.32	32.59
Sub 15	12.46	13.80
Sub 16	84.91	103.52
Sub 19	33.43	42.23
Sub 20	49.39	69.01
Sub 23	60.52	18.20

Raw data set 2: Front thigh pressure tolerance (lb/cm^2)

High Estrogen Group (H)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 1	9.9	11.8	10.1	9.8	11.1	13.3		13.0
Sub 6	10.8	8.3	9.1	8.1	8.4	7.9	8.9	9.5
Sub 9	16.9	11.4	13.0	14.7	17.8	13.2	17.2	17.2
Sub 10	15.1	12.8	12.4	12.8	13.1	11.3	12.9	12.8
Sub 18	10.5	8.7	7.4	8.4	10.3	10.2	10.1	10.8
Sub 22	5.8	4.8	5.9	5.6	6.7	4.9	4.9	5.9

Low Estrogen Group (L)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 3	10.8	9.7	10.3	9.7	12.4	12.3	12.3	10.3
Sub 5	8.3	7.1	5.9	6.1	10.2	8.4	8.5	8.5
Sub 15	7.6	5.0	4.9	5.1	8.6	6.1	6.3	6.1
Sub 16	10.1	10.7	10.2	12.9	15.4	9.3	9.6	11.7
Sub 19	7.4	4.5	4.6	5.4	5.6	5.8	5.8	6.3
Sub 20	9.5	8.7	8.3	7.5	9.1	8.4	8.5	10.2
Sub 23	14.7	8.5	8.9	10.4	11.6	10.3	10.3	12.8

Raw data set 3: Shin pressure tolerance (lb/cm^2)

High Estrogen Group (H)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 1	11.8	12.1	10.9	11.0	12.5	12.1		12.8
Sub 6	16.0	11.9	9.4	9.2	12.3	12.4	10.8	11.9
Sub 9	19.8	13.1	13.5	16.3	22.0	17.7	22.8	21.7
Sub 10	15.2	15.2	11.7	14.9	13.1	13.5	11.8	14.5
Sub 18	11.8	8.5	9.5	10.5	11.3	9.5	12.5	13.0
Sub 22	10.0	6.1	7.3	7.9	8.4	6.9	5.5	7.4

Low Estrogen Group (L)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 3	10.7	9.8	10.5	13.1	21.0	11.9	17.1	19.7
Sub 5	9.7	7.1	7.4	8.1	11.6	9.1	11.3	9.0
Sub 15	10.9	8.9	14.2	10.7	11.5	11.0	12.4	9.3
Sub 16	14.5	9.4	11.7	13.9	14.8	7.9	9.6	12.2
Sub 19	10.8	7.0	6.0	6.1	7.6	6.9	7.4	8.4
Sub 20	12.7	7.9	8.7	9.4	10.7	10.2	9.2	12.6
Sub 23	10.8	8.3	9.6	11.2	11.6	10.9	10.7	11.7

Raw data set 4: Calf pressure tolerance (lb/cm^2)

High Estrogen Group (H)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 1	7.0	8.0	7.9	7.1	8.6	8.0		9.5
Sub 6	9.9	8.7	8.1	8.8	8.7	8.4	7.3	8.7
Sub 9	12.7	11.7	12.6	14.1	14.9	13.3	16.2	18.5
Sub 10	12.6	11.5	11.8	12.5	9.5	10.8	12.1	13.3
Sub 18	9.8	8.7	8.4	8.4	10.8	10.5	9.9	9.4
Sub 22	7.1	6.2	6.1	6.1	7.7	6.9	5.6	6.5

Low Estrogen Group (L)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 3	9.3	8.9	8.3	9.4	14.2	11.0	10.0	11.5
Sub 5	7.9	6.7	4.9	6.0	8.6	7.5	7.7	7.9
Sub 15	8.1	7.8	6.0	8.8	9.0	8.7	8.9	7.7
Sub 16	15.8	9.1	11.9	13.5	14.4	10.1	12.2	11.4
Sub 19	8.5	5.4	5.7	6.0	7.6	6.7	6.7	7.0
Sub 20	10.8	10.3	10.0	11.6	11.3	11.7	10.7	11.9
Sub 23	9.9	7.8	9.0	9.8	10.9	10.3	9.4	11.3

Raw data set 5: Front thigh muscle soreness score

High Estrogen Group (H)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 1	4	5	4	3	4	3		2
Sub 6	3	5	3	1	2	4	0	0
Sub 9	4	4	2	2	3	3	4	2
Sub 10	2	4	3	2	2	2	3	3
Sub 18	3	5	6	4	0	2	3	1
Sub 22	2	4	3	2	1	4	4	2

Low Estrogen Group (L)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 3	3	5	5	2	1	4	3	2
Sub 5	2	5	4	3	2	4	3	4
Sub 15	2	2	1	1	1	4	5	4
Sub 16	1	5	5	3	0	5	5	3
Sub 19	3	5	3	2	2	3	2	1
Sub 20	1	2	2	2	2	3	2	0
Sub 23	2	6	6	2	3	4	2	0

Raw data set 6: Shin muscle soreness score

High Estrogen Group (H)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 1	2	1	1	1	1	0		0
Sub 6	0	1	1	0	0	2	0	0
Sub 9	2	4	3	1	1	2	2	2
Sub 10	2	4	3	2	1	1	2	2
Sub 18	0	1	0	0	0	0	0	0
Sub 22	1	1	1	0	0	1	1	1

Low Estrogen Group (L)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 3	3	1	2	1	0	3	2	0
Sub 5	1	3	4	3	2	3	2	2
Sub 15	2	5	5	3	1	0	1	1
Sub 16	0	5	5	2	0	5	3	3
Sub 19	0	4	4	3	1	2	3	2
Sub 20	2	2	0	0	0	2	1	1
Sub 23	1	4	3	0	1	3	2	0

Raw data set 7: Calf muscle soreness score

High Estrogen Group (H)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 1	2	1	1	1	2	1		1
Sub 6	1	4	2	1	1	1	1	1
Sub 9	1	4	4	1	2	4	1	1
Sub 10	2	4	3	2	1	1	2	2
Sub 18	2	1	0	0	0	2	0	0
Sub 22	3	5	4	3	1	4	4	2

Low Estrogen Group (L)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 3	2	1	0	1	0	3	3	1
Sub 5	1	3	6	4	4	5	4	3
Sub 15	3	4	4	3	1	2	4	2
Sub 16	1	1	2	0	0	3	4	3
Sub 19	1	3	2	1	2	3	1	0
Sub 20	4	5	4	4	4	4	4	3
Sub 23	1	3	3	0	1	4	1	1

Raw data set 8: CK activity (U/L)

High Estrogen Group (H)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 1	32.3	124.9	42.9	62.6	34.9	40.1		26.6
Sub 6	34.4	133.8	73.8	70.1	57.9	127.9	64.1	43.6
Sub 9	39.2	238.2	225.6	328.6	52.0	71.3	48.1	39.2
Sub 10	49.5	742.7	328.1	315.5	25.7	104.2	50.3	42.5
Sub 18	111.8	382.6	235.1	454.2	61.7	487.3	172.3	152.5
Sub 22	46.1	231.2	133.4	238.3	32.6	71.3	49.4	49.4

Low Estrogen Group (L)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 3	44.4	330.1	188.5	179.8	78.8	151.9	95.0	75.3
Sub 5	234.5	382.9	185.5	138.2	213.9	255.0	146.9	141.5
Sub 15	128.3	983.6	501.0	274.1	142.0	337.1	252.0	142.8
Sub 16	38.1	170.2	98.8	58.4	36.0	85.2	44.4	44.6
Sub 19	39.2	122.5	82.6	192.7	32.6	56.1	58.0	71.7
Sub 20	139.3	860.2	452.2	495.9	186.9	647.5	336.9	217.2
Sub 23	75.9	1057.4	420.7	177.8	75.2	248.1	179.3	93.0

Raw data set 9: Thigh circumference (cm)

High Estrogen Group (H)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 1	57.2	56.3	56.0	56.3	56.7	56.6		56.5
Sub 6	45.9	45.5	48.1	45.7	45.7	45.2	45.0	45.1
Sub 9	48.2	48.4	48.3	48.4	48.2	48.7	48.8	49.3
Sub 10	49.8	50.3	49.7	50.0	48.7	49.5	48.8	48.9
Sub 18	47.2	47.1	46.9	47.6	47.9	47.7	47.5	47.5
Sub 22	49.6	49.4	49.0	48.8	48.5	48.8	48.8	48.6

Low Estrogen Group (L)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 3	45.7	46.1	46.6	48.6	46.3	47.4	46.9	47.4
Sub 5	42.4	42.7	43.2	43.0	42.7	42.8	42.7	43.2
Sub 15	39.2	39.9	41.0	40.9	40.1	40.2	40.6	40.0
Sub 16	45.6	45.5	45.6	45.8	45.4	46.2	46.0	46.9
Sub 19	44.9	44.7	45.1	45.1	46.8	45.6	46.3	45.1
Sub 20	49.9	50.6	51.0	50.3	49.8	49.3	48.9	49.2
Sub 23	63.6	64.4	64.2	63.9	64.0	64.3	64.8	64.7

Raw data set 10: Calf circumference (cm)

High Estrogen Group (H)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 1	42.1	42.0	41.6	41.6	41.4	41.2		41.4
Sub 6	36.7	36.9	36.7	36.9	35.2	35.6	35.2	35.6
Sub 9	37.4	38.0	37.3	37.4	37.3	37.6	37.5	37.5
Sub 10	35.1	35.8	35.5	35.4	35.5	35.8	35.3	35.8
Sub 18	34.8	34.6	34.8	34.5	35.1	35.5	34.9	35.2
Sub 22	38.2	38.2	38.8	38.5	38.2	37.8	38.2	37.7

Low Estrogen Group (L)

Sub ID	D1PRE	D1T24	D1T48	D1T72	D2PRE	D2T24	D2T48	D2T72
Sub 3	34.4	34.2	34.5	34.4	33.9	33.8	34.0	34.1
Sub 5	33.5	33.3	33.5	33.3	33.7	33.5	33.7	33.6
Sub 15	31.6	32.3	32.1	32.1	32.1	32.7	32.6	32.1
Sub 16	36.1	36.5	36.2	36.5	36.0	36.2	36.3	36.6
Sub 19	33.9	33.7	34.3	33.9	33.5	33.6	34.0	33.7
Sub 20	35.5	36.1	36.4	36.1	36.4	36.8	36.3	36.1
Sub 23	43.4	43.5	43.4	43.0	43.1	42.8	43.4	43.1

Raw data set 11: Knee extension peak torque (ft-lbs)

High Estrogen Group (H)

Sub ID	D1PRE	D1T72	D2PRE	D2T72
Sub 1	88	87	87	86
Sub 6	99	55	84	73
Sub 9	91	87	81	84
Sub 10	86	69	61	70
Sub 18	71	73	70	65
Sub 22	59	59	70	74

Low Estrogen Group (L)

Sub ID	D1PRE	D1T72	D2PRE	D2T72
Sub 3	91	98	97	92
Sub 5	69	75	60	88
Sub 15	64	48	48	46
Sub 16	52	57	41	53
Sub 19	55	67	65	68
Sub 20	100	84	79	90
Sub 23	113	93	108	96

Raw data set 12: Ankle dorsiflexion peak torque (ft-lbs)

High Estrogen Group (H)

Sub ID	D1PRE	D1T72	D2PRE	D2T72
Sub 1	21	36	39	36
Sub 6	19	21	19	27
Sub 9	30	30	33	24
Sub 10	21	24	20	18
Sub 18	39	43	51	41
Sub 22	5	9	12	26

Low Estrogen Group (L)

Sub ID	D1PRE	D1T72	D2PRE	D2T72
Sub 3	39	41	48	33
Sub 5	24	28	25	59
Sub 15	8	10	8	11
Sub 16	16	13	18	22
Sub 19	36	42	43	43
Sub 20	32	43	48	61
Sub 23	50	22	28	26