Protective mechanism of Estradiol on eccentrically induced muscle damage

Charles Lee Dumke
The University of Montana
Permission is granted by the author to reproduce this material in its entirety, provided that this material is used for scholarly purposes and is properly cited in published works and reports.

** Please check "Yes" or "No" and provide signature **

Yes, I grant permission
No, I do not grant permission

Author's Signature

Date

Any copying for commercial purposes or financial gain may be undertaken only with the author's explicit consent.
PROTECTIVE MECHANISM OF
ESTRADIOL ON ECCENTRICALLY
INDUCED MUSCLE DAMAGE

by:

Charles Lee Dumke

BS. University of Wisconsin-Madison

Madison, Wisconsin  1988

Presented in partial fulfillment of the
requirements for the degree of

Master of Science

The University of Montana

Missoula, Montana

summer, 1995

Approved by:

Chairperson

Graduate Dean

July 14, 1995

Date
The mechanisms that tie the descriptive parameters associated with delayed onset muscle soreness (DOMS), including an increase in serum creatine kinase (CK) activity, and subsequent damage to the contractile filaments of the myofibrils remain unknown. Females exhibit a lower serum CK activity post-exercise compared to males, this was later shown to be the effect of the female sex hormone estradiol (E2). The effects of E2 on CK release and muscle damage was assessed in female rats following eccentric treadmill exercise. Female rats were ovariectomized prior to sexual maturity and treated with an E2 (n=5) or placebo (n=4) pellet insert for 21 days prior to the exercise bout. Exercise consisted of one hour of downhill (-10°) treadmill running at a speed of 19 m/min. Serum samples for CK activity were obtained at pre, 0, 2, 6, 12, 24, and 48 hours post-exercise. The soleus muscle was prepared for light and electron microscopy 48 hours post-exercise. CK release in the placebo rats was significantly (p<.05) greater than pre CK levels at times 0, 2, 6, and 24 hours, with the peak response occurring at time 0. CK release in E2 rats was significant at time 0, and 2 hours post, with the peak response at 2 hours. Placebo rats exhibited greater CK activities at all time points except for 2 hours post-exercise. Microscopic damage was only located in two placebo treated animals, therefore it is difficult to speculate as to the precise protective mechanisms of E2. The effects of E2 seem to be limited to CK release and not directly related to that of myofibril damage. This supports the work of others who have suggested that the blood marker CK is not necessarily a direct measure of observed skeletal muscle damage.
ACKNOWLEDGMENTS

The author wishes to thank Dr. Brent Ruby for his continued patience and enthusiasm throughout this study. His energy is contagious and pulled me through many periods of ‘thesis doubt’. It is an honor to be his ‘first graduate’. Due to the large amount of interdepartmental sharing involved with this study the list of thanks for support is long, but nonetheless important. I would like to thank Dr. Keith Parker for his assistance with the estradiol assay. His unselfishness was inspiring and very much appreciated. Phil Bowman and the rest of the staff at the animal facility were constantly having to address my inane questions and I am thankful. Dr. Vernon Grund was kind enough to donate the use of his lab. Dean McGovern and Joe Harlan provided last minute assistance that may have saved this investigation. Dr. William Granath was kind enough to donate the use of his microscope, and his knowledge of histology. Isaure DeBuron was a beacon of patience within my flood of impatience. She was an invaluable source for the histological aspects of this study. I would like to acknowledge Dr. Gene Burns for his unfailing support throughout my two years at the University of Montana. And of course the members of my committee, Drs. Craig Johnston and Sharon Dinkel Uhlig, were excellent sources for the critical reading of this thesis. I also have to mention my mother for pestering the heck out of me until I finished. Stephen Wolf and Ted Fuller are amazing friends to add to the lifelong list. I also want to thank Team Stampede for making my Montana experience that much more LARGE.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ABSTRACT .......................................................................................</th>
<th>ii</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS ......................................................................</td>
<td>iii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS ...................................................................</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES .........................................................................</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES .......................................................................</td>
<td>vi</td>
</tr>
<tr>
<td>Chapter I: INTRODUCTION</td>
<td></td>
</tr>
<tr>
<td>Problem Statement ..................................................................</td>
<td>4</td>
</tr>
<tr>
<td>Significance of the Problem .............................................</td>
<td>4</td>
</tr>
<tr>
<td>Hypotheses ............................................................................</td>
<td>6</td>
</tr>
<tr>
<td>Limitations ...........................................................................</td>
<td>7</td>
</tr>
<tr>
<td>Delimitations ........................................................................</td>
<td>8</td>
</tr>
<tr>
<td>Definition of Terms .........................................................</td>
<td>8</td>
</tr>
<tr>
<td>Chapter II: REVIEW OF LITERATURE</td>
<td></td>
</tr>
<tr>
<td>Muscle damage and DOMS ....................................................</td>
<td>11</td>
</tr>
<tr>
<td>Histological damage ..........................................................</td>
<td>14</td>
</tr>
<tr>
<td>Sarcolemma damage ..................................................................</td>
<td>15</td>
</tr>
<tr>
<td>Enzyme release .......................................................................</td>
<td>16</td>
</tr>
<tr>
<td>Factors effecting enzyme release .......................................</td>
<td>17</td>
</tr>
<tr>
<td>Gender differences ..................................................................</td>
<td>19</td>
</tr>
<tr>
<td>Muscle damage and enzyme release ......................................</td>
<td>27</td>
</tr>
<tr>
<td>Human and Animal models ...................................................</td>
<td>30</td>
</tr>
<tr>
<td>Conclusions ............................................................................</td>
<td>33</td>
</tr>
<tr>
<td>Chapter III: METHODOLOGY</td>
<td></td>
</tr>
<tr>
<td>Animals ..................................................................................</td>
<td>34</td>
</tr>
<tr>
<td>Hormone replacement ...........................................................</td>
<td>34</td>
</tr>
<tr>
<td>Exercise protocol .....................................................................</td>
<td>35</td>
</tr>
<tr>
<td>Blood sampling .......................................................................</td>
<td>36</td>
</tr>
<tr>
<td>Microscopy ............................................................................</td>
<td>37</td>
</tr>
<tr>
<td>Research design and statistical procedures .......................</td>
<td>38</td>
</tr>
<tr>
<td>Chapter IV: MANUSCRIPT (as submitted to Medicine and Science in Sports and Exercise)</td>
<td></td>
</tr>
<tr>
<td>Title page ..............................................................................</td>
<td>39</td>
</tr>
<tr>
<td>Abstract .................................................................................</td>
<td>40</td>
</tr>
<tr>
<td>Key words ...............................................................................</td>
<td>41</td>
</tr>
<tr>
<td>Text .......................................................................................</td>
<td>42</td>
</tr>
<tr>
<td>Methods ..................................................................................</td>
<td>44</td>
</tr>
<tr>
<td>Results ...................................................................................</td>
<td>48</td>
</tr>
<tr>
<td>Discussion ...............................................................................</td>
<td>50</td>
</tr>
<tr>
<td>Acknowledgments .....................................................................</td>
<td>57</td>
</tr>
<tr>
<td>References ...............................................................................</td>
<td>58</td>
</tr>
<tr>
<td>Figure legends ........................................................................</td>
<td>65</td>
</tr>
<tr>
<td>REFERENCES .............................................................................</td>
<td>72</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1. Postexercise serum creatine kinase activities (U/l) in the rat.
Adapted from Amelink and Bar, 1986 ........................................ 22

Table 2. Postexercise CK activities (U/l) in different hormonally
manipulated rats. Adapted from Bar et. al., 1988 ..................... 23

Table 3: E2 levels of experimental and control rats .......................... 66
LIST OF FIGURES

Figure 1: CK release in rats and humans. Adapted from Armstrong, (1990) and Rodenburg et. al., (1993) ........................................................ 31

Figure 2: Post-exercise CK release ................................................................. 67

Figure 3: Rodent treadmill............................................................................... 68

Figure 4: Rat tail lateral vein blood draw procedure.................................... 69

Figure 5: Z line streaming found in placebo rat. Distal region of soleus muscle. (LM) 1000X................................................................. 70

Figure 6: A band disruption found in placebo rat. (TEM)....................... 71
Chapter One

INTRODUCTION

The first day of skiing, an exceptionally long run, hiking in difficult terrain for many days, first day of lifting weights, doing anything new and unaccustomed to our bodies and muscles can elicit a response most of us have experienced - muscle soreness. Muscle soreness also can occur in elite athletes. The mode of exercise need only be different from the athletes training, for example a trained cyclist going for a long run. It appears that a high fitness level is no protection against muscle overuse (Kuipers, 1994). This may relieve the 'weekend warrior' who attributes Monday's soreness to a failing fitness level. The mechanism of such a reaction that results in muscle damage and soreness has been the focus of many investigators' research. Many relationships remain unclear, and in fact little has been elucidated beyond descriptive indices.

A wide body of literature has been dedicated to describing both acute-, and delayed onset muscle soreness (for reviews see Armstrong et al., 1991; Armstrong, 1990; Evans et al., 1991; 1987). Muscle soreness and damage can be characterized by a number of parameters: increased stiffness, electrically silent muscle shortening, a decreased range of motion, tenderness, decline in force, swelling, ultrastructural damage to contractile filaments, and release of muscle enzymes into the blood (Kuipers, 1994). The delayed nature of this soreness, or delayed-onset muscle soreness (DOMS), has confused
investigators as to its direct cause. Investigators have suggested that the soreness is a result of the summation of many other processes, and cannot therefore be a direct result of any one process (Rodenburg et. al., 1993). Thus, the only well described parameters of muscle include circulating levels of muscle proteins, disruption of muscle fibers, and changes in voluntary strength and contractile properties (Newham et. al., 1987).

Certain intracellular enzymes found in the blood have been used in the diagnosis of muscular damage, including creatine kinase, aminoaspartate transferase, lactate dehydrogenase, and myoglobin (Janssen et. al., 1989; Balnave and Thompson, 1993; Armstrong, 1990; Van der Meulen et. al., 1991). Creatine kinase activity in serum has been used as a diagnostic tool to determine damage to heart muscle as well, or myocardial infarction (Grande et. al., 1982). The leakage of these enzymes into the blood occurs as a result of damage to the muscle membrane, or sarcolemma. This membrane disruption is thought to occur as the result of muscle damage, although the cause-effect relationship has yet to be determined.

The functional contractile filaments of the skeletal muscle cell can become disrupted in response to exercise-induced muscle damage. This is thought to be the initiating step in cell necrosis, or the degeneration-regeneration stage (Armstrong et. al., 1991). As the name implies, actual damage to the muscular architecture can be observed under light or electron microscopy (Ogilvie et. al., 1988; Armstrong et. al., 1983). For example, Z and/or A-line patterns can become destroyed resulting in the loss of
contractile properties for that muscle cell. The cause of this damage is thought to be a result of the high mechanical forces brought on by eccentrically balanced exercise, and exercise of long duration (Armstrong et al., 1991; Armstrong et al., 1990; Evans et al., 1991; Janssen et al., 1989; Evans, 1987). The large tensile forces created during this type of contraction are particularly efficient at creating ultrastructural damage. Fewer motor units are recruited during eccentric contractions compared to concentric contractions of equal force, which results in a greater force per fiber ratio (Armstrong et al., 1983; Newham et al., 1987; Ogilvie et al., 1988). These high forces then result in destruction of, or disruption to, the normal architecture. It still remains unclear how the sarcolemma and the contractile filaments relate to each other. The integrity of the sarcolemma, and thus enzyme release, may or may not have an effect on disruption to the contractile filaments.

One of the more interesting factors affecting sarcolemma disruption and thus enzyme release is gender. Research indicates that females are subject to much lower basal levels and exercise-induced levels of CK release (Shumate, 1979; Thomson and Smith, 1980; Van der Meulen et al., 1991). It was later discovered that this gender difference resulted from skeletal muscle exposure to estradiol, one of the female estrogens (Amelink and Bar, 1986; Bar et al., 1988; Amelink et al., 1988; Amelink et al., 1990). Estradiol appears to ‘protect’ the muscle from enzyme release during eccentrically balanced exercise. This relationship has also been observed when male rats were
treated with estradiol over 21 days (Bar et. al., 1988). Similarly, the muscle remained 'protected' after treatment in an *in vitro* model, eliminating central nervous system effects, differences in clearance by the liver, or effects of circulating estradiol (Amelink et. al., 1990). What remains to be shown is how this 'protected' sarcolemma translates to the muscle architecture. There seem to be two possibilities: estradiol protects the sarcolemma, and by doing so also protects the contractile filaments, or the sarcolemma integrity is unrelated to any resulting ultrastuctural damage. A previous study by Amelink et. al., (1991) suggested that females incur a lower CK response and a lower incidence of muscle damage post-exercise. In contrast, Van der Meulen et. al., (1991), suggest that males and females incur the same amount of muscle damage, but that females have a suppressed CK response post-exercise.

**Problem Statement**

The purpose of this study is to determine the effect of estradiol on the exercise-induced release of CK into serum, and histological muscle damage in rats.

**Significance of Problem**

Even with the rather extensive literature base on muscle damage, the relationship between muscle damage and creatine kinase release, edema, contractile filament damage, and DOMS has yet to be determined. This study
has provided additional information about the relationship between CK release, and contractile filament damage in the presence of estradiol (E2). This study has therefore, provided a link between the CK release into the blood, thus sarcolemma damage, and the damage incurred by the contractile fibers of the muscular architecture. It has helped resolve the discrepancy in the existing literature as to how the incidence of CK in the blood directly relates to muscular damage.

Factors that may alter the serum CK activity can be important in clinical situations and are of interest in disease mechanisms. If E2 protects both the sarcolemma and the fibers from damage, there may be a number of therapeutic uses. Several muscular diseases, such as Duchenne dystrophy, are characterized by elevated basal CK levels (Shumate, 1979). Hormone therapy may provide an alternative therapeutic measure to reduce muscle fiber breakdown.

The E2 mechanism may have an effect on the rate of actin-myosin turnover. Muscle regeneration is dependent on the infiltration of the muscle by phagocytes and neutrophils. If the sarcolemma remains intact, this regenerative phase may be hindered. Greater muscular hypertrophy has been known to occur in males. Whether this may be partly explained by the lack of the ‘protective effect’ elicited by E2, remains unclear. The decreased potential for hypertrophy in females may be explained from a reduced actin-myosin turnover due to the maintenance of the sarcolemma. This would imply that
the maintenance of sarcolemma integrity has an effect on muscle fiber regeneration, muscle cell necrosis, and the subsequent hypertrophy process.

The length of treatment of E2 required to ensure the ‘protective effect’ on muscle fibers suggests that the mechanism is mediated by a steroid hormone receptor. Twenty one days of hormone supplementation elicited the maximum effect on enzyme release blockage, whereas 24 hour hormone treatment was ineffective. Further studies could aid in the understanding of how this steroid hormone affects transcription. If the mechanism is found to be receptor mediated, the proteins that are involved could be isolated and used as another method of treatment, or drugs could mimic the E2-receptor complex to elicit the desired effect.

Applied applications of this research could effect edema, muscular recovery, and the importance of warm-up and cool-down. Certainly the integrity of the sarcolemma must have an effect on edema or serum infiltrates such as macrophages and other phagocytic cells. Whether this has an effect on muscular regeneration and recovery from exercise remains to be seen.

**Hypothesis I:**

The CK activity in the serum of the placebo rats will be significantly higher than that of the estradiol treated rats at all time points following the eccentric exercise bout.
Justification:

E2 has been shown to decrease the release of CK into serum following an eccentric exercise bout (Amelink and Bar, 1986; Bar et. al., 1988; Shumate, 1979; Amelink et. al., 1990).

Hypothesis II:

There will be a significantly greater amount of post-exercise contractile fiber damaged area in the soleus muscle of the placebo rats compared to the E2 treated rats as evidenced from light microscopy.

Justification:

This controversial relationship has yet to be determined. Amelink et. al., (1991) found a higher incidence of histological damage in males versus females following exercise. Conversely Van der Meulen et. al., (1991) saw identical amounts of damage between males and females.

Limitations
1. The sample size was not optimal for the purpose of statistics due to the involved handling of the biological material.
2. Tissue samples were only obtained at 48 hours post exercise.
3. Only female rats ovariectomized before sexual maturity were used in the study.
4. Electron microscopy to examine sarcolemma integrity was limited to a portion of the experimental and control rats in the experiment due to cost.
Delimitations

1. Although animal models have been shown to exhibit similar responses to exercise, the application to humans will still need to be shown.
2. Morphological studies were not completed on entire muscle samples due to time and manpower limitations.
3. Quantification of histological studies was done in a systematic yet somewhat subjective manner.

Definition of Terms

1) A line disruption: The A line (or band) is the distance equal to the length of the myosin filament within the sarcomere. The length of the A line does not change during contraction. After disruption the A line distance increases, thus not allowing effective contact between heavy, and light chains. It can appear under light microscopy as a "smearing" of the ultrastructure. This is the result of high forces placed on the contractile unit during eccentrically balanced exercise.

2) Creatine kinase (CK): A cytosolic enzyme found in many tissues. One of its isozymes is found primarily in skeletal muscle. It catalyzes the reaction:

\[
\text{CK} \quad \text{phosphocreatine} + \text{ADP} \quad \text{ATP} + \text{creatine}
\]

It serves as a marker of muscle damage, increasing in the serum following intense exercise.
3) **Concentric contraction**: The shortening of the muscle to opposing forces caused by the overlapping of the myosin and actin myofilaments.

4) **DOMS**: Delayed onset muscle soreness is the result of eccentrically balanced unaccustomed exercise when peak soreness is reached 2-4 days afterward.

5) **Eccentric contractions**: Muscular contractions opposing forces, often called the lengthening, or negative part of a contraction.

6) **Estradiol (E2)**: One of three estrogens produced by the ovaries in female mammalian species. Its target organ is normally the uterus, and other secondary sex organs. Estradiol has been shown to have an effect on other tissues including skeletal muscle which contains a cytosolic receptor specific to estradiol.

7) **Estrogens**: A group of 18-carbon steroids secreted primarily by the ovary and, to a lesser extent, the adrenals in females and by the adrenals and testes in males. They are responsible for differential secondary sexual characteristics among males and females and maintenance of the females reproductive organs. The major estrogen is estradiol (E2), while other less potent estrogens include estrone (E1) and estriol (E3) (Bunt, 1990).

8) **Ovariectomy**: The surgical removal of the estrogen producing ovaries, as well as the uterus in the female.

9) **Sarcolemma**: The specialized plasma membrane of skeletal muscle cells. It is thought to become disrupted during intense exercise allowing intracellular enzymes (CK) to leak into the serum.
10) **Z line streaming:** The result of high tensile forces on the contractile units of muscle cells. The once distinct vertical Z line within the sarcolemma becomes fuzzy, and often disappears.
Chapter Two
REVIEW OF LITERATURE

Muscle damage and DOMS

Skeletal muscle reacts to intense unaccustomed exercise in a number of ways: creating localized soreness, both acute and delayed, edema, structural damage to the muscle cell, enzyme release into the blood, and a possible loss of contractility. These descriptive indices have been thoroughly studied, yet the underlying mechanism of how the exercise actually causes the muscle damage, how these resulting parameters relate to each other, and how this may cause soreness is not fully understood.

The study of muscle damage began at the turn of the century, when it was suggested that soreness resulted from ruptures within the muscle (Hough, 1902). This phenomena was dismissed for decades until the interest increased dramatically in the 80’s and early 90’s (for reviews see Kuipers, 1994; Armstrong et. al., 1991; Armstrong 1990; Evans 1991; Clarkson 1992). The initial thrust of the interest lay in discovering the cause of delayed-onset muscle soreness. DOMS is the muscular soreness resulting from an eccentrically balanced exercise bout. It is “delayed” because the peak soreness is usually felt 48 to 72 hours post-exercise (Newham et. al., 1987; Rodenburg et. al., 1993; Rodenburg et. al., 1994; Hagerman et. al., 1984; Balnave and Thompson, 1993). Investigations centered around correlating a time course of DOMS with that of one of the morphological, biochemical, or functional
changes resulting from muscle damage. If one of these changes occurred, or precedes the time course with the onset of muscular soreness, a cause effect relationship could be established. DOMS has yet to be highly correlated to any morphological, biochemical or functional change within the muscle. Initially DOMS was thought to be a direct result of the structural damage induced on the muscle cell (Clarkson et. al., 1986). However, the exact mechanism by which the muscle damage results in soreness and why the pain is delayed in nature has yet to be completely answered. Rodenburg et. al., (1993) studied the biochemical and functional outcomes of muscle damage in an attempt to predict other outcomes by only measuring one parameter. Little correlation between DOMS and CK, myoglobin, and maximal force was found. This was partially attributed to the qualitative nature of the soreness scale the subjects used to quantify DOMS. An example of the soreness scale used by Rodenburg et. al., (1994):

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no soreness</td>
</tr>
<tr>
<td>1</td>
<td>dull feeling of soreness</td>
</tr>
<tr>
<td>2</td>
<td>light continuous soreness</td>
</tr>
<tr>
<td>3</td>
<td>more than light soreness</td>
</tr>
<tr>
<td>4</td>
<td>annoying soreness</td>
</tr>
<tr>
<td>5</td>
<td>very sore</td>
</tr>
<tr>
<td>6</td>
<td>intolerable soreness</td>
</tr>
</tbody>
</table>

Subjects were allowed to score in half points. Soreness scores were also obtained upon palpation. Individual differences and perceptions of pain can create a wide variance in these scores. The low correlation between DOMS and other outcomes of eccentric exercise suggest that some care must be taken in using any soreness scale. The probable conclusion from this was that
DOMS is the result of several factors each with their own time course, the sum of which results in the unique time course for DOMS. The authors thus suggest that measurement of functional and biochemical measures is to be preferred above measurement of DOMS when one plans a study to investigate whether differences exist between groups.

Even without a clear definition of the cause-effect relationship of DOMS and other parameters of muscle damage, there seems to be a consistent response to certain exercise stimuli. One of the first suspects in the cause of DOMS is the mode of exercise. It was found that exercise with a large eccentric component was particularly efficient at creating both DOMS and muscle damage (Armstrong et. al., 1983; Friden et. al., 1983; Newham et. al., 1987; Evans et. al., 1991; Ogilvie et. al., 1988). Due to the high tensile forces created during eccentric exercise, it is particularly efficient at creating muscle damage. Eccentric contractions of skeletal muscle are unique in that they produce great force with a much lower metabolic cost than concentric contractions. When a weight is lifted muscles contract and shorten to produce the required force. When the same weight is lowered, the force is generated by the muscles as they lengthen (Evans et. al., 1991). Evans et. al., (1991) also discovered that eccentric exercise had a ~4 fold reduction in metabolic cost, yet was much more likely to result in muscle damage and subsequent DOMS. Other studies have also discovered that equal bouts of concentric or eccentric work, balanced by force production, were not equal in the resulting muscle damage. Eccentric contractions were found to create
more muscle damage (Evans, 1987; Clarkson et. al., 1986; Newham et. al., 1987; Rodenburg et. al., 1994; Armstrong et. al., 1983; Hagerman et. al., 1984; Armstrong, 1990). This reduces the possibility that a reduction in metabolic capabilities is a contributing factor to muscle damage and DOMS (Armstrong et. al., 1983). The result of this disproportionate increase in muscle damage with eccentric exercise and its direct correlation with DOMS is not clear. However, it has become the method of choice for studying the phenomenology of these conditions. Therefore the mechanism by which eccentric exercise creates muscle damage is unclear, yet we can see the results with the aid of light and electron microscopy in the contractile fiber damage.

**Histological Damage**

Morphological damage to skeletal muscle is defined as microscopic ruptures or tears in the contractile filaments (Hagerman et. al., 1984). Armstrong (1990) suggests that this ultrastructural damage is the initial event after exercise that stimulates the degenerative processes that may lead to DOMS. Mechanical forces result in disruption of the actin-myosin architecture. Two typical responses to these high mechanical forces are Z-line streaming and A-band disruption (Manfredi et. al., 1991; Armstrong et. al., 1983; Staron et. al., 1992; Amelink et. al., 1991; Ogilvie et. al., 1988). These disruptions in the skeletal muscle structure are thought to be a direct result of the high mechanical forces created during eccentric exercise. This led to the theory that the areas most likely to incur damage are the weakest link in the muscle architecture (Friden et. al., 1981, Armstrong et. al., 1991). Newham et.
al., (1987) then took this a step further and suggested that these "weak links" were the apparent cause of the confusing training effect of eccentric exercise. It has been observed that as few as one exercise bout is capable of reducing the enzyme efflux during subsequent bouts of similar exercise (Evans, 1987; Newham et. al., 1987; Donnelly et. al., 1992; Balnave and Thompson, 1993). Newham et. al., (1987) suggest that these weak areas are damaged and repaired then less susceptible during subsequent bouts of eccentric exercise. This training effect will be discussed in further detail later.

**Sarcolemmma Damage**

A significant correlate to the mechanical disruption of the contractile architecture is the disruption of the sarcolemma. At first, the disruption of the muscle cell membrane was thought to correlate with the mechanical disruption of the contractile fibers, but recent investigations have challenged this proposal (Van der Meulen et. al., 1991). A strong correlation is, however, expected between sarcolemmma disruption and intracellular enzyme release into the blood, for this is the only mode of passage for these large, charged proteins. Surprisingly, studies have found that enzyme release can be evident with or without morphological damage, and that a proportional increase in enzyme release does not consistently result in corresponding increases in fiber damage (Kuipers, 1994; Van der Meulen et. al., 1991). Although the cause of sarcolemmma disruption remains questionable, it does appear as a direct result of the muscle damage process.
Enzyme Release

The release of myocellular enzymes into the blood as the result of sarcolemma disruption is generally perceived as an indication of muscle damage (Evans and Cannon, 1991). Enzymes typically assayed include CK, aspartate aminotransferase (AST), myoglobin (Mb), and lactate dehydrogenase (LDH) (Amelink et. al., 1988; Van der Meulen et. al., 1991; Balnave and Thompson, 1993; Armstrong et. al., 1983; Rodenburg et. al., 1993; Bar et. al., 1988). AST activity in the serum following exercise was found to increase, but the magnitude of that increase does not lend itself to accurate determinations (Amelink et. al., 1988; Van der Meulen et. al., 1991). Mb is a sensitive marker of muscle damage, thought to be a result of its lower molecular weight (18k) compared to the other enzymes (CK=80k) (Balnave and Thompson, 1993). Gradations of sarcolemma ruptures would allow smaller proteins to pass through the membrane more easily during this initial process. This is illustrated by the fact that Mb release has a distinct pattern; it reaches a peak of release at ~4 hours post exercise (Balnave and Thompson, 1993). Its magnitude of release is also smaller than that of CK. Because it does not have different isozymes specific to skeletal muscle, like CK and LDH, it is more difficult to determine the source of the release. CK and LDH have this advantage of being able to pinpoint the tissue of release by assaying for the specific skeletal muscle isozyme. Amelink et. al., (1988) and Miles and Schneider (1993) both isolated the CK skeletal muscle isozyme to be the cause of the increase in total CK activity following intense eccentric exercise.
Because of CK's sensitivity of response (upwards of 350% over baseline), ease of determination, and ability to pinpoint the tissue of release, it has been accepted as the best marker for sarcolemma disruption due to muscle damage.

The large increase in CK, and relative ease of measurement in the blood following muscle damaging exercise has made it a useful tool in the study of this phenomenon. It has other diagnostic uses as well. Clinicians have long been using CK release to clarify the incidence of myocardial infarctions (Grande et. al., 1982). It has a dose response relationship, again lending itself as a useful diagnostic tool. Elevated basal levels are often used to pinpoint carriers and early sufferers of many muscular diseases such as Duchenne dystrophy (Shumate et. al., 1979). CK release has a far-reaching influence, its mode of release following exercise may be the model by which many fields are affected. The model that best lends itself to the future investigation of this phenomena is exercise-induced muscle damage.

Factors Effecting Enzyme Release

As mentioned earlier, CK release increases disproportionately with an increase in exercise time (Van der Meulen et. al., 1991; Janssen et. al., 1989). This relationship can be expected since sarcolemma disruption will increase over time, and the additive effect of increasing damage will result in a disproportionate increase in muscle damage. The reason CK release responds more readily to eccentric versus concentric contractions remains unclear. This answer must lie in the mechanism of the sarcolemma disruption which is not yet understood. It is known that eccentric contractions generates greater
intramuscular pressure compared to concentric exercise (Evans and Cannon, 1991). Eccentric contractions also have a different integrated electromyograph (IEMG) than concentric exercise (Evans and Cannon, 1991). The IEMG response for eccentric contractions is smaller and does not increase with the increasing force production that is seen in concentric contractions. Most significantly, Newham et. al., (1983) found that fewer motor units were recruited during eccentric exercise, and that unlike concentric contractions, the IEMG rose progressively during exercise. They summarized that greater tension is created per muscle fiber. Although relatively few number of fibers were recruited, large forces were produced. These extreme forces per fiber result in extreme intermuscular pressure exerted on the sarcolemma and may influence patterns of enzyme release.

The 'training effect' is a reduction in CK release and DOMS during subsequent bouts of eccentric exercise. The training effect that is seen in the reduction of CK release can be caused by a single bout of exercise (Byrnes et. al., 1985; Newham et. al., 1987; Evans, 1987; Donnelly et. al., 1992; Balnave and Thompson, 1993). Newham et. al., (1987) suggested three possible explanations for the training response. 1) There may be a change in the motor unit recruitment pattern. Submaximal eccentric contractions may allow other fibers to be preferentially recruited later on. As discussed above, the IEMG of eccentric contractions is less than concentric, possibly reserving motor units for recruitment during subsequent contractions. 2) There may be some adaptation in the muscle fibers that makes them resistant to the
damaging effects of eccentric exercise. 3) As mentioned earlier eccentric contractions may preferentially damage weak fibers nearing the end of the cycle of growth and replacement. Therefore, a large amount of DOMS, loss of force, and rise in serum CK activity would occur only after the first exercise bout, but would be absent after this initiating exercise (Armstrong, 1984; Friden et. al., 1983). Newham et. al., (1987) used maximal contractions to eliminate the possibility that a change in motor unit recruitment was the cause for the reduction in CK release. Changes in strength and contractile properties were also monitored. A near abolishment of serum CK levels occurred after the initial bout of exercise, and no CK release was seen in subsequent bouts, indicating a change in the sarcolemma disruption mechanism. DOMS and maximal voluntary contractile force, however, only progressively diminished with each ensuing exercise trial. From this data the investigators support the theory proposed by Armstrong (1984), and Friden et. al., (1983) that suggests a population of weak fibers prone to necrosis are eliminated during initial bouts of exercise, thus leaving the sarcolemma more resilient.

Gender Differences

One of the most interesting factors affecting sarcolemma disruption and CK release is gender. Females exhibit much lower CK release following eccentrically balanced exercise compared to males. This gender difference was first described by Shumate et. al., (1979); they found a difference in both resting and post exercise levels of CK. Basal levels of CK were ~2 fold less in
females, while post exercise levels were ~7 fold lower. Depending on activity level, resting levels of CK have been shown to vary among individuals. Nevertheless, females tend to oscillate at lower circulating levels (Staron et al., 1992; Van der Meulen et al., 1991). This relationship has been illustrated by many investigators (Bar et al., 1985; Amelink et al., 1988; Staron et al., 1992; Van der Meulen et al., 1991). Van der Meulen et al., (1991) suggested that females possess a greater ability to clear CK from the serum. The hepatic portal system acts as the clearing mechanism in both males and females. This hypothesis was based on three observations, lack of histological damage difference between the sexes, a measured difference in CK clearance, and a lack of difference in skeletal muscle content of CK in males and females. The method of measurement for the CK clearance is questionable for they did not normalize for the resting values, which are generally lower in females. Also the male female difference in clearance was small, and even the authors suggest that it can only partially explain the difference in serum CK activities between genders. Amelink et al., (1990) were able to reproduce the gender difference using an in vitro preparation suggesting that CK production, and not an increase in clearance accounts for the sex-linked variation. In another article, Amelink et al., (1988) demonstrated that the skeletal muscle contents of the CK isozyme were identical in the liver, soleus, extensor digitorum longus, and rectus abdominis muscles in both males and females. In this same study they were still able to reproduce the gender difference in serum
CK activities. Clearly there is an underlying mechanistic gender difference which is somehow protecting the sarcolemma of the female skeletal muscles.

Another mechanistic hint was based on observations that pregnant females were less susceptible to CK release following exercise than both males and nonpregnant females (Thompson and Smith, 1980). It also appears that resting CK levels in pregnant Duchenne muscular dystrophy carriers is markedly reduced (Blyth and Hughes, 1971; Emery and King, 1971). The hormonal surge that accompanies pregnancy was then thought to play a role in this "protective mechanism". Estrogens were logically targeted as they are primarily responsible for the maintenance and development of the entire female reproductive tract, and incur the largest increases during pregnancy.

Initially it was found that the presence of estrogen, or estradiol (E2) the most reactive of the three estrogens, can markedly reduce the release of intracellular protein from the erythrocytes of women (Thomson and Smith, 1980). Amelink and Bar (1986) who have done the majority of work in this area, further solidified this theory through some clever experiments. In these experiments males exhibited a much greater CK release post-exercise, an increase of 335% above resting value, while no change was seen in females. In the same study they looked at the CK release of hormonally manipulated rats. Four groups of rats were studied, males, females, ovariectomized-females-before-sexual-maturity (OBSM), and ovariectomized-females-after-sexual-maturity (OASM). The pattern of CK release from these four groups was unequivocal. (See Table I)
Table 1: Postexercise serum creatine kinase activities (U/l) in the rat. Adapted from Amelink and Bar, 1986.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Males</th>
<th>Females</th>
<th>OBSM</th>
<th>OASM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>67.4</td>
<td>51.5</td>
<td>96.6</td>
<td>54.0</td>
</tr>
<tr>
<td>2</td>
<td>226</td>
<td>50.1</td>
<td>265</td>
<td>121</td>
</tr>
<tr>
<td>8</td>
<td>58.3</td>
<td>39.6</td>
<td>57.6</td>
<td>49.8</td>
</tr>
<tr>
<td>26</td>
<td>59.8</td>
<td>39.0</td>
<td>60.0</td>
<td>53.3</td>
</tr>
<tr>
<td>50</td>
<td>81.3</td>
<td>53.0</td>
<td>76.0</td>
<td>103</td>
</tr>
</tbody>
</table>

Males and OBSM females had a 335% and 275% increase in CK activity respectively following exercise. OASM females showed a more moderate 225% increase. Normal females showed no increase in postexercise CK levels. The preexercise levels of these groups were interesting as well. The OBSM females had significantly higher preexercise levels than both the females and OASM females. The males had moderately high levels between those of OBSM, and normal females. The authors suggest that a long lasting effect of estrogen on the muscle membrane somehow protects it from the damaging effects of exercise. In the case of the OBSM females illustrates that this protective effect can be removed.

In a follow-up study Bar et. al., (1988) more clearly demonstrated estrogen as the source of the protective effect on muscle damage in females. They again used OBSM females, and intact males and females. In this study however they used direct hormone replacement as a means to see if the effect is recoverable in either the OBSM females or males. (see Table II)
Table 2: Postexercise CK activities (U/l) in different hormonally manipulated rats. Adapted from Bar et. al., 1988.

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After 0 hr</th>
<th>After 2 hr</th>
<th>E2 (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fem</td>
<td>52</td>
<td>50</td>
<td>46</td>
<td>N.A.</td>
</tr>
<tr>
<td>Ovx</td>
<td>78</td>
<td>314</td>
<td>125</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Ovx + E2</td>
<td>48</td>
<td>88</td>
<td>52</td>
<td>1048</td>
</tr>
<tr>
<td>Male</td>
<td>83</td>
<td>314</td>
<td>111</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Male + E2</td>
<td>79</td>
<td>92</td>
<td>70</td>
<td>554</td>
</tr>
</tbody>
</table>

A 390% increase in CK levels was seen following exercise in both the males and OBSM females. The intact females again showed no post-exercise increase. The estrogen treatment caused a reduction in the basal CK levels in the males and OBSM females, as well as nearly abolishing any post-exercise rise. The authors were able to reproduce the protective effect of estrogen on muscle damage in male rats, indicating that males also possess the necessary secondary players in this unknown mechanism. An interesting subproblem of this study was the time dependence of the hormone replacement. The duration of E2 treatment varied from 1 hour to 21 days. The effect of 1 hour treatment on post-exercise CK was not significant. Although, the 24 hour and 7 day treatments were significant, the greatest reduction in post-exercise CK release was seen in the 21 day E2 treated rats. This implies a delayed mechanism by which the E2 relays the protective effect on the sarcolemma.

Steroid hormones have a specific mechanism by which they induce the desired changes in the target tissue. Unlike other hormones which bind an
extracellular receptor inducing intracellular changes by means of second messengers, hydrophobic steroid hormones are free to pass through the plasma membrane of cells. They then bind their intracellular cytosolic receptors which allow them to pass into the nucleus of the cell. Once here they bind specific codons on the DNA “turning on” genes that eventually accelerates protein synthesis of certain targeted proteins. The effect of the steroid hormone mechanism is thus long term, increasing the amounts of certain functional proteins. Estradiol has a specific receptor found mainly in the reproductive tissues of the female. This same receptor has been identified in the skeletal muscles of females and males (Dahlberg, 1982). The delayed time it takes for estradiol to induce the protective effect on the sarcolemma indicates that the mechanism is more than likely receptor-mediated. A direct physical-chemical interaction between estradiol and the sarcolemma is possible yet less likely.

Amelink et al., (1988), further demonstrated that males and females contained the same relative amounts of skeletal muscle CK isozyme. This eliminated the possibility that even with rats of the same weight the E2 was not influencing the expression of the CK gene, and thus resulting in a difference in absolute amounts. They also found that the skeletal muscle isozyme was the cause of the increase in total serum CK levels following intense exercise. This eliminated contributions from the stress possibly put on the liver, brain and heart, in response to treatment or exercise.
As previously discussed, the causative factors of muscle damage may alter the motor unit recruitment. This mechanism may further explain the training effect. Amelink et. al., (1990) addressed this and other issues using an *in vitro* rat skeletal muscle preparation. In an isolated muscle, the effect of a difference in the central nervous system functioning is eliminated. Electrical stimulation was used to mimic exercise, causing the desired stress on the sarcolemma. They were able to exhibit the same gender differences in the isolated preparations that were seen *in situ*. This eliminates the effect of the central and peripheral nervous systems, circulating E2, or other secondary effects. The investigators concluded that the mechanism by which E2 influences the sarcolemma has left its mark on the muscle, and cannot be removed. Ovariectomized females and intact males again showed large CK increases in response to exercise. This post-exercise increase was blocked in these same rats who received long duration hormone treatment. A 24 hour treatment of estradiol did not induce the protective effect. In a pilot experiment, the investigators attempted to induce the protective effect by bathing the isolated muscle in an estradiol solution. This preparation failed to block the post-exercise increase in CK, again supporting the receptor mediated mechanism versus the physical-chemical interaction directly with the sarcolemma.

Following logical progression, these investigators then tried to block this protective mechanism with a known E2 receptor antagonist, tamoxifen (Koot et. al., 1991). Rats received 3 weeks of tamoxifen treatment, followed by
in vitro electrical stimulation to mimic sarcolemma damaging exercise. The tamoxifen antagonist effects on target tissues was evidenced by the marked reduction in uterine weight. This occurred as a result of the antagonistic effect tamoxifen exerts on the E2 receptor in the target tissues. However, in skeletal muscle, tamoxifen was found to mimic E2's protective mechanism, acting as an E2 agonist. In both males and females, a reduction in postexercise CK release was observed with tamoxifen treatment. Some unexplained mechanistic difference is thus causing tamoxifen to act as an antagonist in the target tissues, and as an agonist in skeletal muscle. The authors proposed a mechanism which suggests in target tissues tamoxifen requires a antiestrogen binding site in conjunction with the E2 receptor to induce the antagonist effects. In skeletal muscle, this antiestrogen binding site is absent, allowing tamoxifen to bind directly to the E2-receptor and cause the observed agonist effects. This hypothesis is supported by the identification of the E2-receptor in skeletal muscle. The E2-receptor isolated in skeletal muscle is identical to that of the E2-receptor in classical target tissues (uterus, ovaries) (Dahlberg, 1982). The antiestrogen binding site was discovered to be less ubiquitous. It is found in rat uterus, ovaries, liver, kidney, spleen, brain, and colon, but only in negligible amounts of the skeletal muscle, heart and serum (Sutherland et. al., 1980; Sudo et. al., 1983).

An alternative explanation for the unexpected agonist effects of tamoxifen protection of the sarcolemma is a different receptor altogether. The authors neglected to mention that E2 has been shown to up regulate the
synthesis of the progesterone receptor in certain tissues (Sadovsky et. al., 1993; Moudgil, 1985). Progesterone receptor is found in many types of smooth muscle (Zhuang et. al., 1993; Moudgil, 1985); its incidence, however, in skeletal muscle is not known. The antiestrogen, tamoxifen, has been shown to not directly bind the progesterone receptor (Borras et. al., 1994); yet it does seem to have a proliferating effect on the expression of this receptor (Laugier et. al., 1991). This mechanism is a possibility however, and should not be ignored.

**Muscle damage and Enzyme Release**

The mechanism by which E2 protects the sarcolemma is still unclear. The integrity of the sarcolemma seems to somehow be preserved. It has not been shown whether E2 also protects the muscle from ultrastructural damage. It has also to be determined whether the protection mechanism is mediated through a receptor-mediated process, or through some physical-chemical interaction of E2 with the sarcolemma. If the mechanism is mediated through the interaction of the steroid hormone-receptor complex, then the proteins that are regulated will be important in the mechanism of protection. These proteins may then become important in the clinical treatment of many muscular diseases that are characterized by elevated plasma proteins.

Several studies have made reference to the notion that E2 can or cannot simultaneously protect the ultrastructure of the contractile filaments, and the sarcolemma. Newham et. al., (1987) first suggested that CK release does not correspond with muscular pain, DOMS or (hypothesized) structural
damage. They claim that even though subsequent bouts of damage do not elicit a CK response, pain and DOMS are only reduced in a stepwise manner, indicating to these authors that the CK response and structural damage are mutually exclusive. Janssen et. al., (1989) and Van der Meulen, et. al., (1991) both supported this idea by directly showing a gender difference in CK response, but a less pronounced difference in fiber damage between the sexes. Van der Meulen, et. al., (1991), saw a gender difference in the CK release, but no noticeable difference was shown in microscopic analysis of muscle infiltrates between the sexes. Cross sections (which make it difficult to observe A and Z line disruptions) of the muscles were taken, multiple vacuoles plus the infiltration by inflammatory cells were used as the criteria for muscle damage. The inflammatory response may or may not be directly related to disruptions in the contractile filaments. Predictions of muscle damage from the CK release were made by these authors and found to be large overestimates of the actual fiber damage. Janssen et. al., (1989) was also able to elicit a gender difference in the amount of CK release during longer running events. This was significant even considering the difference in muscle size between genders. In a corresponding article Kuipers et. al., (1989) showed that in both female and male participants the pathological changes were similar in nature and frequency. Armstrong et. al., (1983) were successful at creating a CK response without inducing any significant amounts of muscle damage. Again indicating that sarcolemma permeability and fiber damage are two separate mechanisms, both influenced by exercise
but somehow mutually exclusive. Manfredi et. al., (1991) saw elevated CK release in two groups of men, young and older. CK response was similar between the groups, whereas ultrastructural damage to the fibers was much higher in the older men. The authors suggest that the post-exercise rise in CK activity is a manifestation of muscle damage but not a direct indicator of it.

Amelink et. al., (1990) and Amelink et. al., (1991), however tend to disagree. In Amelink et. al., (1991) virtually no CK release or histological damage occurred in the female rat after an exercise bout. Males meanwhile expressed a greater than 3-fold increase in CK activity, and a significant increase in the total disrupted area of histological damage. It may be important to note that these investigators quantified the muscle damage from both cross and longitudinal sections. In another study Amelink et. al., (1990), treated male rats with dantrolene sodium, a muscle relaxant that also seems to protect the sarcolemma. With the introduction of dantrolene sodium they were able to both markedly decrease the CK release and significantly reduce histological muscle damage. The mechanism by which the dantrolene sodium protects exercise-induced muscle damage is unknown but thought to be similar to that of E2. The authors propose that the damage to the sarcolemma that results in CK release eventually, 48 hours later, also causes the damage to the contractile filaments. This is thought to occur by the calcium influx mechanism. This is described by Armstrong et. al., (1991) as the calcium overload phase. When the sarcolemma becomes damaged, calcium is allowed to flow down its electrochemical gradient into the cell. If
the calcium influx overwhelms the cells normal exclusion pumps the injury becomes 'irreversible'. A large rise in intracellular calcium activates a number of calcium-dependent proteolytic and phospholipolytic pathways that are indigenous to the muscle fibers, which degrade structural and contractile proteins and membrane phospholipids. Even with this theory in mind it still remains unclear whether enzyme release and histological damage are directly correlated. Therefore, increases in plasma CK activity do not always reflect structural damage to the fiber contents (Kuipers, 1994).

Human and Animal Models

During this discussion of the literature, the research models have not been discussed. Of the studies mentioned, both rat and human models have been used. All of the phenomenology discussed has been shown in both of the models. DOMS studies were performed on humans using a 7 or 10 point scale to quantify soreness and pain (Newham et. al., 1987; Rodenburg et. al., 1993; Evans, 1987; Hagerman et. al., 1984; Balnave and Thompson, 1993). Histological studies have been done on both models and evidence of Z-line streaming and A-line disruption have also been demonstrated in both. Staron et. al., (1992) found that successive biopsies can greatly affect focal damage to the muscle, making the technique less than optimal for histological studies on humans. For this reason, most histological studies of muscle damage are done in the rat (Ogilvie et. al., 1988; Armstrong et. al., 1983). Regardless, the changes in the muscle architecture as the result of eccentric exercise are assumed to be similar.
Enzyme release in the rat and human does differ slightly. (see Figure 1) The difference resides in the time of peak CK activity following the exercise bout. Rats tend to have a peak in CK activity immediately following the exercise, from 0 to 2 hours (Van der Meulen et. al., 1991; Armstrong et. al., 1983; Amelink and Bar, 1986; Amelink et. al., 1988; Bar et. al., 1988).

Figure 1: CK release in rats and humans. Adapted from Armstrong, (1990) adapted from Rodenburg et. al., (1993)
Armstrong et. al., (1983) also demonstrated a second peak of release later at 36 hours post exercise in the rat. However, this second peak was only demonstrated as a result of downhill running. Level running showed a less severe initial peak with no evidence of a later rise in enzyme release. This second rise in CK activity corresponds with the peak seen in human serum following exercise. This initial acute stage of enzyme release difference in the human and rat has been proposed as the difference in sedentary rats kept in cages and comparatively more active humans (Amelink and Bar, 1986). This difference is most likely a result of the strong relationship between duration and intensity of exercise, and the subsequent CK leakage from muscle (Amelink and Bar, 1986). The human CK response to exercise is significant but delayed from 48 hours to 12 days (Rodenburg et. al., 1993; Donnelly et. al., 1992; Newham et. al., 1987; Balnave and Thompson, 1993).
CONCLUSIONS

It is apparent that E2 has an effect on the sarcolemma integrity during exercise induced muscle damage. However, data from previous research contains conflicting results. The sarcolemma protection has been seen to result in a lower amount of histological damage by some investigators (Amelink et. al., 1990; Amelink et. al., 1991), and no significant difference in others (Van der Meulen et. al., 1991; Janssen et. al., 1989; Kuipers et. al., 1989). This study was undertaken to contribute to the literature by establishing a relationship between sarcolemma integrity and contractile filament damage.
Chapter Three

METHODOLOGY

Animals

Female Spraque Dawley (Simonsen Distributors, Bozeman, Montana) rats ovariectomized before sexual maturity (25 days old) were used in all the experiments. All rats were obtained at 6 weeks of age. One week was allowed for the rats to become accustomed to their surroundings. A weight record was begun immediately in order to monitor weight gain and to aid in spotting health problems. Weight gain and loss during hormone treatment was monitored. Animals were housed in an NIH approved facility at the University of Montana, Pharmacy building, room 000P. Rats were given ad libitum food and water.

Hormone replacement

Ten rats were divided into two groups: control and experimental. Three weeks prior to the exercise trial, rats were subcutaneously implanted with 17β-estradiol containing pellets (0.05 mg pellet⁻¹, 3 week release) (experimental group), or placebo pellets (control group) using a stainless steel precision trochar (Innovative Research of America, Toledo, Ohio). These pellets deliver hormone without the stress of repeated injections, or the peaks and valleys associated with injections; Control rats received a placebo pellet which contains all the components of the hormone pellet except the active
ingredient itself. The carrier-binder compounds of the matrix include cholesterol, lactose, cellulosics, phosphates and stearates. The rats were anesthetized with an intramuscular injection of a mixture of 10 mg/kg xylazine and 50 mg/kg ketamine hydrochloride. When the rats no longer responded to stimuli (toe pinch), blood was drawn from the tail vein to determine pretreatment levels of E2 (Hurwitz, 1978). An area on the anterior surface of the animal at the base of the cervical vertebrae, between the scapulae was shaved and sterilized with alcohol and betadine. An incision was made through the skin with a scalpel; the trochar was then inserted into the subcutaneous space where the pellet was dispensed. The wound was closed with a single stitch and treated with an aerosol antibiotic. The animals were closely monitored during the recovery from the surgical procedure.

*Exercise protocol*

Untrained rats were used since exercise training reduces the amount of enzyme release and morphological damage (Armstrong et. al., 1983; Van der Meulen et. al., 1991; Newham et. al., 1987) At the end of the three week hormone treatment the rats were exercised eccentrically in order to create stereotypical ultrastructural skeletal muscle damage. A rodent treadmill powered by a variable speed drill was used to exercise the rats (see Figure 3). Eccentric exercise is most successful in creating muscle damage, therefore an downhill grade of 10° (-17%) was used. During the first few minutes of running the speed was increased gradually to start the animals running. The
animals exercised for 1 hour at a speed of 19 m·min⁻¹. The literature indicates that the metabolic cost of this workload enables the animals to complete the test, and that lactate levels will not rise at this workload (Amelink and Bar, 1986).

**Blood sampling**

Blood samples were taken in order to quantify creatine kinase activity and E2 levels. Blood samples were obtained by temporarily anesthetizing the animal with the inhalant isoflurane, and using a 25 gauge needle with a 1 cc syringe to extract blood from the tail vein (Hurwitz, 1978) (see Figure 4). E2 was monitored twice: first on the day of hormone pellet implant to determine pre-treatment levels, and again at the end of the three week treatment period to determine post-treatment levels. The E2 change during this three week period was determined in order to ensure physiological levels of ~500 pmol/L, and also a large experimental/control ratio. Estradiol levels were monitored using a commercial radioimmunoassay kit from Diagnostics Products Corporation (Los Angeles, CA). Serum was mixed with ¹²⁵I labeled E2 which then competes for antibody sites in antibody coated tubes. Separation of bound and free is achieved by decanting the tubes. The tubes are then counted in a gamma counter, the counts being inversely related to the amount of E2 present in the serum sample. The sensitivity of this assay is >29.4 pmol/L (8 pg/ml).
Serum samples for creatine kinase activity were taken from all rats previous to exercise, and at 0, 2, 6, 12, 24, and 48 hours post. Creatine kinase activity was monitored in the serum by using a commercial enzymatic Sigma assay kit (Sigma Diagnostics, St. Louis, Mo.). Serum CK activity was linked to NADH production and absorbance measured at 340 nm using a Spectronic 401 spectrophotometer (Milton Roy Co., Rochester, N.Y.).

Microscopy
Rats were sacrificed by carbon dioxide saturation and cervical dislocation at 48 hrs post exercise. The soleus muscle was chosen for microscopy since it sustains the majority of eccentric contractions during downhill exercise and has been shown to develop structural damage following this mode of exercise (Armstrong et. al., 1983; Van der Meulen et. al., 1991). The distal regions of the soleus muscles have been found to contain more areas of disruption compared to the proximal portion of the muscle, therefore this was used in both the light microscopy (LM) and transmission electron microscopy (EM) (Ogilvie et. al., 1988). The belly region of the soleus was also prepared for microscopy for comparison. The soleus muscle was immediately dissected; ~1.5 mm by ~3 mm samples from the distal and belly regions were placed in fixative (2.5% glutaraldehyde in a 0.1 M sodium phosphate, 4% sucrose buffer at pH 7.2-7.4). Samples were fixed overnight and then removed into 0.1 M sodium phosphate buffer pH 7.2-7.4. Samples were rinsed at least two times before preceding with embedding. Post fixation was done in 1% osmium
tetroxide in water. Dehydration of samples was completed in a series of alcohol steps up to 100% EtOH. Samples were oriented longitudinally and embedded in Poly-Bed 812 (Polysciences, Inc.). Thick sections (1 μm) for LM were obtained using a glass knife, while thin sections for TEM were obtained using a Diatome diamond knife using a Sorval MT 500 ultramicrotome. Thin sections were recovered on copper/palladium grids. LM sections were stained with toluidine blue, while the TEM sections were stained with uranyl acetate and lead citrate. LM was done on an Olympus microscope. TEM was completed with a Hitachi 7100.

Research Design and Statistical Procedures

Only the dependent variables associated with the main hypotheses were analyzed. Microscopy data were analyzed for descriptive purposes only to better explain the variation in creatine kinase relative to circulating E2. All E2 and CK data were analyzed using a priori planned comparisons to determine the differences between cell means. All analyses were tested at the p<0.05 level.
Effects of estradiol on creatine kinase release and subsequent muscle damage in the rat

CHARLES L. DUMKE and BREN T C. RUBY

Department of Health and Human Performance
University of Montana
Missoula, MT 59812

Send correspondence to: Dr. Brent Ruby
University of Montana
Department of Health and Human Performance
McGill Hall, Rm 121
Missoula, MT 59812
(406) 243-2117
ruby@selway.umt.edu
ABSTRACT

The mechanisms that tie the descriptive parameters associated with delayed onset muscle soreness (DOMS), including an increase in serum creatine kinase (CK) activity, and subsequent damage to the contractile filaments of the myofibrils remain unknown. Females exhibit a lower serum CK activity post-exercise compared to males, this was later shown to be the effect of the female sex hormone estradiol (E2). The effects of E2 on CK release and muscle damage was assessed in female rats following eccentric treadmill exercise. Female rats were ovariectomized prior to sexual maturity and treated with an E2 (n=5) or placebo (n=4) pellet insert for 21 days prior to the exercise bout. Exercise consisted of one hour of downhill (-10°) treadmill running at a speed of 19 m·min⁻¹. Serum samples for CK activity were obtained at pre, 0, 2, 6, 12, 24, and 48 hours post-exercise. The soleus muscle was prepared for light and electron microscopy 48 hours post-exercise. CK release in the placebo rats was significantly (p<.05) greater than pre CK levels at times 0, 2, 6, and 24 hours, with the peak response occurring at time 0. CK release in E2 rats was significant at time 0, and 2 hours post, with the peak response at 2 hours. Placebo rats exhibited greater CK activities at all time points except for 2 hours post-exercise. Microscopic damage was only located in two placebo treated animals, therefore it is difficult to speculate as to the precise protective mechanisms of E2. The effects of E2 seem to be limited to CK release and not directly related to that of myofibril damage.
KEY WORDS

DELAYED ONSET MUSCLE SORENESS, ECCENTRIC EXERCISE, MUSCLE DAMAGE, SARCOLEMMA DISRUPTION, A-BAND DISRUPTION, Z-LINE STREAMING, OVARECTOMY, ENZYME RELEASE, GENDER DIFFERENCES
Exercise for which our muscles are unaccustomed can result in soreness, disruption of muscle fibers, an increase in the circulating levels of muscle enzymes, and changes in voluntary strength and contractile properties (8, 9, 13, 17, 19, 21, 26, 27, 28, 29, 30). A number of descriptive studies have been completed on delayed onset muscle soreness (DOMS) and its parameters (13, 17, 19, 21, 27, 28, 29, 30). Damage to the sarcolemma results in leakage of sarcoplasmic enzymes into serum. The release of creatine kinase (CK) into serum is a known response to muscle trauma (20, 23, 27, 28, 34). Histological damage to the contractile filaments is thought to be a contributing factor to DOMS (17, 19, 29). Disruptions to the regular pattern of the sarcomeres in the form of A band widening or Z line streaming contribute to the loss of contractile strength during DOMS (6, 17, 30, 35). The relationship between these descriptive indices remains unclear. It has yet to be described how these parameters relate, and which mechanistic details they share.

Several factors contribute to DOMS and enzyme release: duration (28, 37), mode of exercise (7, 14, 11, 18, 19, 32), training (11, 13, 16, 17, 29), and gender (1, 2, 3, 34, 35, 37). Enzyme release is a direct measure of sarcolemma damage, therefore, as exercise time increases the amount of CK release tends to rise disproportionally (28, 37). Eccentric contractions are associated with increased muscle damage and subsequent enzyme release (7, 11, 14, 18, 19, 32). Although the metabolic cost of eccentric contractions is less than concentric, the mechanical force is greater (11, 18). Newham et. al., (29) found that fewer
motor units were recruited during eccentric exercise to exert large forces, resulting in a large force per fiber ratio. These large forces per fiber result in extreme intramuscular pressure exerted on the sarcolemma and may influence patterns of enzyme release. The 'training effect' is a reduction in CK release and DOMS during subsequent bouts of similar eccentric exercise. This training effect can be observed after only a single bout of exercise (11, 13, 16, 17, 29). A number of postulates have been suggested for this effect, yet the mechanism remains unclear.

One of the more interesting factors affecting sarcolemma disruption, and thus enzyme release, is gender. Research indicates that females are subject to much lower basal levels and exercise-induced levels of CK release (34, 36, 37). It was later discovered that this gender difference resulted from skeletal muscle exposure to estradiol, one of the female estrogens (1, 2, 3, 12). Estradiol appears to 'protect' the muscle from enzyme release during eccentrically balanced exercise. This relationship has also been observed when male rats were treated with estradiol over 21 days (12). Similarly, the muscle remained 'protected' after treatment in an in vitro model, eliminating central nervous system effects, differences in clearance by the liver, or effects of circulating estradiol (3). What remains to be shown is how this ‘protected’ sarcolemma translates to the skeletal muscle architecture. There seem to be two possibilities: estradiol protects the sarcolemma, and by doing so also protects the contractile filaments, or the sarcolemma integrity is unrelated to any resulting ultrastuctural damage. Previous work by Amelink
et. al., (4, 5) suggested that females incur a lower CK response and a lower incidence of muscle damage post-exercise. Others, in contrast, suggest that males and females incur the same amount of muscle damage, but that females have a suppressed CK response post-exercise. (7, 37).

The purpose of this study is to establish a mechanistic link between sarcolemma disruption, and thus enzyme release, with actual damage to the skeletal muscle architecture.

METHODS

Animals. Female Spraque Dawley rats (Simonsen Distributors, Bozeman, MT) ovariectomized before sexual maturity (25 days old) were used in all the experiments. These animals produce no natural E2 yet possessed the ability to respond to E2 transcriptionally. This may be important since it is suspected that E2 imposes an upregulation in protein synthesis in the muscle cell. Rats were obtained at 6 weeks of age. One week was allowed for the rats to become accustomed to their surroundings prior to hormone or placebo pellet implantation. A weight record was initiated immediately in order to monitor weight gain and to assist health monitoring. Weight gain and loss during hormone treatment was also monitored. Rats were given food and water ad libitum. Animals were housed in an NIH approved facility at the University of Montana, and maintained in accordance with the Medicine and Science in Sports and Exercise policy statement (6).
Hormone replacement. Three weeks prior to the exercise trial, rats were subcutaneously implanted with 17β-estradiol containing pellets (0.05 mg pellet⁻¹, 3 week release) (experimental group), or placebo pellets (control group) using a stainless steel precision trochar (Innovative Research of America, Toledo, Ohio). These pellets deliver hormone without the stress of repeated injections, or the dramatic fluctuation in circulating hormone as a result of daily injections. Control rats received a placebo pellet which contains all the components of the hormone pellet except the active ingredient itself. Rats were anesthetized with an intramuscular injection of a mixture of 10 mg·kg⁻¹ xylazine and 50 mg·kg⁻¹ ketamine hydrochloride. Blood was drawn from a lateral tail vein to determine pretreatment levels of circulating E2 (22). An area on the anterior surface of the animal at the base of the cervical vertebrae, between the scapulae was shaved and sterilized with alcohol and betadine. A small incision was made through the skin with a scalpel; the trochar was then inserted into the subcutaneous space where the pellet was dispensed. The wound was closed with a single stitch and treated with an aerosol antibiotic. Each animal was closely monitored during recovery from surgery.

Exercise protocol. Untrained rats were used since exercise training has been shown to reduce the amount of enzyme release and morphological damage (8, 29, 37). Eccentric exercise is most successful in creating muscle damage, therefore an downhill grade of 10° (-17%) was used. A rodent treadmill powered by a variable speed drill and controlled with a voltmeter was used to
exercise the rats. During the first few minutes of running the speed was gradually increased to familiarize the animals with running. The animals exercised for 60 min at a speed of 19 mm min\(^{-1}\). Previous data has demonstrated that the metabolic cost of this workload enables the animals to complete the 60 min without a significant increase in blood lactate levels (2).

**Blood sampling.** Blood samples were obtained by temporarily anesthetizing the animal with an inhalant, isoflurane. A 25 gauge needle was inserted into the lateral tail vein to obtain \(\sim 400 \mu l\) of whole blood (22). Samples were allowed 30 min to clot, they were then spun (3000 rev min\(^{-1}\) at 20°C) to extract serum. Serum was stored at -30°C for subsequent enzyme assays. E2 was monitored twice: first on the day of hormone pellet implant to determine pre-treatment levels, and again at the end of the three week treatment period to determine post-treatment levels. Serum E2 levels were monitored using a commercial radioimmunoassay kit (Coat a Count) from Diagnostics Products Corporation (Los Angeles, CA), with a sensitivity of \(\pm 29\) pmol L\(^{-1}\) (8 pg ml\(^{-1}\)). Serum samples for creatine kinase activity were taken from all rats previous to exercise, and at 0, 2, 6, 12, 24, and 48 hours post. Creatine kinase activity was monitored in the serum by using a commercial enzymatic Sigma assay kit (Sigma Diagnostics, St. Louis, Mo.). Serum CK activity was linked to NADH production and the increase in absorbance measured at 340 nm using a Spectronic 401 spectrophotometer (Milton Roy Co., Rochester, N.Y.).

**Microscopy.** Rats were sacrificed by carbon dioxide saturation and cervical dislocation at 48 hrs post-exercise. The distal soleus muscle was chosen for
microscopy since it sustains the majority of eccentric contractions during downhill running and has been shown to develop structural damage following treadmill exercise (7, 30, 37). The belly region of the soleus was also prepared for microscopy for purposes of comparison. The soleus muscle was immediately dissected; ~1.5 mm by ~3 mm samples from the distal and belly regions were placed in fixative (2.5% glutaraldehyde in a 0.1 M sodium phosphate, 4% sucrose buffer at pH 7.2-7.4). Samples were fixed overnight and then removed into 0.1 M sodium phosphate buffer pH 7.2-7.4. Samples were rinsed at least two times with buffer before preceding. Post fixation was done in 1% osmium tetroxide in water. Dehydration of samples was completed in a series of alcohol steps up to 100% EtOH. Samples were oriented longitudinally and embedded in Poly-Bed 812 (Polysciences, Inc.). Thick sections (1 μm) for LM were obtained using a glass knife, while thin sections for TEM were obtained using a Diatome diamond knife using a Sorval MT 500 ultramicrotome. Thin sections were recovered on copper/palladium grids. LM sections were stained with toluidine blue, while the TEM sections were stained with uranyl acetate and lead citrate. LM was done on an Olympus microscope. TEM was completed with a Hitachi 7100.

Research Design and Statistical Procedures. All data were analyzed using apriori planned comparisons to determine the differences between cell means. All analyses were tested at the p<0.05 level.
RESULTS

Post treatment weight changes. Before hormone implantation weights were not significantly different between the groups, 234.8 ± 11.0 g and 236.0 ± 13.1 g for the E2 and placebo groups respectively (p=0.67). Immediately after pellet implantation the E2 rats began losing weight, whereas placebo rats continued to gain weight at a previously determined normal rate of ~20 g·week⁻¹. The animals all appeared to be in good health and expressed no lethargy or apparent behavior change as a result of the weight change. At the end of the 21 day treatment period the placebo rats were significantly heavier than the E2 treated rats, 290.2 ± 19.4 g and 223.2 ± 9.2 g respectively (p=0.0001). Over the 21 day treatment period, the placebo group showed a net gain in body weight of +54.2 g, whereas the E2 treated rats showed a net loss of -11.6 g.

Circulating E2. Serum E2 levels were determined pre and post hormone treatment for both groups (Table 3). The ovariectomized female rats prior to E2 treatment exhibited no detectable concentrations of circulating E2. Following the 21 day treatment period, the placebo group still had no detectable E2 concentration. However, the E2 treatment group had significantly higher serum levels of E2 compared to placebo, 784.8 ± 164.3 pmol·l⁻¹ vs <29.4 pmol·l⁻¹ (p=0.0001). These hormonally manipulated circulating levels are comparable to previously reported serum E2 levels in rats by another investigator using similar delivery (12). These levels were also similar to the circulating E2 concentrations during the luteal phase in a normal, ovulating female rat (3).
Post-exercise CK release. Samples for serum CK activity were obtained prior to exercise, immediately following, and at 2, 6, 12, 24, and 48 hours post-exercise (Figure 2). Activities were normalized to 30° C. Serum CK activity was not significantly different prior to the exercise trial (p=0.50). However, immediately post-exercise, the placebo group experienced a near three fold increase in CK activity. In contrast, the E2 treatment group expressed nearly a two fold increase in CK activity. The E2 group continued to rise, reaching a peak at 2 hours post-exercise. The E2 group then expressed a dramatic return to baseline levels of CK. Significant elevations above baseline were observed at only 0, and 2 hours post-exercise in the E2 treated rats. In contrast, the placebo group exhibited significantly greater activities over pre-exercise values at time points, 0, 2, 6, and 24 hours post. This may be an indication of a later peak in the placebo group as noted in other investigations using exercise with a large eccentric component in rats (2, 3, 7, 12, 37).

Structural Damage. Limited evidence of contractile filament damage of the soleus muscle was observed under light and electron microscopy. Stereotypical examples of A band disruptions and Z-line streaming were found in only two rats within the placebo group (see Figure 5 & 6). The evidence of the myofibrilar damage did not appear proportional to the release patterns of CK. This supports the observations of other investigators that suggest that CK release is not linked directly to histological skeletal muscle damage (7, 33, 37).
DISCUSSION

Post treatment weight changes. The differences in mass and muscle surface area between gender has been controlled for by other investigators and found to have no effect on the amount of post-exercise CK release (2, 4). Amelink et al., (2) also showed that the presence of E2 does not effect the expression of CK enzyme in the skeletal muscle, and that this same skeletal muscle CK is responsible for the post-exercise increase in the serum. This shows that the difference in weight brought on by the E2 treatment is not causing an indirect effect on CK release confusing the mechanism of protection at the sarcolemma.

The weight changes brought on by the hormone treatment is interesting and may affect the metabolism of the animal. Some studies have shown that gender, and subsequently E2, appear to affect substrate utilization at rest and exercise in both humans and rats (for review see 31). It is suggested that the presence of E2 increases lipolysis and thus preferentially fat is used as a energy substrate. However, this would not necessarily result in a net weight loss due to the density of the lean body mass component. The weight loss experienced by the rats must, at least in part, be explained by a loss in lean mass, and therefore lower basal metabolic rate. It may also result from a suppressed appetite, which may result in an increased degree of protein catabolism. Level of activity can also affect lean body mass. However, it is difficult to make any assumptions since energy intake and production were not measured in this study. It is even more difficult to explain since the
animals began losing weight almost immediately. The above suggested explanations would be chronic, yet the E2 treated rats showed weight losses of ~7 g after only three days. E2 has wide ranging effects on both target and nontarget tissues. The results presented here provide fodder for future investigations on the effects of E2 on metabolism.

Circulating E2. Bar et. al., (12) also used crystalline pellets promoting similar levels of circulating E2. Repeated subcutaneous injections were used in the other studies involving hormonal manipulation (3, 24). Bar et. al., (12), using their similar pellet mode of E2 delivery, reported a mean of $1048 \pm 499$ pmol·L$^{-1}$ for circulating serum E2 (n=15). They also reported undetectable levels of E2 in ovariectomized female rats (<10 pmol·L$^{-1}$). This is very comparable to the results from this study, considering the large standard deviations reported for both studies. Amelink and Bar, (1) measured serum E2 levels in both ovariectomized female rats, and intact females. The ovariectomized rats showed <30 pmol·L$^{-1}$ of circulating E2, whereas the normal cycling females had levels ranging from 110.2 to 1101.7 pmol·L$^{-1}$.

Post-exercise CK release. Previous investigators have shown similar CK responses to eccentric exercise in ovariectomized female or male rats, an immediate peak post-exercise, which is then followed by a latent rise in serum enzyme activity (1, 2, 3, 7, 12, 37). Our study does differ with some in that we were able to induce a post-exercise peak in E2 treated animals. Clearly E2 has an effect on CK release; the response is both delayed and lessened by an E2 induced mechanism. Also, the amount of time spent above pre-exercise
levels is reduced in E2 treated rats, thus abolishing the latent rise in activity. This is in contrast to the initial study by Amelink and Bar, (1) which showed no post-exercise rise in CK in hormonally manipulated rats. However, in an in vitro model, females did show a diminished rise in CK in response to electrical stimulation (3). Therefore, in conjunction with other studies, it is suggested that females have an affected CK release mechanism, and that its messenger of change is related to circulating E2 (34, 35, 36, 37).

A number of issues arise to explain the delayed CK peak shown in the E2 treated rats. The method and amount of hormone delivery may effect the protective mechanism E2 influences on the sarcolemma. In fact it has been found that a large dose of E2 24 hours prior to exercise was less effective in reducing post-exercise CK release (3, 12). The largest reduction in CK release has been seen after 21 days of hormonal treatment (1, 3, 12, 24). This indicates a possible effect of E2 on the expression of a protein which then may cause the sarcolemma to be less vulnerable to damage. The mediator of such a mechanism may include the E2 receptor found in target tissues such as the ovaries, uterus and other sex organs. However, this receptor has also been isolated in male and female skeletal muscle (15). Current investigations as to the role of E2 receptor on sarcolemma protection are being completed. With such a mechanism in mind it is possible that the amount of circulating E2 may effect protection of the sarcolemma. As indicated above, however, the rats in this study exhibited circulating levels of E2 similar to those previously reported (12). Of physiological importance, hormone levels in the present
study were similar to that of a normal ovulating female rat (2). It was also considered that the delivered 17β-estradiol may be mechanistically inferior to that of naturally occurring E2 in its ability to protect the sarcolemma. However, this possibility was eliminated for two reasons: Bar et. al., (12) used similar pellets and showed a negligible post-exercise CK response. Also, the assay used in this study has low cross reactivity with other naturally occurring steroid hormone derivatives. Therefore, it is unlikely that the process of hormonal manipulation was the cause of the delayed peak observed in the E2 treated animals.

Another explanation for this previously unobserved phenomena is the type of exercise used to induce damage. The exercise may have been so severe as to override the protective mechanism induced by E2. Indeed, it was necessary to encourage the naive rats to complete the exercise bout. This is not unusual however, and in fact previous investigators have used an electric shock to keep the animals running (2, 7, 37). In our study 200-300 g rats ran at 19 m·min⁻¹ for 60 min down a 10° grade. Armstrong et. al., (7) ran their 400-500 g rats for 90 min at 16 m·min⁻¹ down a 16° decline. Van der Meulen et. al., (37) ran rats for 2.5 hours at 30 m·min⁻¹ on a 10° incline, increasing the metabolic cost significantly. In studies involving hormonal manipulation, 19 m·min⁻¹ for 2 hrs with 0° grade has also been used (1, 2, 12). Considering the exercise protocols in these studies it is difficult to conclude that the present protocol was so severe as to override the protective effects of E2.
The delayed peak in CK release seen in this study may be mainly a function of the post-exercise sampling intervals (see Figure 1). Several studies using rats observed a diminished rise in CK in females, yet obtained fewer samples at different time points (2, 12). This may have caused the investigators to 'miss' the post-exercise CK peak, since it seems to spend less time elevated above baseline as seen in the experimental group of this study. Other investigators have observed a rise in the CK response of females that is lower, or temporally variant from that of males (23, 33, 34, 37). Amelink et. al., (3) in an in vitro female rat model showed a diminished yet significant rise in CK post-electrical stimulation. This may suggest a mechanism induced by E2 that delays the CK response to exercise, and hastens the return to baseline levels. The speculation of such an observation can be confusing. One may conclude that recovery from an exercise bout may be hastened, yet it is necessary that plasma infiltrates such as macrophages and lymphocytes invade the muscle in order to initiate damage repair (8, 9). Van der Meulen et. al., (37) also suggest that CK clearance may be partly responsible for the diminished response to exercise in females. This model fits the data presented here in that the CK response is both slowed and precipitates the return to pre-exercise levels. This cannot fully explain the gender difference in CK response since it is maintained in an in vitro model, which removes the effect of clearance by the hepatic portal system (3, 24). It may also be possible that sarcolemma integrity may be partly responsible for a reduction in overall protein turnover and thus hypertrophy observed in females.
However, from the addition of this data, it can be established that the mechanism of CK release is altered in the presence of E2.

**Structural Damage.** The effects of E2 on histological damage remains unclear. It cannot be concluded that the mechanism by which E2 effects CK release also effects the disruption of the contractile filaments. The post-exercise CK response was significantly greater for both groups all rats, yet damage to the soleus muscle was noted only in small areas of two of the rats from the placebo group. Clearly, histological examination all the involved muscles in the eccentric exercise is impossible. A representative sample is necessary making quantification difficult. Systematic examination of the distal and belly regions of the soleus muscle was made and no clear trends in muscle damage were observed for either group.

The lack of evidence for histological damage in the presence of a large CK response is not unusual. Other investigators have made note of a low level or lack of histological evidence following an exercise bout (4, 7, 37). The lack of evidence in this study for a direct link between CK release and muscle damage is supported by others (7, 23, 25, 27, 37). Armstrong et. al. (7) were successful at creating a large CK response with an estimated fiber damage of less than 5%. Van der Meulen et. al., (37) found a much lower actual histological damage when compared with an estimated amount of muscle damage from the CK release. This study is further evidence that CK release is not directly proportional to histological damage, and therefore is a inconsistent and questionable marker for skeletal muscle damage. It is also
possible that E2 may independently affect the sarcolemma integrity and the
contractile filamental damage. This mechanistic link remains to be made, yet
E2 may be the emissary of this discovery.

Gender comparisons of histological damage remain unclear. Amelink
et. al., (5) has reported a gender difference in post-exercise induced muscle
damage. They suggest that females are protected by the same E2 induced
mechanism which affects CK release. Van der Meulen et. al., (37) however
found no histological difference between genders, but did show a difference in
CK release. The present study shows a difference in enzyme release, but an
unclear difference in muscle damage corresponding to circulating levels of E2.
This suggests that sarcolemma permeability and contractile fiber damage are
two separate mechanisms, both influenced by exercise with no established
direct mechanistic link.
ACKNOWLEDGMENTS

The authors wish to thank Dr. Keith Parker for his assistance with the estradiol assay. Isaure DeBuron is thanked for her technical assistance on both the light and electron microscopy. The critical reading of this manuscript by Drs. Craig Johnston and Sharon Dinkel Uhlig was very helpful. This work was completed with support from the office of Research Administration, The University of Montana.

Address for correspondence: Dr. Brent Ruby, University of Montana, Department of Health and Human Performance, McGill Hall, Missoula, MT 59812, (406) 243-2117, ruby@selway.umt.edu.
REFERENCES


FIGURE LEGENDS

Table 3: E2 levels of experimental and control rats ................................. 66

Figure 2: Post-exercise CK release ............................................................ 67

Figure 5: Z line streaming found in placebo rat. Distal region of soleus muscle. 1000X................................................................. 70

Figure 6: A band disruption found in placebo rat. (TEM)...................... 71
Table 3:  E2 levels of experimental and control rats.  (Pmol/L)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>pre-treatment</th>
<th>post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>&lt;29.4^</td>
<td>&lt;29.4^</td>
</tr>
<tr>
<td>E2</td>
<td>&lt;29.4^</td>
<td>784.8 ± 164.3^</td>
</tr>
</tbody>
</table>

^ due to sensitivity of assay; * p<.05 E2 vs placebo group; † p<.05 pre vs post treatment.
Figure 2: Post-exercise CK release.

* p<.05; E2 vs placebo; † p<.05; effect of time
Figure 3: Rodent treadmill
Figure 4: Rat tail lateral vein blood draw procedure
Figure 6: A band disruption found in placebo rat. (TEM)
Figure 5: Z line streaming found in placebo rat. Distal region of soleus muscle. (LM) 1000X
REFERENCES


