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# T2 Relaxation Time in Men and Women Following Eccentric Elbow Flexor Exercise

Patrick A. Moull
*The University of Western Ontario*

Supervisor
Dr. Marsh
*The University of Western Ontario*

Graduate Program in Kinesiology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science
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(Thesis format: Monograph)

by

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The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

# Abstract

Previous research suggests that estrogen exhibits a protective effect on skeletal muscle and may help curb the effects of induced damage. Eccentric (ECC) exercise has been shown to provide the greatest levels of exercise induced muscle damage (EIMD) and with indirect markers of muscle damage including decreases in strength, increased inflammation, and higher magnitudes of delayed onset muscle soreness (DOMS). Studies have shown that elevations of the T2 relaxation times of activated muscles coincide with these other indirect measures of muscle damage. The purpose of this study was to compare the effects of a high intensity ECC elbow flexor exercise in both a group of men and women on T2 relaxation time and coinciding indirect measures of muscle damage to determine if sex provided any differences in results. No significant differences were found in T2 times within or between groups in the 3 regions of interest except for the brachialis measures in the women group (81.9% increase) and average measures of the men (38.6% increase) from baseline to 72 hours post protocol. These findings coincided with no significant relative differences in cross-sectional area (CSA) (14.5%; 32.6%), strength (-33.6%; -15.2%), or pain measures in men and women respectively from baseline to 72 hours post protocol. Following high intensity ECC exercise, women did not appear to demonstrate a protective effect on skeletal muscle. T2 relaxation time may not be a suitable measure to detect differences in muscle damage between the sexes, however, the small size of the groups in this study, especially the women, could potentially have resulted in low statistical power therefore affecting the significance of the results.

# Keywords

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# List of Abbreviations

| | |
|---|---|
| 1RM | 1-repitition maximum |
| CK | Creatine kinase |
| CON | Concentric |
| CSA | Cross-sectional area |
| DOMS | Delayed-onset muscle soreness |
| ECC | Eccentric |
| EIMD | Exercise induced muscle damage |
| HRT | Hormone replacement therapy |
| MRI | Magnetic resonance imaging |
| MVC | Maximum voluntary contraction |
| VPS | Visual pain scale |
| WBC | White blood cell |

# Chapter 1

# 1    Introduction

Muscle injuries are one of the most common injuries occurring in sports, with an incidence of 10% to 50% of all sustained injuries (T. Järvinen, H. Järvinen, Kääriäinen, Kalimo, & Järvinen, 2005). Exercise induced muscle damage (EIMD) has been experienced by the majority of those participating in physical activity whether they be recreational or professional. Out of these injuries, muscle lacerations are the least common as 90% of these injuries are characterized as contusions or strains. A contusion is the result from a sudden direct blow to the muscle that generally occurs during various contact sports whereas sprinting and jumping are the main activities associated with muscle strains (Crisco, Jokl, Heinen, Connell, & Panjabi, 1993; Järvinen, Kääriäinen, Järvinen, & Kalimo, 2000). Unlike bone, which heals by way of a regenerative process, skeletal muscle repairs by way of a process involving a destruction phase and subsequent repair and remodeling phase (Järvinen et al., 2005). The determination of the specific degree and severity of induced muscle damage is not an exact science. Markers of muscle damage such as strength loss, pain, and specific metabolite behaviors help to tell some, but not all of the story. While external discolor or disfigurement allows for an estimate of the severity and type of muscle injury sustained, the use of Magnetic Resonance Imaging (MRI) allows for detailed view at the internal structures at the area of injury occurrence.

MRI is the standard for many clinical diagnostic applications as it provides unparalleled visualization of anatomic detail of soft tissues such as muscle, tendon,

cartilage, and various organs (Adams, Duvoisin, & Dudley, 1992). The practical usage of MRI to observe and diagnose physical abnormalities of the brain, heart, spinal cord, tumors, cysts, and various other parts of the body makes it a critical tool to allow physicians to diagnose and monitor a whole variety of medical conditions. Skeletal muscle is the largest non-adipose tissue body composition component and the need to accurately measure muscle in vivo is apparent in several disciplines (Mitsiopoulos et al., 1998).

In terms of skeletal muscle analysis, MRI of skeletal muscle have shown exercise-induced contrast enhancement between protagonist and antagonist muscles which appears to be graded with exercise intensity (Fisher, Meyer, Adams, Foley, & Potchen, 1990). Along with these findings it has been observed that transverse (T2) relaxation times of recruited muscle in proton magnetic resonance images increase during exercise and appears to arise from changes in intracellular water chemistry and seems to be accentuated following a muscle lengthening eccentric (ECC) versus shortening concentric (CON) exercise (Ploutz-Snyder, Nyren, Cooper, Potchen, & Meyer, 1997). This suggests that there may be some relation between the level of exercise induced muscle damage and the T2 relaxation times of these muscles.

When performed at a high enough intensity, ECC can induce muscle damage and delayed pain, often defined as delayed-onset muscle soreness (DOMS) (Armstrong, 1984). There is evidence that estrogen and by extension a person's sex has a significant influence on post tissue damage muscle leukocyte invasion and inflammatory response as well as evidence of a protective effect in neurological diseases after acute injury in human and animal models (Stupka et al., 2000; Suzuki, Brown, & Wise, 2009; Tiidus,

2001). It has also been demonstrated that women possess greater protection than men when it comes to muscle membrane disruption following intense exercise as a result of higher levels of circulating estrogen (Tiidus, 2001).

## 1.1    Research Goal

The purpose of this study was to compare the T2 relaxation time values of the elbow flexors of men and women following high intensity ECC resistance exercise of the biceps muscles.

The majority of research in this area has focused on the use of MRI to look at relationship between T2 relaxation time with the activation and potential damage of the muscle at various points of time following some form of exercise. An area of this research that has not yet been deeply explored are the potential differences between men and women in T2 relaxation time values following high intensity ECC damage. ECC strain has been shown to cause increased levels of fiber disruption and greater reductions in tetanic force relative to isometric and concentric activity (Lieber & Fridén, 1999). Compared to animal studies, estrogen's influence on human skeletal muscle contractile function or post-exercise muscle damage is not as clear (Enns & Tiidus, 2010). These inconsistencies have been attributed to various factors including the age and fitness levels of the subjects, and the type and intensity of the exercise protocols. Studies on animals have demonstrated that estrogen reduces muscle atrophy as well as accelerates recovery from experimentally induced atrophic conditions (Fisher, Hasser, & Brown, 1998; McClung, Davis, Wilson, Goldsmith, & Carson, 2006).

MRI T2 is considered a sensible, reproducible, widely available, and clinically

relevant magnetic resonance measure for observing damaged and diseased skeletal muscle (Carlier, 2014). T2-mapping is known to detect inflammatory and necrotic sites in muscle which are characterized by elevated T2 relaxation times resultant from the increase of fluid relative to muscle tissue (Araujo & Carlier, 2014). It has been demonstrated that percent of maximum voluntary contraction (MVC) decreases post ECC exercise and T2 relaxation time values elevate post ECC exercise ( Fisher et al., 1990; Piitulainen, Holobar, & Avela, 2012; Proske & Allen, 2005). Using MRI measures of T2 relaxation times of muscle may provide a non-invasive, direct measure of the functionality of either healthy or damaged muscle.

Fast twitch fibers experience the greatest levels of disruption following ECC exercise (Lieber & Fridén, 1988). The elbow flexors are comprised of the deep brachialis, and the superficial short/long heads of the biceps brachii. Animal studies have shown that there is a greater ratio of fast to slow twitch muscles in more superficial muscles compared to deeper muscles (Neufuss et al., 2014). In human studies, similar relationships have been found with the superficial gastrocnemius and deep soleus muscles (Gollnick, Sjödin, Karlsson, Jansson, & Saltin, 1974). As it appears that muscle damage can result in regional differences dependent on fiber type, an examination of the T2 relaxation times of the brachialis and both heads of the biceps muscle were performed.

Therefore, in this study I attempted to answer the following questions:

1. Is there a sex dependent effect on the resultant T2 relaxation times of skeletal muscle following high intensity ECC of the elbow flexors?

2. Are the T2 relaxation times observed related to the functionality and muscle

damage caused by the ECC protocol?

3.  Will T2 relaxation time values be affected by the muscle fiber type

    composition of the brachialis and heads of the biceps?

## 1.2    Hypotheses

The following hypotheses were tested:

1.  Men will experience a larger increase in T2 relaxation time values relative to

    baseline compared to women.

2.  There will be an exhibited increase in T2 relaxation time alongside decreased

    MVC and appearance of indirect markers of muscle damage relative to

    baseline following the ECC damage protocol.

3.  The T2 relaxation time values will increase more relative to baseline in the

    superficial biceps than the deep brachialis.

# Chapter 2

## LITERATURE REVIEW

## 2.1    Characteristics of Muscle

Skeletal muscle comprises almost half of the total body mass of humans and thus is the most abundant tissue of the human body.  Muscle coordinates movement by way of shortening contractions and its location of attachment on the skeleton. Their specific shape and insertion points determine the specific movements that the muscle is able to perform.

Skeletal muscle is composed of many bundles of myofibers, within each myofiber contains many myofibrils, which are then composed of repeating sarcomeres (Grefte, Kuijpers-Jagtman, Torensma, & Von Den Hoff, 2007).  A sarcomere is an arrangement of contractile proteins myosin and actin, which form the thick and thin filaments respectively (Grefte et al., 2007).  Following depolarization of the muscle myofiber due to nerve action, intracellular Calcium is released by the sarcoplasmic reticulum which results in the binding of myosin to actin and a contraction of the skeletal muscle.

The highly organized arrangement of muscle fibers contributes to a variety of functional capabilities. Multiple types of fibers adds to the heterogeneous nature of skeletal muscle and these types can be classified according to differences in structural and functional properties (Pette & Staron, 2000).  Skeletal muscle is made up of many motor units. Each motor unit is comprised of a single motor neuron and each muscle fiber that it innervates.  The functional properties and size of the motor units help to determine the overall ability of each muscle (Ciciliot, Rossi, Dyar, Blaauw, & Schiaffino, 2013).

Three types of motor units were initially identified: type 1 slow, type 2A fast fatigable, and 2B fast fatigue resistant (Burke et al., 2015). A fourth motor unit composed of type 2X fibers has been observed in rat skeletal muscle (Larsson, Edström, Lindegren, Gorza, & Schiaffino, 1991). These fibers are distinguishable based on the presence of a specific myosin heavy chain, as well as their oxidative and glycolytic metabolisms (Schiaffino & Reggiani, 2011). Type 1 and 2A fibers are more oxidative and type 2B fibers are more glycolytic. Intermediate muscle fibers containing type 1 and 2A, 2A and 2X, or 2X and 2B myosin heavy chains are also common in normal muscle (Smerdu, Karsch-Mizrachi, Campione, Leinwand, & Schiaffino, 1994).

## 2.2.1  Muscle Injury

Muscles injuries can be caused by way of contusion, strain, or laceration. Regardless of the cause of muscle injury, the physiology of the healing response is relatively similar (Tiidus, 2010). One exception to this is that a major contusion or laceration injury to skeletal muscle can result in localized connective tissue-based scar formation during the healing process (Tiidus, 2010).

The most frequent traumatic muscle injury is a contusion produced by the impact of a blunt non-penetrating object, which often leads to an increase in muscle weight due to edema and hemorrhage (Crisco et al., 1993). Following contusion skeletal muscle exhibits a significant drop in tetanic tension and function, but is regained over time (Crisco et al., 1993).

Strain injury occurs in skeletal muscle when an excessive pulling force is applied resulting in overstretching. These injuries are often located near the

myotendinous junction of the superficial muscles working across two joints such as the rectus femoris, semitendinosus, and gastrocnemius muscles (Järvinen et al., 2000). Mild strains are classified as a tear of a few muscle fiber with minor swelling and minimal or no loss in strength; moderate strains classified by a greater degree of damage with a clear loss in strength; sever strain classified as a tear extending across the whole muscle belly resulting in a total loss of function (Järvinen et al., 2000)

## 2.2.2 Exercise Induced Muscle Damage

EIMD occurs in humans frequently after unaccustomed exercise, particularly if the exercise involves a large amount of ECC contractions (Clarkson & Hubal, 2002; Schoenfeld, 2012). Symptoms of EIMD include DOMS and loss of physical function. Damage can be specific to just a few macromolecules of tissue or manifest as large tears in the sarcolemma, basal lamina, and supportive connective tissue, as well as altering the contractile elements of the cytoskeleton (Vierck et al., 2000).

## 2.2.3 Delayed Onset Muscle Soreness

Muscle soreness is a frequently reported consequence of EIMD. This has been termed as DOMS as the associated symptoms of pain and tenderness generally peak 24-48 hours post exercise (Schoenfeld, 2012). This phenomena is believed to be the result of a sensitization of nociceptors by tissue breakdown products and noxious chemicals such as histamines, bradykinins, prostaglandins, and free radicals (Clarkson & Hubal, 2002). The experienced soreness may also involve mechanoreceptors, including muscle

spindle afferents that are able to access the pain pathway at the level of the spinal cord (Proske & Allen, 2005).

## 2.3    Mechanisms of Muscle Injury

## 2.3.1  Mechanical Factors

ECC contractions cause more damage to muscle than CON contractions because fewer motor units are recruited during ECC exercise and therefore a smaller cross-sectional area of muscle is activated to handle the same load as would be handled in a CON contraction (Clarkson & Sayers, 1999).  Decreases in isometric tension immediately after an injury to the muscle were found to be related to peak force produced during the actions, implying that tension may produce damage (Warren et al., 2007).  It was later found that the extent of muscle injury was related more to the change in length than the amount of force generated by the muscle, and that muscle injury was associated with the magnitude of strain rather than the high tension forces imposed on the muscle (Child, Saxton, & Donnelly, 1998).

Current data suggests that the earliest events associated with skeletal muscle injury are mechanical in nature and may be placed primarily on the sarcomere strain experienced by the muscle (Lieber & Fridén, 1999).  Compared to isometric exercise or passive stretching, ECC exercise appears to elicit a large increase in diameter of muscle fibers, but exhibited only in those of the fast glycolytic type (Lieber & Fridén, 1999).  As muscle fibers do not all simply extend from one tendon plate to the other and are arranged in series throughout the length of the muscle, differential fiber type injury may

just be a result of variable fiber lengths of a certain type and the increased strain on the shorter fiber length of glycolytic fibers (Lieber & Fridén, 1999). Because some sarcomeres may become overextended and are weaker than others, they are unable to handle the stresses of certain mechanical strains and experience disruption, while the stronger ones remain intact following ECC exercise (Newham, McPhail, Mills, & Edwards, 1983).

## 2.3.2  Disturbances in Calcium Homeostasis

In laboratory studies on mice it was reported that muscle fibers injured by way of ECC contractions had elevated free cytosolic calcium concentration and it was hypothesized that the increased strain on the fibers from the forced lengthening would trigger activated calcium channels in the membrane, increasing influx into the muscle cells (Clarkson & Sayers, 1999). Calcium leakage from damaged sarcoplasmic reticulum lumen resultant of eccentric damage was suggested to be a potential factor in muscle damage, but it was later discovered that following the application of a suppressor  of the flux of calcium from the sarcoplasmic reticulum, little was noticed in any effect of preventing exercise induced injury (Bigard, Merino, Lienhard, Serrurier, & Guezennec, 1997).

Calpain is a calcium-activated protease located at the I and Z regions of skeletal muscle and is suggested to play a role in the muscle damage process (Belcastro, Shewchuk, & Raj, 1998). In vitro calpain has been shown to cleave cytoskeletal proteins such as desmin, which serves to attach adjacent myofibrils at the Z-discs (Patel & Lieber, 1997). Increases in calpain activity have been demonstrated following exhaustive and

eccentric exercise with a loss in Z-disc structure and concomitant loss of desmin (Belcastro, Parkhouse, Dobson, & Gilchrist, 1988). Actin and myosin are not substrates for calpain and this suggests that ultrastructural damage to the muscle following ECC exercise is predominantly in the Z-disc region (Clarkson & Sayers, 1999).

## 2.4    Muscle Repair

## 2.4.1  Inflammation

Biochemical changes due to structural disruption of the extracellular matrix and damage to the myofibers facilitates the escape and entrance of intracellular and extracellular proteins and mediates the inflammatory response. Damage to the muscle fibers results in transfers of fluids and cells to the damaged tissue which produces the swelling characteristic observed post injury. White blood cells (WBC) are speculated to be the first cells to infiltrate damaged muscle (Tidball, 1995), while increases in circulating (Cannon et al., 1990; Smith et al., 1989) and accumulating WBC in the skeletal muscle tissue (Fielding et al., 1993) have been reported in humans following eccentric exercise.

Most investigations have shown that macrophages are the predominant inflammatory cell type at all stages of inflammation following the first 12 hours post injury (Orimo, Hiyamuta, Arahata, & Sugita, 1991; Tidball, 1995). Following hind limb suspension and reloading to examine inflammation, it has been demonstrated that macrophages exhibit a large increase in concentration at later stages of inflammation (Krippendorf & Riley, 1993), suggesting that WBC and macrophages together play

important roles in the process (Tidball, 1995).

A prominent role of inflammatory cells is the removing of tissue debris and is necessary for the successful repair of injured muscle.  It has been determined that the efficiency and ability of WBC and other macrophages to perform phagocytosis is crucial in the proper regeneration of skeletal muscle as younger mice show a greater regenerative capacity relative to the old (Zacks & Sheff, 1982).

The overall inflammatory process can be summed up as a quick and early invasion of WBC, followed by increases in macrophages and subsequent removal of cellular debris, allowing for commencement of regeneration of the muscle fiber (Tidball, 1995).

## 2.4.2  Regeneration of Myofibers

While myofibres are generally considered to be irreversibly post mitotic, the regenerative capacity of skeletal muscle by way of satellite cells that lay between the basal lamina and plasma membrane of the muscle fiber helps restore any injured contractile mechanisms.  In response to injury these cells proliferate, differentiate into myoblasts, and form with each other to create multinucleated myotubes (Järvinen et al., 2005).  These newly formed myotubes fuse with parts of the injured myofiber that survived the initial trauma, and eventually the regenerating parts of the myofibers return to their mature form.  The regenerative capacity of skeletal muscle in response to injury is significantly reduced with age.  This decrease is not attributable to the number of active satellite cells but to an overall decline in the regenerative capacity of the aged muscle, as each phase of the repair process slows down over time (Jarvinen, Aho, Lehto, &

Toivonen, 1983).

### 2.4.3  Scar Tissue Formation

Immediately after an injury to the skeletal muscle, a gap formed between the ruptured muscle fibers is filled with a hematoma (Järvinen et al., 2005).  Within the first day inflammatory cells including phagocytes invade the hematoma and begin to dispose of the blood clot (Hurme, Kalimo, Lehto, & Järvinen, 1991; Tidball, 1995).  Blood derived fibrin and fibronectin cross-link to form an initial scaffold and anchorage sites which provides the wound tissue strength to withstand contraction forces applied to it (Hurme et al., 1991; Lehto, Duance, & Restall, 1985), allowing the invading fibroblasts the opportunity restore integrity to the connective tissue framework (Goetsch, Hawke, Gallardo, Richardson, & Garry, 2003; Lehto et al., 1985).

The connective tissue scar produced at the injury site is the weakest point of the injured skeletal muscle early after trauma (Hurme et al., 1991).  As more collagen is produced, the mechanical stability of the scare tissue increases up to approximately 10 days following the trauma, at which point the muscle tissue adjacent to the scar is more likely to rupture if put under stress (Järvinen et al., 2005).  The majority of skeletal muscle injuries heal without the formation of any disabling fibrous scar, but if the injury is severe enough the resultant scar can create a mechanical barrier that considerably delays or even completely restricts the myofibers across the injury gap (Järvinen et al., 2005).

### 2.4.4  Vascularization of Injured Muscle

A crucial process of muscle regeneration to allow functional recovery of skeletal muscle following injury is vascularization.  The restoration of blood supply to the injured area is the first sign of regeneration and a necessary step for the subsequent morphological and functional recovery of the muscle (Järvinen et al., 2005; Turner & Badylak, 2012).  Newly formed capillaries provide an aerobic metabolism for the myofibers, which is crucial as newly formed thin myotubes do not progress towards fully functional muscle absent the required supply of oxygen (Järvinen et al., 2005).

### 2.4.5  Regeneration of Intramuscular Nerves

Along with vascularization of the muscle, neuromuscular connections must be reestablished or regenerating muscle will become atrophic (Turner & Badylak, 2012).  In cases of axon rupture due to injury, reinnervation requires regrowth of a new axon distal to the rupture. Because these axons are usually ruptured within or next to the muscle, the nerve-muscle contact is fairly readily reestablished (Järvinen et al., 2005).

### 2.5    Muscle Strength

Following high intensity ECC exercise of the elbow flexors, there is a dramatic 50% loss in strength which is gradually but not fully restored 10 days post damage (Clarkson, Nosaka, & Braun, 1992).  Muscle soreness is unlikely the cause of this drop in strength, as perceived soreness is not experienced until after the initial immediate drop in strength, and strength loss persists long after soreness diminishes (Clarkson & Sayers,

1999). Decreased contractile protein content of the muscle is also not related to the initial

drop isometric tetanic force but was found to be partly accountable for decreased force at

the point of five days post exercise and beyond (Ingalls, Warren, & Armstrong, 1998).

Following ECC contractions skeletal muscle demonstrated a delay between the start of

muscle stimulation and contraction (Brown, Child, Donnelly, Saxton, & Day, 1996),

suggesting a failure of excitation-contraction coupling (Warren et al., 1993), which has

been shown to account for up to 75% of the drop in isometric force from 0 to 5 days post

exercise (Ingalls et al., 1998).

ECC exercise also produces an impairment in the muscles ability to generate

force when stimulated at low frequencies (Newham et al., 1983), possibly resultant from

a reduction in calcium release due to structural protein damage of the sarcoplasmic

reticulum (Westerblad, Duty, & Allen, 1993). This phenomena has been proposed as the

result of reactive oxygen species (ROS) causing damage to the sarcoplasmic reticulum-

calcium release mechanism (Essig & Nosek, 1997).

A change in the strength-tension relationship of the muscle has been observed

following ECC exercise as the greatest decrease in the elbow flexors ability to generate

force was at the shortest muscle length (Saxton & Donnelly, 1996). This

disproportionate loss of strength at shorter muscle length may be due to an overstretching

of sarcomeres from the lengthening contractions, implying fewer cross bridges formed

between the actin and myosin reducing the ability to generate contractile force (Clarkson

& Sayers, 1999).

## 2.6.1    Estrogen

The term "estrogens" describes a collection of molecules secreted primarily by the ovaries, but also to a lesser extent the testes of men.  They are involved in the development and maintenance of normal sexual and reproductive function (Heldring et al., 2007), but have also demonstrated the ability to exert a wide range of biological effects in physiological systems including the cardiovascular, immune, nervous, and musculoskeletal (Katzenellenbogen et al., 1995).  Estrogen has been shown to attenuate inflammation and damage while enhancing repair in neural and hepatic tissues (Harada et al., 2001; Sribnick, Ray, & Banik, 2004), as well as exert protective effects on cardiac, smooth, and skeletal muscle (Enns & Tiidus, 2010).  The incidence of cardiac disease in populations of post-menopausal women, as well as men of the same age, has shown to be much higher than in pre-menopausal women and their associated higher levels of estrogen (Milne & Noble, 2008).  Skeletal muscle studies pertaining to the study of estrogen in animals and its related benefits have been well documented, while in humans the effects have not yet been clearly precisely determined (Tiidus & Enns, 2009).  In terms of why estrogen may have such a physiological impact has been attributed to three general thoughts:

1. Estrogen possesses a structural similarity to potent antioxidants such as vitamin E, and is thought to have a great ability in stimulating antioxidant enzymes to eliminate free-radicals and limiting oxidative damage (Enns & Tiidus, 2010; Strehlow et al., 2003).

2. Due to a structural similarity to cholesterol, estrogen may have the ability to interact with membrane phospholipids to exert a membrane stabilizing effect

(Enns & Tiidus, 2010; Whiting, Restall, & Brain, 2000)

3. Discovery of three types of estrogen receptors has led to the discovery that estrogen may aid in the regulation of downstream genes and molecular targets (Enns & Tiidus, 2010; Patten et al., 2004)

## 2.6.2  Influence of Estrogen on Skeletal Muscle Function

In animals, estrogen has been shown to affect muscle fatigue as well as twitch-characteristics (Mccormick, Burns, Piccone, Gosselin, & Brazeau, 2004; Schneider, Fine, Nadolski, & Tiidus, 2004), and reductions in skeletal muscle contractility and isometric tetanic force production have been observed in ovariectomized rodents (Moran, Warren, & Lowe, 2006; Wattanapermpool & Reiser, 1999).  A reversal of this decreased maximal tetanic force production was reversed in rats that underwent estrogen replacement (Moran, Nelson, Landisch, Warren, & Lowe, 2007).  It was also discovered that more strong-binding myosin was found in estrogen supplemented animals suggesting an influence on contractile function in skeletal muscle (Moran et al., 2007).

Human studies have been performed examining sex differences in strength and fatigability during exercise but results are inconsistent.  Some studies have demonstrated a greater level of muscular endurance resulting in a longer time to fatigue in women during low to moderate intensity exercise (Fulco et al., 1999; Hunter, Critchlow, Shin, & Enoka, 2004), whereas studies incorporating higher intensity and eccentric exercise have reported no significant differences in relative strength loss or skeletal muscle fatigability between the sexes (Hubal, Rubinstein, & Clarkson, 2008; Rinard, Clarkson, Smith, & Grossman, 2000).

Studies observing the effects hormone replacement therapy (HRT) on postmenopausal women and age related declines in strength and post exercise muscle damage have been inconclusive.  Evidence that HRT can partially overcome these age related effects on muscle have been observed (Phillips, Rook, Siddle, Bruce, & Woledge, 1993; Teixeira et al., 2003), while other studies have determined that HRT has little to no effect on muscle strength and force development in humans (Bemben & Langdon, 2002; Ribom, Piehl-Aulin, Ljunghall, Ljunggren, & Naessén, 2002).

Studies done on the effects of estrogen on skeletal muscle structure and contractile function present conflicting results as so many variables come into play. Factors such as the study type, age of the subjects, types of comparisons made (pre- vs. postmenopausal, men vs. women), size and fiber type composition of the muscles examined, and the type/intensity of the exercise have all shown to play a part in the results found (Enns & Tiidus, 2010).

## 2.6.3  Estrogen's Effect on Muscle Damage and Inflammation

Creatine kinase (CK) is an enzyme expressed by various tissues and cell types that catalyzes the two-way reaction between creatine and phosphocreatine by using adenosine triphosphate.  Muscle protein CK is one of the most common markers of muscle membrane disruption in the bloodstream. Levels of CK have been found to be significantly higher in male compared to female rats following muscle injury (Amelink & Bär, 1986).  Although it has been reported that female rodents and humans clear CK from the blood faster than male mice (Warren et al., 2006), it should be noted that this does not necessarily mean that estrogen and its membrane stabilizer effect does not play any role

in the release of CK following exercise as male mice have demonstrated a direct inverse relationship between estrogen supplementation and CK release (Amelink, Koot, Erich, Van Gijn, & Bär, 1990).

Following an EIMD protocol involving downhill running with rats, females were found to exhibit significantly less muscle fiber structural damage and swelling compared to male rats (Komulainen, Koskinen, Kalliokoski, Takala, & Vihko, 1999). In a human study, postmenopausal women lacking estrogen-replacement experienced greater levels of muscle damage following ECC exercise (Dieli-Conwright, Spektor, Rice, Sattler, & Schroeder, 2009). When cells are exposed to conditions of stress or injury, free-radical reactions can occur leading to membrane disruption. Women rowers demonstrated lower levels of oxidative stress markers compared to men following a 4-week rowing training program (Ayres, Baer, & Ravi Subbiah, 1998). Parallel to those findings, women in the mid-luteal phase of their menstrual cycle had higher blood concentrations compared to men of superoxide dismutase (Chung, Goldfarb, Jamurtas, Hegde, & Lee, 1999), an important antioxidant in any living cell that is exposed to oxygen.

Animal studies have demonstrated that the post-injury muscle infiltration of leukocytes is influenced by sex and estrogen status (St. Pierre Schneider, Correia, & Cannon, 1999; Tiidus et al., 2001), and that ovariectomized rats with estrogen replacement exhibited significantly attenuated neutrophil infiltration 1 hour post exercise compared to non-supplemented rats and simultaneously attenuated calpain activity was observed due to decreased calcium influx into the cell (Tiidus et al., 2001). In human studies, some have reported lower levels of leukocyte infiltration post ECC training in

women relative to men (Stupka et al., 2000), while others reported no differences (MacIntyre, Reid, Lyster, & McKenzie, 2000). These differences may be attributed to differences in protocols and experimental methods, or it may be possible that other sex hormones exert effects on post-exercise leukocyte infiltration (Enns & Tiidus, 2010).

## 2.7    T2 Relaxation Time

The use of MRI has allowed for a significantly greater understanding of skeletal muscle metabolism and physiology. The T2 relaxation time is a quantitative measure of a basic biophysical property that leads to signal contrast on MRI (Mathur et al., 2011). Magnetic resonance signals are generated by placing samples in a powerful static magnetic field and then pulsing them with radio-frequency energy (Adams et al., 1992). Susceptible hydrogen nuclei will be boosted to a higher energy state, producing a detectable signal which declines over time via concurrent processes of "relaxation" (Adams et al., 1992; Meyer & Prior, 2000). T1 relaxation involves the loss of energy to surrounding nuclei with similar resonant frequencies while T2 relaxation results from interactions between excited nuclei and any perturbing magnetic fields (Adams et al., 1992).

The signal intensity of tissue in an MRI depends on the imaging echo time, the concentration of hydrogen nuclei in the tissue, and T2 of those hydrogen nuclei in the magnetic environment (Meyer & Prior, 2000). Tissues like bone and fat possess slower relaxation times than skeletal muscle and will thus appear 'brighter' in images (Meyer & Prior, 2000).

Changes in the T2 of skeletal muscle have been observed during both acute

physiological responses in healthy muscle and under pathophysiological conditions

(Mathur et al., 2011).  Increases in T2 have been observed in activated muscles have been

shown to last approximately 1 hour post-exercise (Larsen, Ringgaard, & Overgaard,

2007; Bickel, Slade, & Dudley, 2004), and although the mechanism resulting in this

increase is not fully known, it has been attributed to a redistribution of water molecules in

the muscle cells (Meyer & Prior, 2000; Saab, Thompson, & Marsh, 2000).  A second T2

increase develops gradually after 1 to 6 days but only following ECC and not CON or

isometric exercise (Fleckenstein, Weatherall, Parkey, Payne, & Peshock, 1989).  The

time course and magnitude of this delayed T2 increase and its association with other

markers of muscle damage have led to the conclusion that T2 reflects edema (Nosaka &

Clarkson, 1996), although some studies monitoring subjects over longer periods of time

have found elevations of T2 up to 2-3 months following a single bout of exercise

(Fleckenstein et al., 1989; Shellock, Fukunaga, Mink, & Edgerton, 1991).  This suggests

muscle T2 may remain long after swelling as subsided and that this residual T2 increase

may not be solely attributed to extracellular water accumulation and could possibly

reflect a long-lasting adaptation (Foley, Jayaraman, Prior, Pivarnik, & Meyer, 1999).

# Chapter 3

## METHODS

## 3.1    Subjects

Nine (6 men, 3 women; age: 20-31) recreationally active individuals volunteered for this study.  All subjects were recruited from the student population at the University of Western Ontario.  Inclusion criteria for both groups of subjects were as follows: recreationally active, absence of neuromuscular degenerative disorders, and no previous history of major injury/surgery to the biceps brachii.  Recreationally active was defined as a minimum of one upper-body resistance training session every two weeks consistently for three months.  In addition, the women must not have been on any birth control or hormonal interventions.  The study protocol was designed to assess T2 relaxation time, MVC, cross-sectional area (CSA), and pain measures between men and women following an eccentric contraction exercise protocol of the elbow flexors.

Prior to enrollment in the study, subjects were asked to fill out an MRI screening questionnaire to determine if they were eligible to participate in the study (Appendix A). The questionnaire was similar to clinical MRI screening and ensured that the subjects were free of any metal fragments such as surgical implants, pins, clips, and shrapnel.  The study protocol was approved by the University of Western Ontario ethics committee (Appendix B).

## 3.2    Anthropometry

Body mass and height were measured, without footwear, to the nearest 0.1 kilogram (Health O Meter, Balance Beam Scale), and nearest 0.01 meter (stadiometer attached to Health O Meter), respectively.

## 3.3    Exercise Protocol

Written informed consent was obtained from all participants prior to the exercise protocol. Subjects were asked to wear unrestrictive clothing before undergoing a standardized ECC exercise protocol. A single-arm preacher curl method was used in which the subject sat resting one arm on the pad as the only method of force production. Prior to the initial max effort testing protocol participants were familiarized with the preacher curl unit and allowed a brief warm-up. 10 sets of 10 repetitions at 110% of the subjects' 1-repitition maximum (1RM) were performed with their non-dominant hand. Subjects were instructed to rest for a one-minute time period in between sets. The CON portion of the exercise was aided by an investigator so that no load was exerted on the subjects. A three-second count for the ECC portion of the contraction was verbally communicated and monitored by an investigator. The protocol was ceased if the subject exceeded the three-second count on more than two occasions consecutively.

## 3.4    Strength, Elbow Angle and Pain Measures

MVC was recorded from each subject with a Cybex Humac Norm Dynamometer (CSMI Medical Solutions, Stoughton, MA. USA) using the Research

Toolkit software.  MVC was measured and analyzed offline using Spike2 software.

Subjects sat in a Hammer Strength unilateral preacher curl machine with their arms

firmly anchored at 90° to their shoulder without any internal or external rotations of the

elbow joint.  Seat height ensured the subject's arm remained at a precise angle to limit

scapula retraction.  Two attempts were completed with a two minute break separation.

The greatest of these values were used as the subjects 1RM.  MVC was determined prior

to the exercise protocol, directly following the exercise protocol, as well as 48 and 72

hours after the protocol.

　　　　Standing elbow angle was determined using a goniometer prior to strength

testing at each data collection point.  The goniometer was aligned to the lateral

epicondyle of the humerus with each end of the apparatus aligned to the acromion

process of the scapula and styloid process of the radius, respectively.  As passive elbow

angle demonstrates the elastic and tensile properties of the biceps brachii muscle and

tendon.  Following damage to a muscle, it will remain partially contracted indicating a

degree of membrane damage at smaller elbow angles.  This protective effect may be a

result of the nervous system trying to prevent further damage or contractures occurring in

the connective tissues fusing together.

　　　　Pain measures were gathered using a visual pain scale (VPS) (Verbal Numerical

Rating Score) as well as a pain algometer (Wagner Instruments Model: FPX 50,

Greenwich, CT. USA) at the time of the MVC measurements.  The VPS was ranked 0-10

(10 being worst pain imaginable) and the algometer measurement was determined

through application to the distal end of the biceps and measured in newtons.  Algometer

measurement was reached following a verbal cue from subjects that pain threshold was

reached.

## 3.5    Cross-sectional Area and T2 Relaxation Time

Elbow flexor cross-sectional area and T2 relaxation times were measured using images acquired from a MRI scanner (Siemens 3T BIOGRAPH mMR) at St. Joeseph's Hospital (Figure 3.1).  All MRI scans were performed by one of two trained operators. Subjects were positioned prone with their non-dominant arm raised above their head. This position was used to ensure that the elbow flexors were as close to the middle of the bore as possible to acquire the best signal.  To ensure minimal movement during the sequence, the participant's arm was wrapped and supported by a flex coil.  Participants' bodies were positioned and supported with foam blocks to assist with proper alignment within the bore and ensure adequate comfort.  Participants were inserted head first into the 60 cm wide bore of the magnet.  Ear plug protection was provided for the subjects to reduce the noise associated with the MRI.  While in the scanner the subject was in constant contact with the operator via an intercom system and a safety device was held in the hand so that the scan could be halted at any point if the subject became too uncomfortable.

The series was comprised of 32 5 mm-thick slices that were acquired perpendicular to the long axis of the humerus.  A 2D radio frequency at 250 hertz/pixel was used with the following parameters: acquisition time 6 minutes and 26 seconds, repetition time 2000 ms; echo time 14.0 ms; flip angle 70 degrees; and voxel size 0.9 x 0.9 x 5.0 mm.  Ten transverse T2 weighted images were acquired for each slice (Figure 3.2).
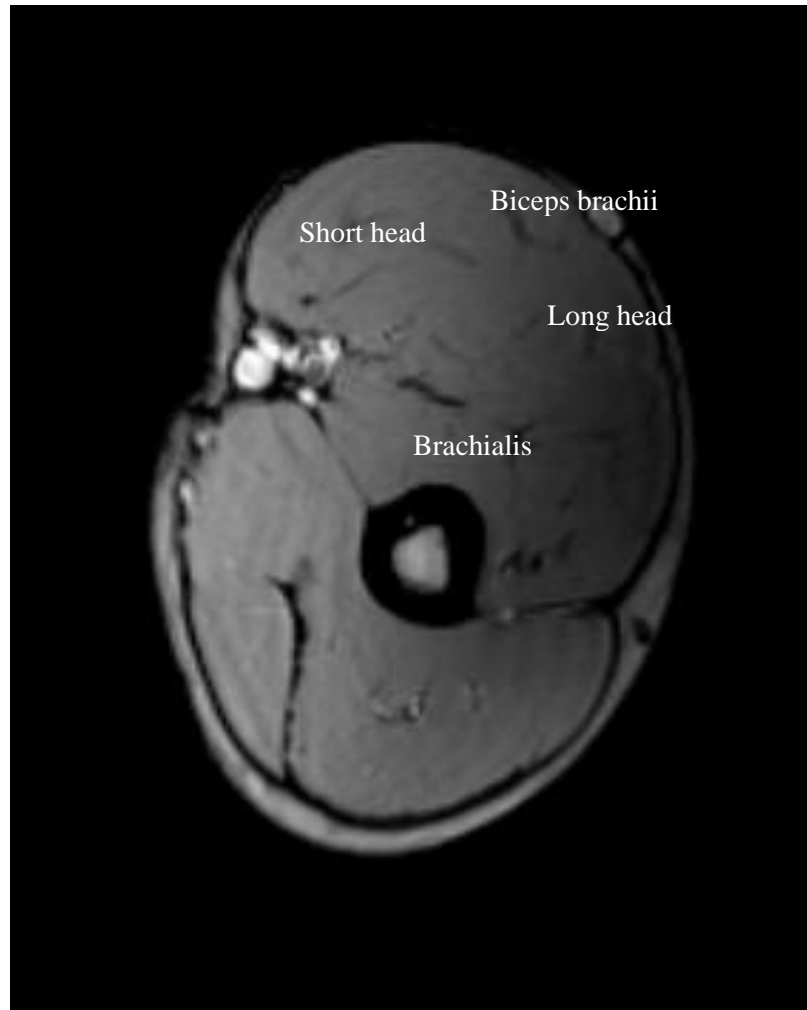
Figure 3.1. Axial Magnetic Resonance image of the elbow flexors and extensors. Image shows the two target muscles for T2 relaxation time analysis: short and long heads of the biceps brachii, and the brachialis.
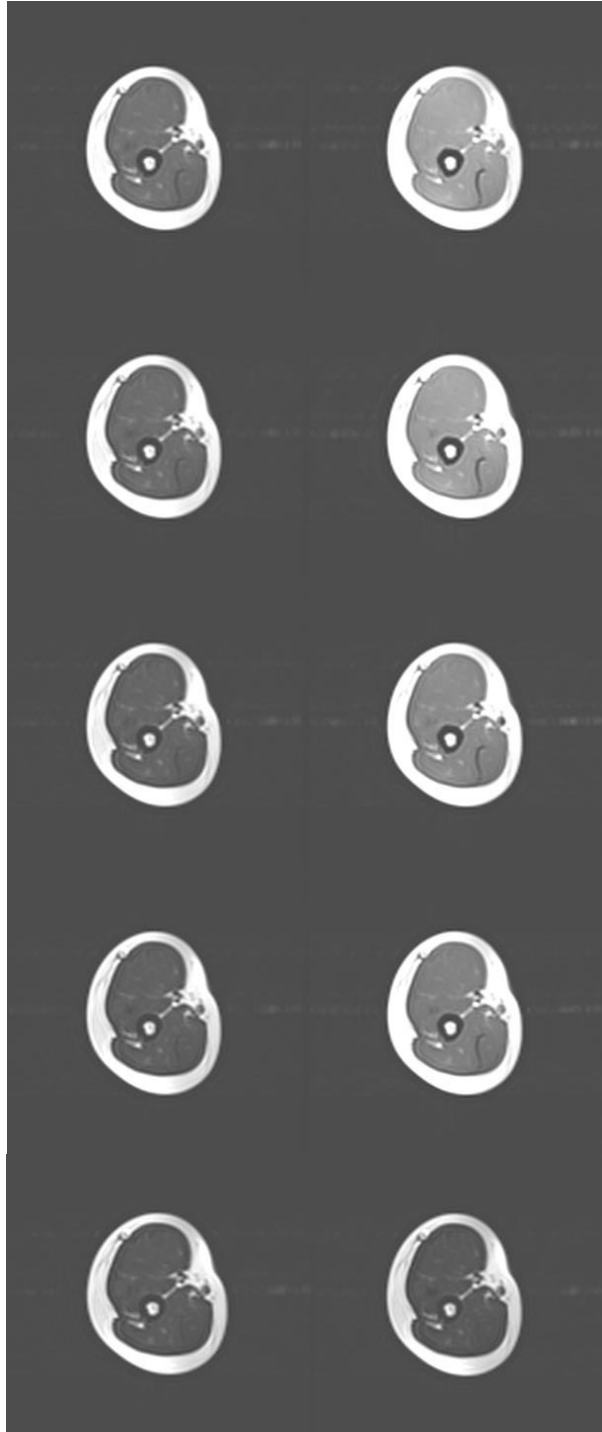
Figure 3.2. Axial magnetic resonance images showing the gradient of the 10 echo T2 relaxation time.

## 3.6    MRI Image Analysis

The images obtained from the MRI scanner were analyzed using OsiriX (Version 5.8.2).  Total elbow flexor CSA ($mm^2$) were measured by a single investigator using visual inspection and manual trace function.  Using three separate locations at the same landmarks, an average CSA was determined for each subject.  T2 relaxation times (ms) were analyzed using the T2FitMap add-on and were gathered from the region of interest (ROI) with the greatest CSA in the muscle belly in each of the brachialis, long, and short head of the biceps.  Connective and adipose tissue were intentionally avoided. It should be noted that 10 echoes were used for the T2 analysis. A single ROI was determined on one of the echo images and then propagated throughout the rest.  Only a single ROI was necessary for analysis per muscle as a single location will retrieve similar T2 measurements as gathering multiple along the length of the same muscle (Mathur et al., 2011).

## 3.7    Statistical Analysis

All statistical analyses were conducted using GraphPad Prism (version 6.01). Separate one-way analysis of variances (ANOVAs) were used to determine differences in MVC, CSA, elbow angle, and T2 relaxation times at the various time points within each of the groups.  Paired-samples t tests were used to determine differences in the pain algometer and VPS measurements within each group.  Unpaired-sample t tests were used to determine differences between groups in age, height, body mass, MVC, CSA, elbow angle, pain algometer, VPS, and T2 relaxation time measures.  All results are reported in

the text as group means ± standard deviations, and the level of significance was $p < 0.05$.

# Chapter 4

## RESULTS

## 4.1     Anthropometry

There were no significant differences in age (p = 0.999), height (p = 0.054), or body mass (p = 0.060) between the group of men and women subjects (Table 4.1).

## 4.2     Strength and Pain Measures

The men exhibited a significant decrease in MVC from baseline directly after the protocol, 48 hours after the protocol, and 72 hours after the protocol (p = 0.001).  The women however did not exhibit a significant decrease in MVC between any of the time points (p = 0.159).  There was no significant difference in MVC values between groups at any of the time points (baseline, p = 0.087; post-protocol, p = 0.845; 48 hours, p = 0.733; 72 hours, p = 0.773).  MVC data presented in Table 4.2, Table 4.4, and Figure 4.1.

Neither the men nor women had shown a significant difference in elbow angle between any of the measured time points (men, p = 0.058; women, p = 0.402).  There was no exhibited difference in elbow angle between the groups at any of the time points (baseline, p = 0.401; post-protocol, p = 0.410; 48 hours, p = 0.926; 72 hours, p = 0.461). Elbow angle data presented in Table 4.2 and Figure 4.2.

There was no significant difference in pain algometer measures in the men (p = 0.455) or the women (p = 0.802) from 48 to 72 hours post ECC protocol.  There were also no significant difference in pain algometer measures between groups at 48 hours (p = 0.915) or 72 hours (p = 0.715) post protocol.  Neither the men (p = 0.708) or the women

(p = 0.192) specified a significant difference in experienced pain by way of their VPS

measurements between 48 and 72 hours post protocol.  No significant difference in VPS

between groups at either time was observed (48 hours, p = 0.136; 72 hours, p = 0.461).

All strength and pain measures were reported in Table 4.2, Table 4.4, and Figure 4.3.

Table 4.1 **Subject Characteristics**

|  | Men (n = 6) | Women (n = 3) |
| --- | --- | --- |
| Age (years) | 24.7 ± 3.6 | 24.7 ± 2.1 |
| Height (m) | 1.79 ± 0.08 | 1.64 ± 0.08 |
| Body mass (kg) | 81.1 ± 15.2 | 66.0 ± 3.5 |

Values are mean ± standard deviation; *n* number of subjects. Differences between groups
* following post hoc testing are significant at p < 0.05

Table 4.2 **Strength and Pain Measures**

| | Baseline (pre-protocol) | 0 hours | 48 hours | 72 hours |
|---|---|---|---|---|
| Men (n = 6) | | | | |
| MVC (Nm) | 84.2 ± 16.4 | 44.7 ± 11.9* | 64.3 ± 17.8* | 63.5 ± 21.2* |
| CSA (cm$^2$) | 22.7 ± 7.0** | | 27.3 ± 7.8** | 26.0 ± 8.6 |
| Elbow Θ (°) | 167.5 ± 4.1 | 148.7 ± 13.8 | 153.3 ± 16.6 | 154.0 ± 10.7 |
| Pain algometer (N) | | | 124.5 ± 74.3 | 131.7 ± 69.7 |
| VPS | | | 5.1 ± 0.5 | 4.8 ± 1.6 |
| Women (n = 3) | | | | |
| MVC (Nm) | 55.2 ± 7.6 | 30.9 ± 4.9 | 36.4 ± 6.6 | 34.9 ± 6.2 |
| CSA (cm$^2$) | 13.8 ± 3.1** | | 16.6 ± 4.3** | 18.7 ± 6.9 |
| Elbow Θ (°) | 163.3 ± 10.4 | 156.7 ± 10.4 | 154.7 ± 8.1 | 146.3 ± 19.8 |
| Pain algometer (N) | | | 119.3 ± 36.0 | 114.9 ± 40.1 |
| VPS | | | 3.7 ± 2.1 | 6.0 ± 3 |

Values are mean ± standard deviation; *n* number of subjects; *Nm* Newton meters; ° degrees; *N* Newtons. Within group differences from * baseline, † the previous time period, or between group differences ** at the same time point following post hoc ANOVA and unpaired t-tests respectively are significant at $p < 0.05$.

## 4.3    Cross-sectional Area

There were no significant differences in CSA between any of the time points in either the men ($p = 0.071$) or the women ($p = 0.159$).  There were however significant differences between CSA in men compared to women at baseline ($p = 0.034$), and 48 hours ($p = 0.033$), but not 72 hours ($p = 0.226$) post ECC protocol.  The results of the measured CSA's are presented in Table 4.2, Table 4.4, and Figure 4.4.  Figure 4.5 demonstrates the time course of the potential swelling in a single subject.

## 4.4    T2 Relaxation Times

Neither the men (p = 0.112) or women (p = 0.069) demonstrated any significant differences in T2 relaxation time between any of the time points in the brachialis muscle except for the time period between 48 and 72 hours in the women.  There were no significant differences between the groups at any of the measurement time points (baseline, p = 0.311; 48 hours, p = 0.816; 72 hours, p = 0.205) (Figure 4.6).

The men (p = 0.118) and women (p = 0.413) exhibited no significant differences in T2 relaxation times in the short head of the biceps between any of the time points. There were no significant differences between the groups at any of the measurement times either (baseline, p = 0.856; 48 hours, p = 0.935; 72 hours, p = 0.778) (Figure 4.7).

The long head of the biceps showed now significant difference of T2 relaxation in either the men (p = 0.089) or women (p = 0.087) between each of the time points. There were no significant differences between the groups at any of the measurement times either (baseline, p = 0.967; 48 hours, p = 0.637; 72 hours, p = 0.292) (Figure 4.8).

The average of the T2 relaxation times for the three elbow flexors was determined. It was discovered that there was a significant difference in the men values between baseline to 72 hours, and 48 to 72 hours post ECC protocol (p = 0.022).  No significant differences between any of the time points were found in the women group (p = 0.441).  Between group differences in the average T2 relaxation times were not found to be significant (baseline, p = 0.252; 48 hours, p = 0.493; 72 hours, p = 0.178) (Figure 4.9).  The results of all the MRI T2 relaxation time measurements are presented in Table 4.3 and Table 4.4.

Table 4.3 **T2 Relaxation Time Measures of Elbow Flexors**

|  | Baseline (pre-protocol) | 48 hours | 72 hours |
|---|---|---|---|
| Men (n = 6) |  |  |  |
| Brachialis (ms) | 37.0 ± 2.8 | 49.7 ± 17.6 | 53.6 ± 18.1 |
| Short head (ms) | 38.2 ± 2.0 | 42.6 ± 9.5 | 49.1 ± 12.8 |
| Long head (ms) | 38.3 ± 2.5 | 47.8 ± 16.7 | 54.5 ± 18.8 |
| Average (ms) | 37.8 ± 2.4 | 46.7 ± 14.5 | 52.4 ± 16.0*† |
| Women (n = 3) |  |  |  |
| Brachialis (ms) | 38.8 ± 0.54 | 52.4 ± 10.9 | 70.6 ± 14.7† |
| Short head (ms) | 38.4 ± 1.1 | 43.1 ± 4.4 | 52.4 ± 22.0 |
| Long head (ms) | 38.3 ± 1.2 | 53.7 ± 17.4 | 68.5 ± 13.1 |
| Average (ms) | 38.5 ± 0.9 | 49.7 ± 11.6 | 63.9 ± 17.1 |

Values are mean ± standard deviation; *n* number of subjects; *ms* milliseconds. Within group differences from * baseline, † the previous time period, or between group differences ** at the same time point following post hoc ANOVA and unpaired t-tests respectively are significant at $p < 0.05$.

Table 4.4 **Relative (%) Differences of Measures from Baseline to 72 Hours**

|  | Men | Women |
|---|---|---|
| Brachialis T2 | 47.6 ± 59.8 | 81.9 ± 38.2 |
| Short head T2 | 29.6 ± 37.4 | 37.5 ± 61.6 |
| Long head T2 | 41.0 ± 42.6 | 78.8 ± 31.0 |
| Average T2 | 38.6 ± 8.76 | 65.8 ± 25.5 |
| CSA | 14.5 ± 22.5 | 32.6 ± 19.2 |
| MVC | -33.6 ± 21.1 | -15.2 ± 20.7 |

Values are mean ± standard deviation. Between group differences ** following post hoc unpaired t-tests are significant at $p < 0.05$.
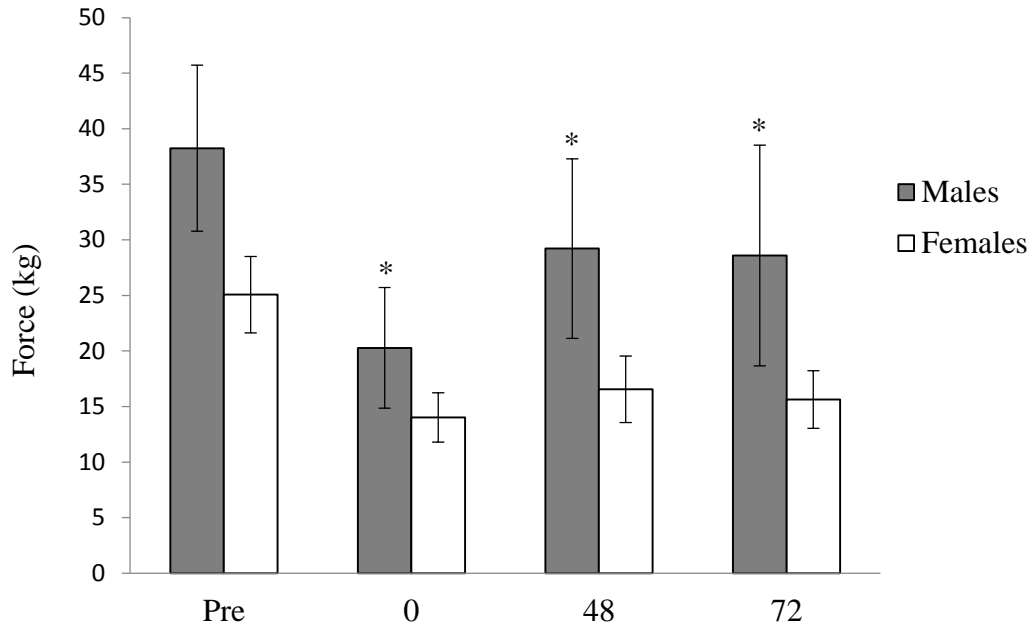
Figure 4.1. MVC in both men and women at baseline, immediately post ECC protocol, 48, and 72 hours post ECC protocol. Within group differences from * baseline, † the previous time period, or between group differences ** at the same time point following post hoc testing are significant at $p < 0.05$.
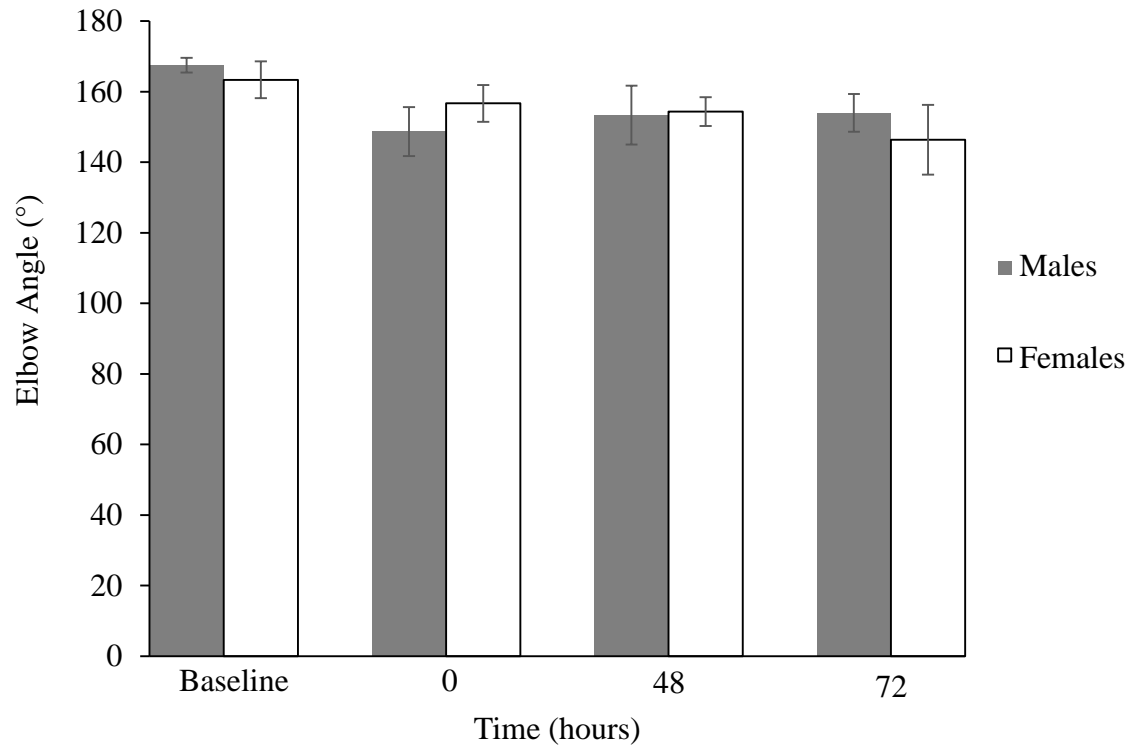
Figure 4.2. Elbow angles of both men and women baseline, immediately post ECC protocol, 48, and 72 hours post ECC protocol. Within group differences from * baseline, † the previous time period, or between group differences ** at the same time point following post hoc testing are significant at $p < 0.05$.
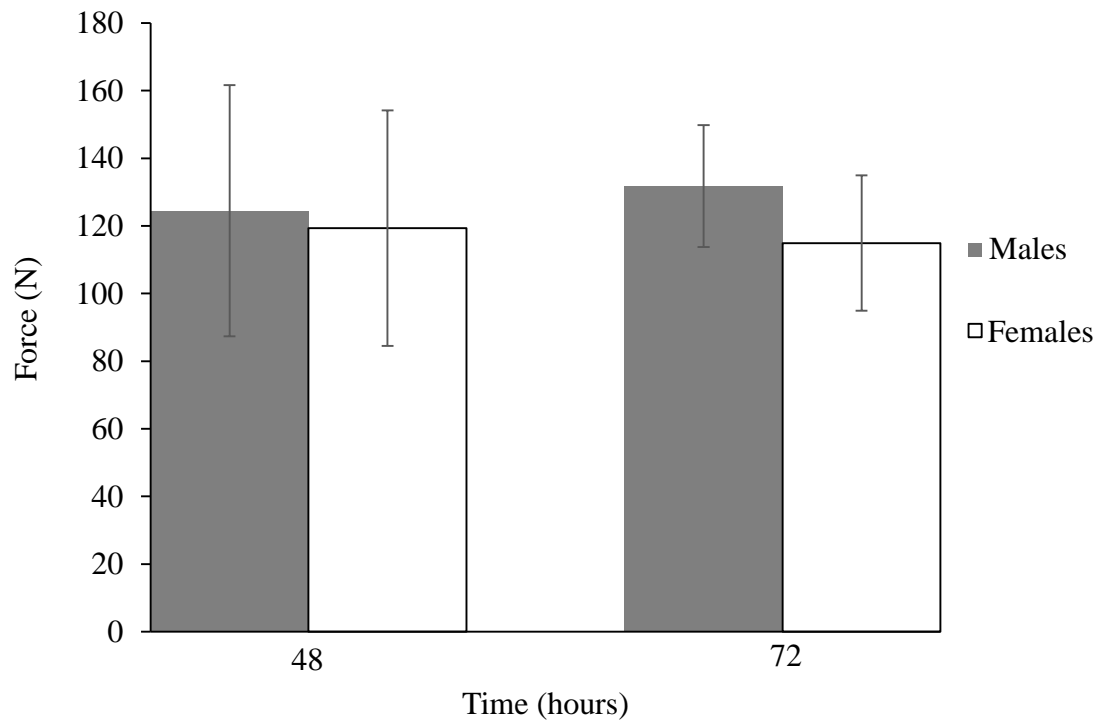
Figure 4.3. Pain algometer values retrieved from distal belly of the biceps brachii in both men and women at both 48 and 72 hours post ECC protocol. Within group differences from † the previous time period, or between group differences ** at the same time point following post hoc testing are significant at $p < 0.05$.

Figure 4.4. CSA in both men and women at baseline, 48, and 72 hours post ECC protocol. Within group differences from * baseline, † the previous time period, or between group differences ** at the same time point following post hoc testing are significant at $p < 0.05$.
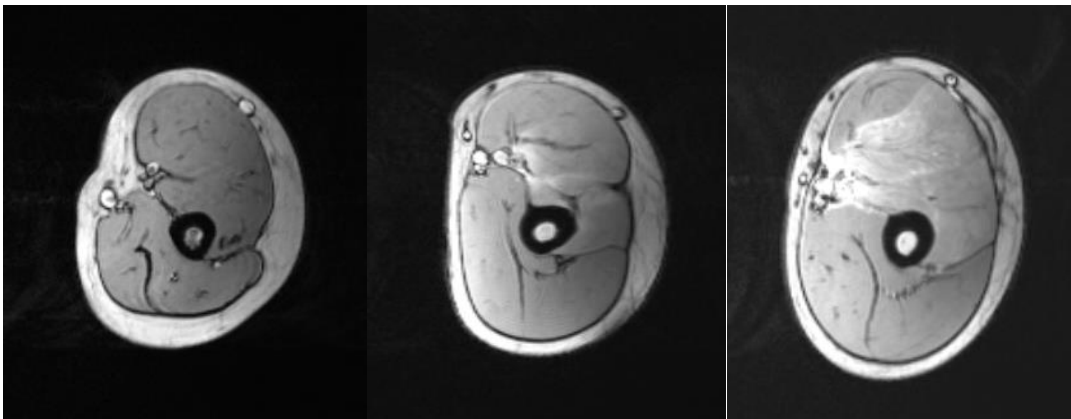


Figure 4.5. Axial magnetic resonance images of the elbow flexors and extensors at baseline, 48 hours post protocol, and 72 hours post protocol respectively.
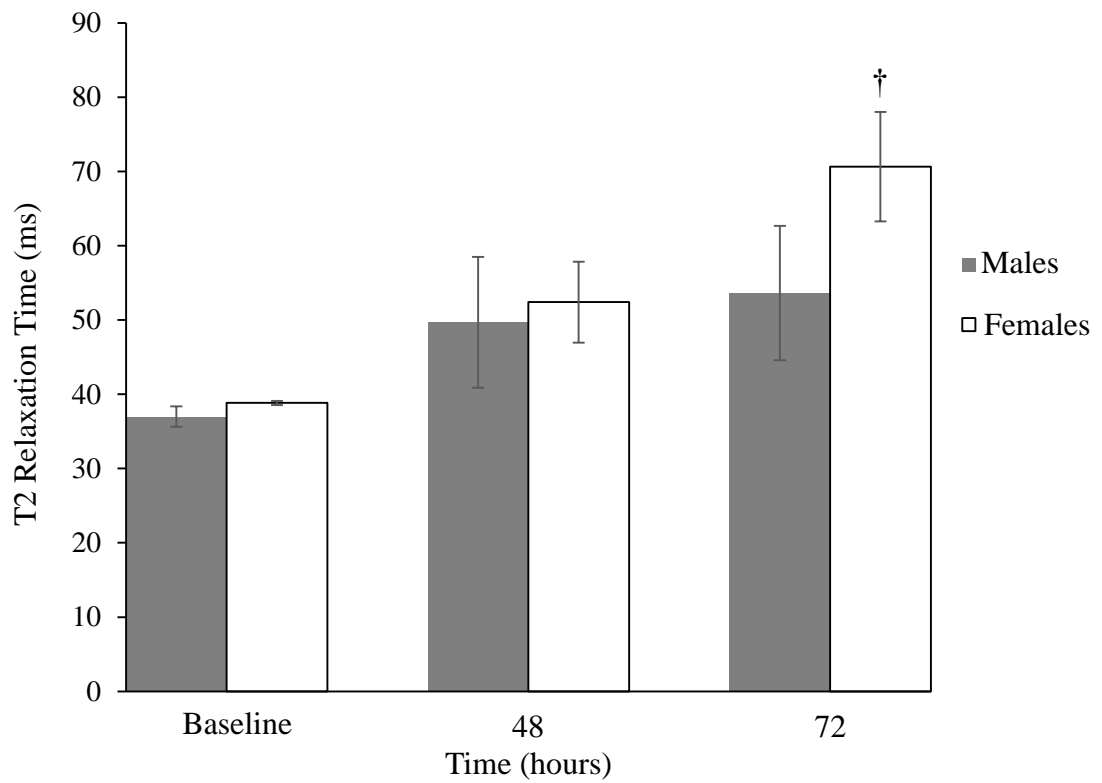
Figure 4.6. T2 relaxation time values of the brachialis in both males and females over the three MRI times. Within group differences from * baseline, † the previous time period, or between group differences ** at the same time point following post hoc testing are significant at $p < 0.05$.
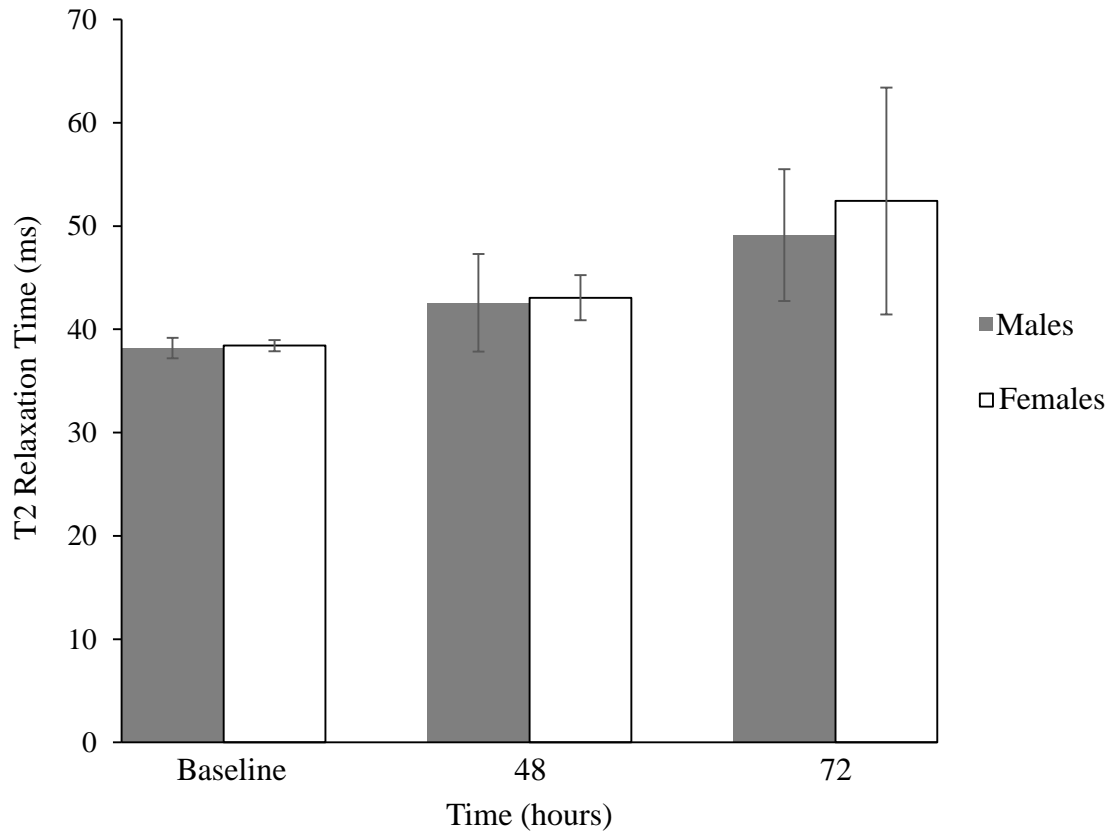
Figure 4.7. T2 relaxation time values of the short head of the biceps in both males and females over the three MRI times. Within group differences from * baseline, † the previous time period, or between group differences ** at the same time point following post hoc testing are significant at p < 0.05.
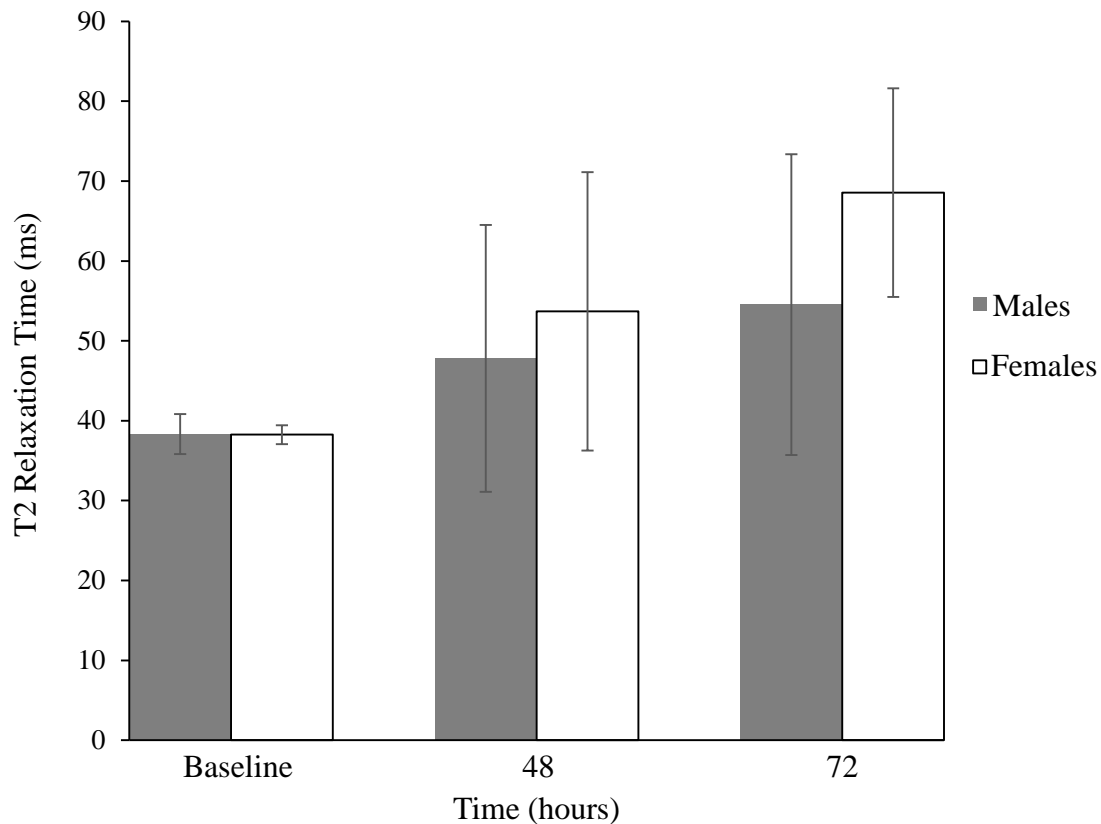
Figure 4.8. T2 relaxation time values of the long head of the biceps in both males and females over the three MRI times. Within group differences from * baseline, † the previous time period, or between group differences ** at the same time point following post hoc testing are significant at p < 0.05.
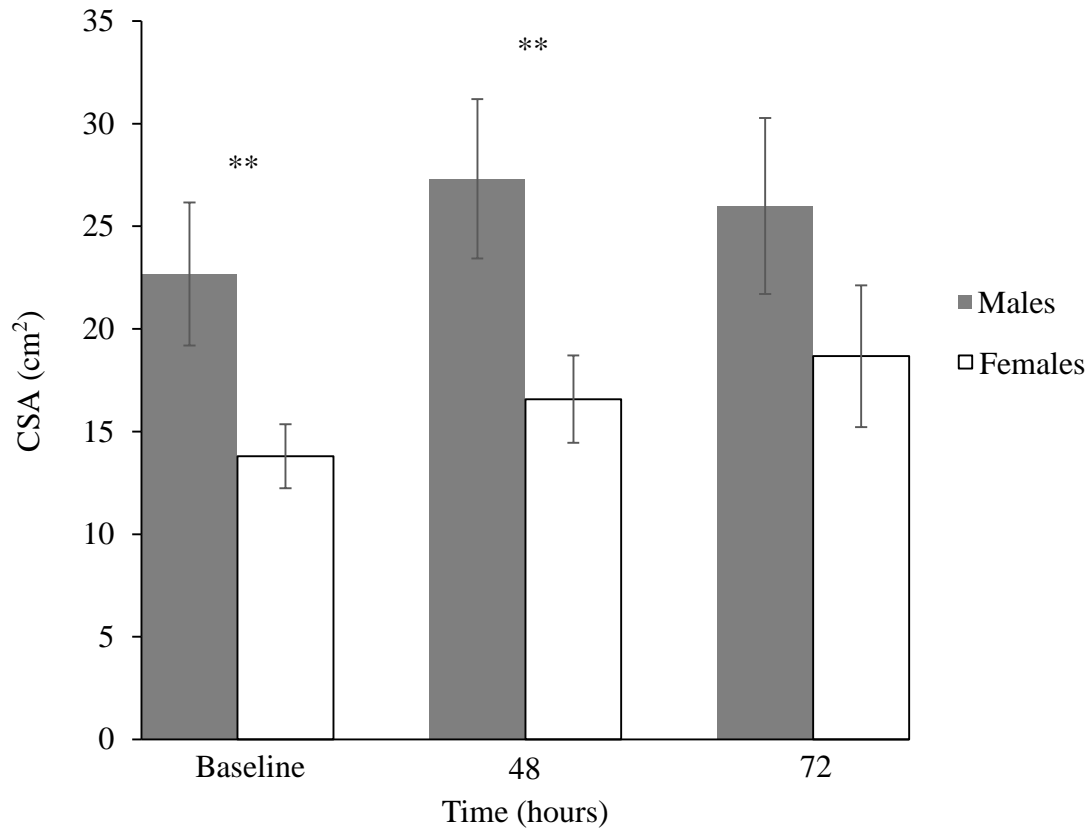
Figure 4.9. Average T2 relaxation time values of the combined brachialis, and the two heads of the biceps in both males and females over the three MRI times. Within group differences from * baseline, † the previous time period, or between group differences ** at the same time point following post hoc testing are significant at $p < 0.05$.
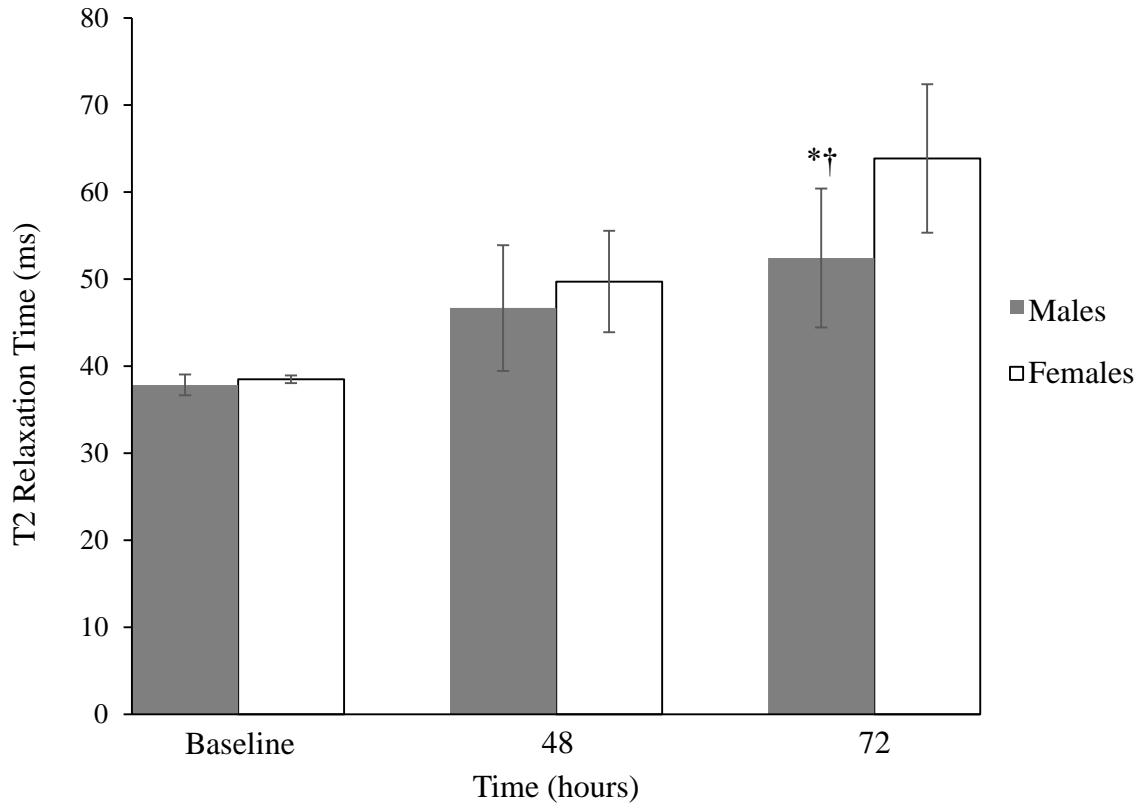
# Chapter 5

## DISCUSSION

The purpose of this study was to compare the T2 measures of men and women following a high intensity ECC exercise protocol and to relate these measures to other markers of muscle damage including muscle strength, CSA, and pain measures.  It was hypothesized that men would exhibit a larger increase in T2 relaxation time, more substantial loss of strength, greater increases in CSA due to inflammation, and higher levels of experienced pain following the ECC protocol due to the potential muscle protective effects of estrogen.  It was also hypothesized that the more superficial biceps would exhibit higher changes in T2 relaxation measures compared to the deeper brachialis.  On an individual muscle level, there were no significant differences between or within groups in terms of T2 relaxation time.  When the averages of the brachialis along with the short and long heads of the biceps brachii were observed together, a significant difference was observed in the men and not the women in measures between baseline and 72 hours following the protocol.  Corresponding with the T2 measures men experienced a significant decrease in MVC values whereas women did not.  There was a significant difference between groups in CSA at baseline and at 48 but no longer at 72 hours post protocol.  No significant differences were observed within or between groups in the measures of elbow angle, pain algometer, or VPS measures at any of the points throughout the study.

The inconsistent T2 relaxation time findings of the present study align with those of previous longitudinal studies documenting increases in T2 measures following ECC exercise (Larsen et al., 2007; Shellock et al., 1991).  Both the men and women

demonstrated an increase in T2 relaxation times over the course of the study following the ECC protocol but with the only significant changes observed in the men from baseline, and the women in the brachialis. For this study, immediate T2 measurements were not taken directly after the exercise. Immediate post-exercise elevations of T2 has been used in past studies as an index of exercise intensity (Adams et al., 1992; Fisher et al., 1990). Identical loads, sets, repetitions, and timing of two exercise regimens yielded identical acute T2 relaxation values (Foley et al., 1999). This study demonstrated a continual, but for the most part insignificant increase up until the 72 hour time period post exercise for most of the T2 measures. The persistent and continual elevation of T2 up to 7 days post ECC exercise outlasts other indicators of injury such as CK, pain levels, and muscle volume suggesting that T2 may reflect a more permanent change in the muscle (Foley et al., 1999). This study attempted to search for differences in T2 measures as an indication of acute muscle damage, but it may be of benefit to monitor T2 levels along with other indicators such as strength and CSA over a longer period of time if feasible.

A protective effect exists conferred by a single bout of eccentric exercise against muscle damage from following bouts known as the "repeated-bout effect". Many studies have demonstrated that peak changes in numerous objective measures of muscle damage were smaller and occurred earlier following the second compared to the initial bout of ECC exercise, extending even to the response of T2 (Foley et al., 1999). Excessive strain produced by bouts of unaccustomed ECC exercise could mechanically disrupt the sarcomere and/or connective tissue enough to result in permanent and irreversible necrosis to the most damaged fibers (Armstrong, Ogilvie, & Schwane, 1983). As

subsequent bouts of ECC exercise produce reduced responses in indicators of muscle damage including a blunted response in T2 relaxation measures helps to confirm the idea and utility of T2 relaxation time as a marker of muscle damage.

Indirect measures of muscle damage were recorded throughout the study to compare to the T2 relaxation time findings as an attempt to correlate and quantify the magnitude of damage relative to the MRI findings. Increases in muscle CSA following unaccustomed exercise and muscle damage is mostly attributed to the invasion of WBC's, macrophages, and extracellular proteins due to the structural disruption of the extracellular matrix and damage to the myofibers. Theoretically, the larger the increase in muscle CSA, the greater degree of the damage. This study was meant to test the idea that women may exhibit some kind of membrane stabilizing effect on skeletal muscle. In past rodent studies, it was found that female rats exhibit less muscle fiber structural damage and swelling compared to males following a downhill running protocol (Komulainen et al., 1999), as well as reduced neutrophil infiltration post-exercise in females with greater levels of estrogen (Tiidus et al., 2001). While a significant difference in absolute CSA was seen between the men and women at both baseline and 48 hours post ECC protocol, these changes have been attributed to the size difference between the two groups, as no significant relative changes in CSA were found. This does not however rule out the potential stabilizing effect, as the protocol was extremely high intensity relative to a more moderate exercise which may cancel out any positive protective effects on skeletal muscle.

Similarly to other high intensity ECC exercise protocol's which demonstrated a 50% strength loss following the damage (Clarkson et al., 1992), this study also exhibited

a similar reduction (men: 52%; women: 30%).  Rodent studies have demonstrated reduced muscle contractility and force production in ovariectomized females (Moran et al., 2006; Wattanapermpool & Reiser, 1999), while human studies have found women demonstrate greater levels of muscular endurance and time to fatigue at moderate intensity exercise levels (Fulco et al., 1999; Hunter et al., 2004).  While the absolute change was significant and greater in the men compared to the women, the relative changes within and between groups were not significant.  This initial drop attributed to the failure of excitation-contraction coupling (Warren et al., 1993) was not found to be significantly different between the sex groups and this data reflects similar findings of past studies that reported no significant differences in relative strength loss between the sexes following high intensity ECC exercise (Hubal et al., 2008; Rinard et al., 2000).  If estrogen exhibits a protective effect on skeletal muscle and strength retention, it may only be at exercise intensities at the low to moderate range of the spectrum.

There were no significant differences within or between the sexes in measures of pain throughout the study.  In past clinical studies, it has been found that women have an overall greater pain sensitivity, enhanced pain facilitation, and reduced pain inhibition compared to men (Bartley & Fillingim, 2013).  Relative pain algometer readings for both men and women in the study remained fairly consistent between the 48 and 72 hour post ECC protocol time points.  While the pain experienced in the men using the VPS remained consistent between time points, the women reported on average a 38% increase in experienced pain between 48 and 72 hours.  While not statistically significant, this trend reflects the findings of past studies.  Sex differences in pain can be affected by a multitude of factors including sex hormones, genetic factors, pain coping, and gender

roles (Bartley & Fillingim, 2013). As an absolute stimulus may be experienced differently from one individual to another regardless of sex, we cannot conclude for certain that the pain measures gathered in the study are a reflection of muscle damage and would provide a weak correlation to T2 relaxation time and muscle damage between men and women.

This study also set out to determine if the damage caused to muscles of varying fiber type would be exposed using MRI T2 relaxation time measures. As fast twitch predominate muscles experience the greatest levels of fiber disruption following ECC exercise (Lieber & Fridén, 1988), it would be expected that the change in T2 measurements in the short and long heads of the biceps would be of a greater magnitude than that of the brachialis. Table 4.4 demonstrates that the changes in brachialis T2 measurements from baseline to 72 hours post exercise protocol were 9% greater in men and 16.1% greater in women than their respective averaged T2 measurements from the three measured muscles. Table 4.4 also demonstrates the relative increases in long and short heads of the biceps compared to the three muscle average from baseline to 72 hours (Men: 6%, -23%; Women: 20%, -43%). These findings do not appear to support the hypothesis that the more superficial muscles would experience a greater relative increase in T2 times following ECC muscle damage.

The present study is not without its limitations. This longitudinal study cannot discount the potential influence of selection bias, genetic differences, varying hormonal measures within and between groups, or total exercise history. Also, the varying sample sizes of the groups may have resulted in insufficient power to detect small, but potentially significant differences in T2 and indirect muscle damage within and between groups

resulting in type II statistical error. While the women population was controlled so that no hormonal birth control would interfere with potential results, the hormonal make-up of the participants did not allow for full control over the groups. It was expected that ten sets of ten ECC contractions at 110% of the subjects MVC or contractions to failure would result in EIMD. Factors such as diet and sleep prior to the ECC protocol were not controlled for and may have affected the performance of each individual and thus the following results acquisition.

In conclusion, contrary to what was expected during a longitudinal study observing the changes in T2 relaxation time of the elbow flexors in men and women following a high intensity ECC exercise protocol, there did not appear to be any significant changes that coincided with significant changes in indirect measures of muscle damage to confidently state that there is a muscle protective effect exhibited by estrogen following EIMD or that using T2 relaxation time is an accurate tool in the quantification of muscle damage. The women did not exhibit significant differences in T2 relaxation times in any of the three observation ROI of the elbow flexor muscles compared to the men except for in the brachialis. While the men did show a significant increase in T2 relaxation time compared to the women, the relative differences between the groups from baseline to 72 hours post protocol in T2 and indirect muscle damage markers like CSA and MVC were not significant.

Although the lack of significance of these results suggest a lack of utility of T2 relaxation times to determine and quantify the magnitude of effect sex has on muscle damage or the absolute measure of muscle damage in general, past studies have demonstrated the physiological benefits of estrogen in elderly women and rodents

subjected to HRT as well as in the preservation of muscular endurance and lower

oxidative stress markers in women rowers relative to men (Ayres et al., 1998; Phillips et

al., 1993; Teixeira et al., 2003).  While this may suggest that sex may exert physiological

benefits on skeletal muscle, the intensity of the study protocol may have surpassed the

ability for any effect to be exerted.  Future longitudinal studies observing the potential

protective effect of sex on muscle and the utility of T2 relaxation times as a marker of

muscle damage should attempt to acquire larger sample sizes to further reduce the

chances of type II error.  Also, possibly increasing the length of the study while

monitoring the participants over a longer period of time as T2 relaxation time has been

shown to potentially be the result of a long-term adaptation of skeletal muscle and not

just due to fluid accumulation in the damaged area (Foley et al., 1999).  Finally,

Zaraiskaya, Kumbhare, & Noseworthy (2006) have found that another MRI technique

called diffusion tensor imaging can be used in the evaluation of muscle damage and may

be another value future studies can use to further determine effectiveness.

# Chapter 6

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# Appendix A

## MRI Screening Form

<table>
<tr>
<td>⚠️</td>
<td><strong>The 3T PET/MRI has a very strong magnetic field that may be hazardous to individuals with certain metallic, electronic, magnetic or mechanical implants/devices. All individuals are required to fill out this form and have it reviewed by a Technologist/Operator BEFORE entering the magnet room. All subjects must change into clothing that has no metal fasteners or underwires and remove all metal on their person. Please be advised that the magnetic field is ALWAYS ON.</strong></td>
</tr>
</table>

NAME: _____     HEIGHT: _

DATE OF BIRTH: _____     WEIGHT: _

**Please answer the following questions:**

❏ YES ❏ NO     HAVE YOU HAD A PREVIOUS MRI?
❏ YES ❏ NO     HAVE YOU EVER HAD A METALLIC OBJECT IN YOUR EYE?
❏ YES ❏ NO     IS THERE ANY CHANCE YOU MIGHT BE PREGNANT?
❏ YES ❏ NO     ARE YOU CLASUTROPHOBIC?

**Do you have any of the following?**

❏ YES ❏ NO     HEART PACEMAKER/WIRES/STENT/DEFIBRILLATOR/VALVES
❏ YES ❏ NO     BRAIN ANEURYSM CLIPS
❏ YES ❏ NO     SHUNT/SURGICAL CLIPS
❏ YES ❏ NO     SHRAPNEL/BULLETS
❏ YES ❏ NO     DENTURES
❏ YES ❏ NO     INTRA-UTERINE DEVICE (IUD)
❏ YES ❏ NO     OTHER IMPLANTED DEVICES (EAR IMPLANTS, EYE IMPLANTS, PROTHESES)
❏ YES ❏ NO     MEDICATION PATCHES
❏ YES ❏ NO     BODY PIERCING
❏ YES ❏ NO     PERMANENET TATTOO/EYELINER

**Please list any surgeries on the following:**

▪ HEAD _____
▪ NECK _____
▪ SPINE _____
▪ CHEST _____
▪ ABDOMEN _____
▪ EXTREMITIES _____
▪ OTHER _____

**I confirm that the above information is correct to the best of my knowledge. I have read and understood the contents of this form and have had the opportunity to ask questions**
**regarding the information on this form**

| Participants Signature: | | Date: | |
|---|---|---|---|
| Technologist/Operator Signature: | | Date: | |

# Appendix B

## Ethics Approval

**Western Research**

Research Ethics

**Western University Health Science Research Ethics Board**
**HSREB Delegated Initial Approval Notice**

**Principal Investigator**: Dr. Gregory Marsh
**Department & Institution**: Health Sciences\Kinesiology,Western University

**HSREB File Number**: 105665
**Study Title**: Degrees of Damage: Quantifying male vs. female exercise-induced muscle damage through magnetization transfer ratios
**Sponsor**: University of Western Ontario

**HSREB Initial Approval Date**: December 04, 2014
**HSREB Expiry Date**: December 04, 2015

**Documents Approved and/or Received for Information**:

| Document Name | Comments | Version Date |
|---|---|---|
| Revised Western University Protocol | | 2014/10/08 |
| Letter of Information & Consent | Revised LOI | 2014/11/27 |
| Advertisement | Revised Advert. | 2014/11/27 |

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Datenoted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB ContinuingEthics Review. If an Updated Approval Notice is required prior to the HSREB Expiry Date, the Principal Investigator is responsible for completing andsubmitting an HSREB Updated Approval Form in a timely fashion.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), theInternational Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical PracticePractices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health CanadaMedical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when theyare presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

_____
Ethics Officer, on behalf of Dr. Joseph Gilbert, HSREB Chair

Ethics Officer to Contact for Further Information

| ___ Erika Basile | ✓ Grace Kelly | ___ Mina Mekhail | ___ Vikki Tran |
|---|---|---|---|
| ebasile@uwo.ca | grace.kelly@uwo.ca | mmekhail@uwo.ca | vikki.tran@uwo.ca |

*This is an official document. Please retain the original in your files.*

**Western University, Research,** Support Services Bldg., Rm. 5150
London, ON. Canada  N6A 3K7  t. 519.661.3036  f. 519.850.2466  www.uwo.ca/research/services/ethics

# CURRICULUM VITAE

**Name:** Patrick Alexander Moull

## Education

2013 – Present   Western University
                   Master of Science in Kinesiology

2008 – 2012     Western University
                   Bachelor of Science
                   Honours Specialization in Kinesiology

## Work Experience

2013 – 2015     Teaching Assistant
                   School of Kinesiology
                   Faculty of Health Science
                   Western University