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DIURNAL EFFECTS OF EXERCISE ON MARKERS OF MUSCLE DAMAGE IN COLLEGE-AGE HEALTHY INDIVIDUALS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The primary aim of this study was to examine differences in muscle damage markers (maximal voluntary isometric contraction, range of motion, soreness, swelling, thickness, urinary titin) between a morning group and an evening group. A secondary aim was to determine a correlation between pre-exercise urinary titin concentrations and performance metrics of muscle damage, serving as a predictor of muscle damage. 28 participants were recruited and randomized into two groups (14 each). Participants either arrived at 7:00 am or 5:00 pm and were instructed to perform 3 sets of 10 eccentric bicep curls at 120% of their established one repetition concentric maximum. MVC was assessed before and after to ensure a 40% decline. ROM, DOMS, swelling, and thickness were assessed before and immediately post-exercise. Performance metrics of muscle damage were assessed, 24, 48, 72 and 96-hours post exercise, and urinary titin was measured 96 hours post-exercise to capture peak concentrations. The primary findings were that time of day did not affect the degree of muscle damage, nor baseline measures of pre-exercise urinary titin. Although post-exercise urinary titin concentrations could not be quantified due to extremely high concentrations that exceeded the assay's top limit of detection, it is clear that the muscle damaging protocol utilized in this project resulted in exaggerated urinary titin response. In the future, we plan to further explore the urinary titin response to damaging exercise and the potential diurnal variations in this response.

CHAPTER I
INTRODUCTION

Exercise induced muscle damage (EIMD) is a phenomenon that occurs following unaccustomed actions or novel stimuli to the muscle. EIMD can be defined as the mechanical disruption and damage to the sarcomere and impairment to the excitation-contraction coupling system [1]. EIMD can occur at the micro-level of muscle tissue, affecting actin and myosin, or, may manifests as large tears in the sarcolemma, basal lamina, connective tissue and cause injury to contractile protein elements and structural proteins [2]. EIMD sets off a cascade of cellular responses including inflammation and disturbed calcium regulation which correspond with increased blood markers of muscle damage and reduced force production [3]. EIMD can be identified using functional metrics such as a decrease in isometric strength (maximal voluntary contraction; MVC) and joint range-of motion (ROM), and heightened pain during movement often termed delayed onset muscle soreness (DOMS). EIMD can also be identified biochemically through an increase in blood biomarkers of cellular damage such as creatine kinase, lactate dehydrogenase, creatinine, or pyruvate dehydrogenase[4]. However, interpretation of elevated biomarkers can be difficult, as early assessment post-exercise may be more reflective of a metabolic disturbance rather than physical damage [4,5].

Emerging evidence suggests a key sarcomeric structural protein, titin, may vary its intracellular concentrations in a circadian manner with levels changing over the course of the day. Given titin's key role in the structural integrity of sarcomeres this may potentially create a vulnerable period for exercise-induced muscle damage at certain times of the day. This potential vulnerable period is explained by reductions in structural attachment of myosin to the Z-disc via reductions in the amount of titin-cap protein (TCAP) present in the sarcomere. Titin (also known as connectin) is a massive single-chain protein abundant in skeletal and cardiac muscle

sarcomeres and serves as a functional scaffold, a mechano-signaler of hypertrophy and for generating passive elastic energy as part of stretch-shortening cycle (SSC) [6]. A single molecule of titin spans the width of the sarcomere from the Z-disc to the M-line (> 1 μm long) [7]. The skeletal muscle isoform of titin is called N2A titin (3.3-3.7 MDa) and is expressed as multiple splice variants. The titin filament is arranged into several immunoglobulin-like domains (Ig-domains), fibronectin-type-3 domains and several unique sequences (US). Of particular interest is the NH_2-terminal end of titin which anchors titin to the Z-disc via TCAP and another protein called nebulin [8]. The NH_2-terminal end is comprised primarily of two proteins: α-actinin 2 and telethonin or TCAP protein. The NH_2-terminal region of titin undergoes vast amounts of splicing with increases or reductions of these repeats eliciting an increase or decreased length of titin respectively, therefore playing a critical role in muscle function.

Recent evidence has indicated that circadian rhythm plays a role in regulating skeletal muscle structure and function. Circadian rhythms are regulated by a feedback loop regulated by cell-autonomous, transcriptional-translational processes that function as a molecular clock[9]. The skeletal muscle molecular clock is regulated by what are termed clock-controlled genes (CCGs). The molecular clock is split into two phases, the active phase, and the inactive phase. In rodent models, the active phase lasts approximately 12 hours and corresponds to the darkness of nighttime while the inactive phase corresponds to approximately 12 hours of light during the daytime. This pattern of active to inactive phase reflects sleep/wake schedule of rodents. The specific CCGs termed CLOCK and BMAL1 have been shown to play a role in the active phase of the molecular clock[10] and may be of key importance for the circadian regulation of titin. For example, BMAL1 has been shown to regulate a downstream gene known as RBM20. Riley and colleagues (2022) demonstrated that mice with a BMAL1 knockout display altered splicing of

titin which led to increased heterogeneity of sarcomere length, which has been suggested to lead to an increased risk of overstretching and muscle damage[11]. Heterogenous sarcomere lengths have also been shown to be disadvantageous for force production at the whole muscle level, as the likelihood that individual sarcomeres working at their optimal length would be diminished, reducing the total number of actin-myosin crossbridge links[11].Overexpression of RBM20, in the BMAL1 knockout model was subsequently shown to rescue titin function[9]. BMAL1 has been shown to have reduced activity during the inactive phase of the skeletal-muscle molecular clock. As such titin splicing is likely altered during the inactive phase of the molecular clock in a manner that will lead to greater heterogeneity and a greater risk of muscle damage. Previous work has also demonstrated that TCAP concentrations vary in a diurnal pattern, with the highest amounts present in the active phase. TCAP levels appear to be controlled by the activity of BMAL1 and CLOCK 1,2. [9] both of which increase their expression during the active phase of the circadian clock. Because of TCAP's structural role in anchoring the myosin thick filament to the Z-disc of the sarcomere, there is a potential vulnerable period of skeletal muscle damage in the inactive phase of the skeletal-molecular when TCAP levels will be reduced.

Given the evidence of circadian variations in TCAP and titin splicing, which plays a key role in the structural integrity of sarcomeres, it is plausible that the susceptibility of sarcomeres to exercise-induced muscle damage might be different at different times of the day. This supposition is supported by previous work comparing diurnal variations in exercise-induced skeletal muscle damage in athletes, with serum biomarkers of muscle damage (creatine kinase and leukocyte infiltration) [12,13] being elevated, likely indicating greater EIMD, following strenuous exercise in the evening (17:00 h) compared to morning (7:00 h). However, these two studies have several important limitations. First, creatine kinase was assessed immediately upon

completion of exercise (it is typically assessed 24-96 hours after exercise to indicate EIMD), therefore making it impossible to determine if the magnitude of the response was due to mechanical damage of the muscle or merely a metabolic disruption. Further, the studies employed a repeated measures design whereby each participant exercised in the morning and the evening in a counterbalanced fashion. This is a limitation because the repeated bout effect was not taken into consideration in this within-subjects design. The repeated bout effect is a "protective" phenomenon where an initial bout of strenuous exercise produces muscle damage, and then limits damage in subsequent bouts that occur of up to 6 months later[14]. It is likely that the initial bout of exercise, whether it occurred in the morning or the evening, functioned to limit the magnitude of damage in the second bout. Therefore, it is difficult to interpret whether the observed differences in EIMD in the morning compared to the evening are due to diurnal timing of exercise, or if they are the consequence of the repeated-bout effect. Previous work from Yamaguchi and colleagues demonstrated urinary titin fragments (UTF) to sensitively reflect the repeated bout effect, with significant reductions in UTF in the second bout of eccentric elbow flexor exercise compared to the first bout [15]. Both visits were separated by a 2-week period.

To date, no study has assessed circadian variation in titin levels prior to performing strenuous eccentric exercise to induce EIMD. This is likely due to the fact that previously, the assessment of sarcomeric titin concentration required a muscle biopsy, gel electrophoresis and Western Blotting or immunoblotting analysis [16]. However, recent advances in proteomic tools offer a non-invasive assessment of titin using urinary concentrations coupled with assessment via enzyme-linked immunosorbent assays (ELISA). While the mechanism underlying urinary excretion of titin NH_2-terminal fragments is not fully understood, it is likely that NH_2-terminal fragments of titin are enzymatically cleaved at the muscle level and excreted into urine through

glomerular filtration [17]. Previous work from Kanda and colleagues validated urinary titin NH_2-terminal ends as a biomarker of muscle damage by demonstrating significant correlations to other serum markers of muscle damage (serum creatine kinase, myoglobin). Further, urinary titin concentrations 96 hours post-EIMD were strongly correlated with functional measures such as range of motion and delayed-onset muscle soreness [17]. They suggest the assessment of urinary titin has potential to be used as a high quality/practical biomarker of muscle damage. This is due to the non-invasive nature of sample collection and does not require specially trained personnel.

1.01 Purpose

Given the biological plausibility of circadian fluctuations in titin levels leading to a vulnerable period for EIMD and the limitations of previous human studies examining diurnal variation in the magnitude of EIMD following a bout of damaging exercise, the aim of this study will be two-fold: 1) to assess diurnal (morning vs. evening) variation in exercise-induced muscle damage in two groups of individuals (limiting the possible effects of the repeated bout effect), and 2) to examine the relationship between pre-exercise urinary titin concentrations with traditional functional measures of EIMD such as decline in MVC, joint range-of-motion, limb circumference, ultrasound assessed muscle thickness, and delayed onset muscle soreness.

1.02 Significance

Assessment of exercise induced muscle damage is important for researchers, coaches, and practitioners alike. Understanding diurnal patterns of muscle damage may be important when trying to optimize periodization schemes for various athletes. Physique and barbell athletes may want to prioritize exercising during the potential vulnerable period to maximize returns on training, whereas “in-season” athletes may want to avoid training during the potential vulnerable period as exercise induced muscle damage and the corresponding soreness may interfere with

sport-specific performance. Assessment of urinary titin and diurnal differences in muscle damage may be useful for clinical populations as well, as determining a time-of-day in which less muscle damage occurs and subsequently less associated soreness, may help with exercise retention rates. Further, assessment of urinary titin may be a non-invasive method of predicting muscle damage and serve as a reliable biomarker of muscle damage compared to commonly used analytes such as creatine kinase and lactate dehydrogenase.

1.03 Research Questions

1. Does eccentric exercise in the evening invoke a greater muscle damage response than morning?
2. Do pre-exercise urinary titin values correlate with MVC, ROM, swelling, DOMS, and thickness of the elbow flexors?
3. Do post-exercise urinary titin values correlate with MVC, ROM, swelling, DOMS, and thickness of the elbow flexors?

1.04 Research Hypotheses

1. Eccentric exercise in the evening will invoke a greater muscle damage response than morning due
2. Pre-exercise urinary titin values will have negative correlation with MVC and ROM, and positive correlation with Swelling, DOMS, and Thickness.
3. Post-exercise urinary titin will have a negative correlation with MVC and ROM, and positive correlation with Swelling, DOMS, and Thickness

1.05 Delimitations

1. All subjects are free of current and past skeletal musculotendinous injuries.
2. All subjects are healthy college-aged resistance naïve men and women (ages 18-30).

3. All subjects are instructed to replicate diet for each training session.
4. All subjects are asked to abstain from resistance training 48 hours from visit 1 and throughout the remainder of the study
5. All subjects are asked to abstain from acute caffeine consumption, β-hydroxymethylbutyrate, creatine, and β-alanine.

1.06 Limitations

1. Findings from this study may not be applicable to populations not specified such as trained athletes and older populations.
2. Findings from this study may not be applicable to other muscle groups or training modalities.

1.07 Assumptions

1. Participants will give maximal effort during exercise protocols.
2. Participants are truthful in reporting dietary intake.
3. Participants are truthful in abstaining from exercise prior to protocol.
4. Urinary N-titin fragments are primarily reflective of skeletal muscle titin turnover and muscle damage and not cardiomyocyte turnover.

1.08 Operational Definitions

1. **TCAP:** Titin-cap or Telethonin, one of the two heterodimer proteins responsible for anchoring titin to the Z-line[7]
2. **Enzyme-Linked Immunosorbent Assay (ELISA)**: An analytical biochemistry assay used to detect presence of ligand in a liquid sample using specific antibodies[18]
3. **Delayed Onset Muscle Soreness (DOMS)**: Phenomenon that occurs after novel training or eccentric contraction overload that results in a delayed increase of pain and soreness[19]
4. **Maximal Voluntary Isometric Contraction (MVC)**: Assessment that looks at the maximal isometric force production of a given muscle[20]
5. **Exercise Induced Muscle Damage (EIMD)**: A phenomenon that occurs following novel training stimulus or eccentric contraction overload that results in damage of the muscle tissue.[21]
6. **CCG**: Clock-Controlled Genes[10]
7. **BMAL1**: Brain and Muscle Arnt-Like Protein-1, one of the positive arm proteins responsible for driving the active phase of the molecular clock and driving transcription of PER1,2 and CRY1,2.[10]
8. **Range of Motion (ROM):** An assessment of a joint that rotation of the moving lever from the stationary lever.[22]
9. **PER1**: Period 1[10]
10. **PER2**: Period 2[10]
11. **CRY1**: Cryptochrome 1[10]
12. **CRY2**: Cryptochrome 2[10]
13. **RBM20**: RNA-Binding Motif Protein-20[9]

CHAPTER II

REVIEW OF LITERATURE

2.01 Outline

Skeletal muscle fibers contain hundreds to thousands of myofibrils, which are composed of many sarcomeres arranged end to end throughout the myofibril. Sarcomeres are the individual functional units of skeletal muscle and are responsible for generating force through myosin thick filament and actin thin filament ATP-sequenced cross-bridge cycling (also known as the sliding filament theory) [23]. Historically, sarcomeres have been understood as a two-filament system, primarily consisting of myosin and actin; however, a third filament comprised of the giant molecular protein titin is now understood as playing roles in both contractile and structural functions of sarcomeres [24]. The primary contractile filaments of the sarcomere consist of the myosin thick filament and the actin thin filament. The Z-lines at the serial end of each sarcomere consists of primarily of actin and serves to anchor both the myosin thick and actin thin filaments to the sarcomere. Titin is responsible for attaching the myosin thick filament to the Z-line and is intertwined with myosin. The NH2-terminal end of titin is the region of titin between the end of the myosin thick filament and the Z-line and is the region of attachment between the two filaments. The titin cap protein (aka Telethonin/TCAP) is the small region of the NH2-terminal end in which titin is anchored to the Z-line[7]. Emerging evidence in animal and human cardiomyocyte models have identified titin to have a circadian rhythm, with lower concentrations of titin-cap (telethonin) protein during the inactive phase of the circadian rhythm [9]. This inactive phase of the circadian rhythm is defined in rodent models as reductions in the clock-controlled gene heterodimer: CLOCK and BMAL1 with concomitant increases in PER1, PER2, CRY1 and CRY2, occurring during light time. Comparative analysis has demonstrated that many physiological rhythms in

nocturnal species are opposite to those in humans. This principle is consistent with the expression of the molecular clock in rodents vs. humans [10]. This inactive phase in rodents is observed during light/day whereas humans it occurs in the dark, when the sun is down [10]. Due to the structural nature of titin, this period of reduced titin-cap concentrations has led to the idea of a possible vulnerable period for skeletal muscle damage. Further, the assessment of skeletal muscle titin is an invasive and time-consuming process. However, advances in proteomic measures offering a potential solution via urinary titin analysis. The following review of literature covers background on skeletal muscle titin physiology, the skeletal muscle circadian rhythm, exercise-induced muscle damage, the assessment of skeletal muscle damage and urinary titin analysis.

2.02 Titin Physiology

Titin (also known as connectin) is a large single-chain mega protein abundant in skeletal and cardiac muscle sarcomeres and spans the width of the sarcomere from Z-disc to the M-line (> 1 μm long) [7]. The skeletal muscle isoform type is called N2A titin (3.3-3.7 MDa) and is expressed as multiple splice variants. The titin filament is arranged into immunoglobulin-like domains (Ig-domains), fibronectin-type-3 domains and several unique sequences (US). Of particular interest is the NH_2-terminal end of titin which anchors titin to the Z-disc via nebulin [8]. The NH_2-terminal end is comprised primarily of two proteins: α-actinin 2 and telethonin (TCAP). The NH_2-terminal end and Ig-domains comprise the I-band region of titin and acts as a viscoelastic spring mechanism [9]. This region of titin undergoes vast amounts of splicing with increases or reductions of these repeats eliciting an increase or decreased length of titin respectively, therefore playing a critical role in muscle function. Skeletal muscle titin has many predominant roles and serves as a functional scaffold, potential exercise-induced mechano-signaler of hypertrophy and an adaptable molecular

spring responsible for generating passive elastic energy through a force-enhancing mechanism known as the stretch-shortening cycle (SSC) [6].

Titin serves a functional scaffolding role in the sarcomere by anchoring the myosin thick filament to the Z-disc of the sarcomere. This functional scaffolding role is further supported by titin's role in providing elasticity to the sarcomere. This role is described by the ability of titin to increase length under applied forces and then shorten to pre-existing lengths when the force is removed. These conformational changes are explained in a multi-phase step. Titin shape changes occur under small, applied forces followed by increases in forces through hierarchical force-responsive unfolding of the polypeptide. The exact pattern of these changes has yet to be fully elucidated [25].

Equivocal findings have also shown titin to have potential hypertrophic signaling properties. During active skeletal muscle contraction, myosin heads bind to actin filaments in an ATP-controlled sequence of steps and propagates along the filament. The relative load placed on the myosin head is either under internal (shortening) or external (lengthening) load and is dependent on the relative compliance of the contractile proteins [26]. Recent evidence suggests myosin to be more compliant than originally thought, with speculations that M-line titin is subsequently extended and loaded under force. [27]. There are two main classes of force receptors. The first type of mechanosensor responds immediately to force, a primary example are mechanosensitive ion channels in which mechanical signals are transformed into chemical signals via ion signaling [26]. Of particular interest is the second class of force receptors which can indirectly measure external force acting on a molecule. Focal adhesion kinase (FAK) is an example that senses substrate stiffness via the integrin-talin-actin force chain [28]. Titin utilizes a second-class force receptor known as titin kinase (TK). Titin kinase in its activated form has been shown to

directly interact with the ubiquitin-associated zinc-finger protein neighbor of-BRCA1-gene-1 (Nbr1) which forms a signaling complex with p62/SQSTM1 and the muscle ring finger proteins 1, 2 and 3 (MuRF1, MuRF2, MuRF3) [29]. Under applied lengthening forces, titin kinase opens and phosphorylates, removing its stimulatory effects on MuRFs [26]. Knockout of MuRF1 and MuRF2 results in increases in skeletal muscle hypertrophy by reductions in muscle protein breakdown.

Titin may play a role in the force-augmenting mechanism known as the stretch shortening cycle (SSC). The stretch-shortening cycle is a combination of a rapid eccentric contraction followed by a brief latency period and rapid concentric muscle action [6]. The comprehensive mechanisms surrounding the SSC are not fully understood but are associated with the passive elements of titin. Because of the structural and viscoelastic spring-like roles of titin and its ability to modulate length and stiffness upon force application, it is theorized to be the primary contributor to the augmented force production following this phenomenon.

Previous work in rodent cardiomyocytes has demonstrated that titin-cap protein (T-cap), a section of the NH_2-terminal end of titin, exhibits diurnal variation with the lowest concentrations present in the inactive phase of the circadian rhythm [9]. T-cap functions to anchor titin to the Z-line by binding titin's Z1Z2 domain and allows the sarcomere to resist strong stretching forces. [30,31]. The NH_2-terminal region of titin (comprised of T-cap and α-actinin heterodimer) is intimately involved in the formation of Z-lines during nascent myogenesis [31]. Overexpression of zeugmatin, a protein located in the NH2-terminal end of titin in chick skeletal muscle cultures causes disruption in myofibril integrity by eliminating binding sites for titin, further demonstrating the important role of the NH2-terminal end of titin in regulating myofibril integrity. [32]. Due to the multi-faceted roles of titin and its circadian variation, previous work in cardiomyocytes found with genetic ablation of T-cap in mice, T-tubules were altered and subsequently led to contractile,

stretch-sensing defects, and dilated cardiomyopathy. T-cap Z-disc complex disfigurations in humans have also been associated with heart failure [33,34]. If such model of T-cap diurnal fluctuation exists in human skeletal muscle tissue, it would allow skeletal muscle fibers to modulate titin length on a frequent circadian basis. This notion for a potential vulnerable period is supported by previous studies assessing diurnal variation in muscle damage with measurements of CK and leukocyte infiltration [12,13].

2.03 Skeletal Muscle Circadian Rhythm

The skeletal muscle circadian rhythm is a newly discovered field and yet to be fully elucidated. The skeletal muscle circadian rhythm is regulated by the molecular clock. The molecular clock is a cell autonomous feedback loop regulated by transcriptional-translational processes [35]. The molecular mechanisms underlying the sarcomere's circadian rhythm is understood as a 24-hour phasic cycle with the active phase regulated by core clock-controlled genes identified as CLOCK and BMAL1. It is estimated that CLOCK and BMAL1 regulate over 4000 genes. These clock-controlled genes are then inhibited by PER1, PER2, CRY1 and CRY2 to initiate the inactive phase of the circadian rhythm. BMAL1 is one of the primary clock-controlled genes responsible for regulating the active phase of the circadian rhythm and regulates a clock-controlled gene known as RBM20 [10]. Riley and colleagues demonstrated in mice with BMAL1 knockout that titin splicing was altered with subsequent disruptions in sarcomere length. Overexpression of RBM20 in BMAL1 knockout mice ameliorated the deleterious effects of BMAL1 knockout on titin splicing [9].

Historically, it was understood that the phase of the molecular clocks across all peripheral tissues were regulated by the suprachiasmatic nuclei (SCN), however the molecular clock has been identified as a self-sustaining 24-hour phasic cycle that can operate independent of signals from

the environment and SCN. Recent evidence suggests that time of feeding and time of physical activity are quintessential cues that can modify the phase of the molecular clock in peripheral tissue independent of changes in the central clock [10].

2.04 Exercise-Induced Muscle Damage

Exercise induced muscle damage (EIMD) is a common occurrence following a bout of intense exercise particularly with novel muscle action stimulus. EIMD can affect a few macromolecules of muscle tissue or result in large tears in the sarcolemma, basal lamina, connective tissue or as injury to contractile proteins and cytoskeletal structure[2]. EIMD sets off a cascade of events including but not limited to; local inflammation, disturbed calcium regulation, activation of ubiquitin-proteosome pathways, and secretion of biomarkers of damage into the blood stream[3]. EIMD displays a continuum ranging from favorable adaptive cell signaling with mild-to-moderate levels of damage spanning to maladaptive responses such as pervasive membrane damage, tissue necrosis and severe myocellular disruptions[36]. EIMD is highly influenced by muscle action with a greater response elicited by eccentric contractions compared to concentric and isometric contractions [37]. Mechanistically this can be explained by a reduced demand of motor units to maintain a given amount of force, as cross-bridges do not need to be detached during eccentric contractions, increasing the force per motor unit at a given load. EIMD leads to mechanical disruption of the actin-myosin cross-bridges, titin, desmin, and deformation of T-tubules leading to subsequent disruptions in calcium homeostasis [38,39] . EIMD is eventually repaired, soreness is dissipated, and force production returns to baseline or above in a super-compensatory manner over several days to weeks [5].

2.05 Assessment of Skeletal Muscle Damage

Assessment of skeletal muscle damage can be performed biochemically, histologically, and functionally. Histological evidence of skeletal muscle damage can be observed from muscle biopsy samples analyzed under light and electron microscopy via observing Z-line streaming, and loss of lateral registration of sarcomeres however, this method primarily serves as a confirmational rather than quantification tool. Indirect evidence of muscle damage can also be observed through magnetic resonance imaging (MRI). Direct evidence is the preferred assessment method but is limited by cost of materials and the invasive nature of it [40]. Skeletal muscle damage can also be assessed biochemically through assessments of creatine kinase (CK), myoglobin, lactate dehydrogenase (LDH), aspartate amino transferase (AST) and myosin heavy chain (MHC) in the blood [41]. The appearance of creatine kinase in the blood has generally been considered an indirect marker of muscle damage but has limitations. Creatine kinase has been shown to be affected by nonmodifiable factors such as ethnicity, age, and gender with elevated levels at low-to-moderate intensity exercise. The appearance of CK in serum following low intensity exercise represents a disturbance to muscle metabolism rather than skeletal muscle damage [4]. Functional assessments of muscle damage include assessing maximal voluntary contraction, range of motion (ROM), soreness and edema.

2.06 Urinary Titin Analysis

The assessment of skeletal muscle titin concentrations requires a complex and invasive process of collecting muscle tissue via biopsy and analyzing the sample under electron microscopy. Recent advances in proteomics offer a non-invasive assessment of N-terminal fragment of titin, which has become measurable using an enzyme-linked immunosorbent assay kit (ELISA) with a urinary sample [42]. The assessment of urinary titin concentrations must be standardized with the assessment of urinary creatinine to account for dilution of the urine sample

and rate of glomerular protein filtration. Urinary creatinine is the most widely used analyte to correct urinary titin concentrations because it is an inert biproduct of non-enzymatic metabolism of skeletal muscle creatine. Further, urinary creatinine has been shown to be produced at a constant daily rate and has been used as a biomarker to assess renal function [43]. The direct mechanism underlying the transference of skeletal muscle titin to urinary filtrate is unknown. It is reasonable to assume an inverse relationship between skeletal muscle titin concentration and urinary titin concentration. Previous work has demonstrated a negative relationship between urinary titin concentration and femoral muscle volume in ICU admitted patients [44]. Mechanistically, it is thought that eccentric contractions cause the proliferation of calpain-3, a calcium dependent protease. Calpain-3 has been shown to degrade and cleave titin, resulting in the release of the N-terminal fragment into the urine through glomerular filtration [43,45]. Previous work from Yamaguchi and colleagues compared the urinary titin fragment (UTF) response between concentric and eccentric contractions and found a significantly greater UTF response after eccentric contractions compared to concentric [46]. The ubiquitin-proteosome pathway may also play a role in titin breakdown. Previous work from Lang and colleagues found gradual increases in ubiquitinated titin levels in mice with denervation-induced muscle atrophy, as well as upregulations in the autophagy-lysosome pathway [47]. This mechanism of glomerular filtration of UTF can be partially explained by the delayed increase in titin fragments 96 hours after eccentric exercise, which may reflect the complicated urinary excretion of titin fragments [17]. Work from Kanda and colleagues demonstrated a high negative correlation between urinary titin and range of motion and maximal voluntary contraction and a positive correlation between urinary titin concentrations and delayed onset muscle soreness.

Urinary titin concentrations do not entirely reflect skeletal muscle titin turnover, as the diaphragm and cardiac cells possess titin. It is reasonable to assume urinary titin concentrations primarily reflect skeletal muscle titin turnover, as previous work from Nakanishi and colleagues examined urinary titin concentrations in critically ill patients with diaphragm atrophy versus a control group and found no significant difference in urinary titin concentrations [48]. Urinary titin concentrations were also found to have a significant negative correlation with erector spinae muscle mass and pectoralis major muscle mass in patients with interstitial lung diseases [49]. Further, skeletal muscle mass accounts for roughly 40% of human body mass and the heart accounts for less than 1% of human body mass [50,51].

2.07 Literature Summary

Circadian rhythms have a significant effect on the structural integrity of the sarcomere via changes in titin concentrations. Circadian fluctuations in titin have been observed in humans and mice with altered molecular clock genes. Due to the structural nature of titin in the sarcomere, this circadian fluctuation in titin creates a potential vulnerable period for skeletal muscle damage. Previous studies have looked at time of day exercise induced muscle damage on other blood biomarkers of muscle damage such as CK and leukocyte infiltration, but no study has looked at diurnal variations in muscle damage and subsequent skeletal muscle titin concentrations. Further, no study has looked at the diurnal effects of exercise induced muscle damage on urinary titin concentrations in humans. Additional research is needed to clarify the extent of these changes in urinary titin concentrations and the potential relationship to skeletal muscle damage in humans.

CHAPTER III

METHODOLOGY

3.01 Purpose

The purpose of this study was to assess differences in performance metrics of muscle damage following eccentric muscle damaging protocols conducted either in the morning or in the evening. A secondary purpose was to determine if a relationship exists between baseline urinary titin concentrations prior to exercise and post-exercise measures of muscle damage.

3.02 Participants

All participants recruited were healthy college aged men and women (aged 18-30 year old) who were resistance-training naïve (~6 months resistance training free) and free of musculoskeletal injury. A power analysis, using G power, performed for a mixed-model ANOVA (2 groups—morning vs evening x 6 assessments of EIMD) with a projected effect size of 0.60 SD in the magnitude of EIMD revealed a total sample size of 28 participants, with 14 in each group would be sufficient with a beta of 0.80 and an alpha of $p < 0.05$. The sample selected included women as previous work has suggested that men and women experience similar symptoms of EIMD [52,53]. Further, since urine volume and related renal components are affected by physiological changes such as sex, dehydration and kidney function, creatinine was used as an analyte to correct urinary titin volumes for sex differences[54]. Estrogen has been demonstrated to have anabolic effects regarding glycogen metabolism, and progesterone has been demonstrated to have antagonistic effects on estrogen. During the luteal phase of menstruation, both estrogen and progesterone are high, thus all women in this study were tested in the luteal phase. This was done to limit negative performance effects of low estrogen, and super compensated effects of estrogen due to low progesterone [55]. Women on hormonal contraceptives who participated did so

at any point during their cycle as long as they were not actively menstruating. Prior long-term ICU admittance was also an exclusion criterion that will be evaluated during the initial visit. Previous work has also demonstrated a phenomenon called Intensive Care Unit Acquired Weakness (ICU-AW). This phenomenon whereby admittance into the ICU under critically ill conditions leads to loss of muscle mass and physical function persisting up to 5 years after discharge [42]. All participants recruited were free of renal dysfunction as pathologies of the nephrons and podocytes such as focal segmental glomerulosclerosis can affect the passing of filtrate and may skew urinary titin readings [56]. Sampling was localized to the University of Oklahoma Norman campus and University of Oklahoma Health Science Center campus. Recruited participants were finalized after meeting the inclusion criteria detailed in this chapter.

Inclusion Criteria	Exclusion Criteria
• Healthy college-aged individuals (18-30). • Resistance training naïve in upper extremities (~6 months resistance training free). • Free of skeletomuscular injuries. • Free of renal dysfunction. • No history of ICU admittance	• Use of the following ergogenic aids (β-hydroxymethylbutyrate, creatine, acute caffeine usage, β-alanine) • Use of prescription pain or psychiatric medication • Regular third shift work schedule (~11 pm – 7 am) • Regular upper body resistance training or endurance training • Abnormal/irregular sleep schedules • Irregular fad diets (ketogenic, carnivore, etc.)

3.03 Research Design

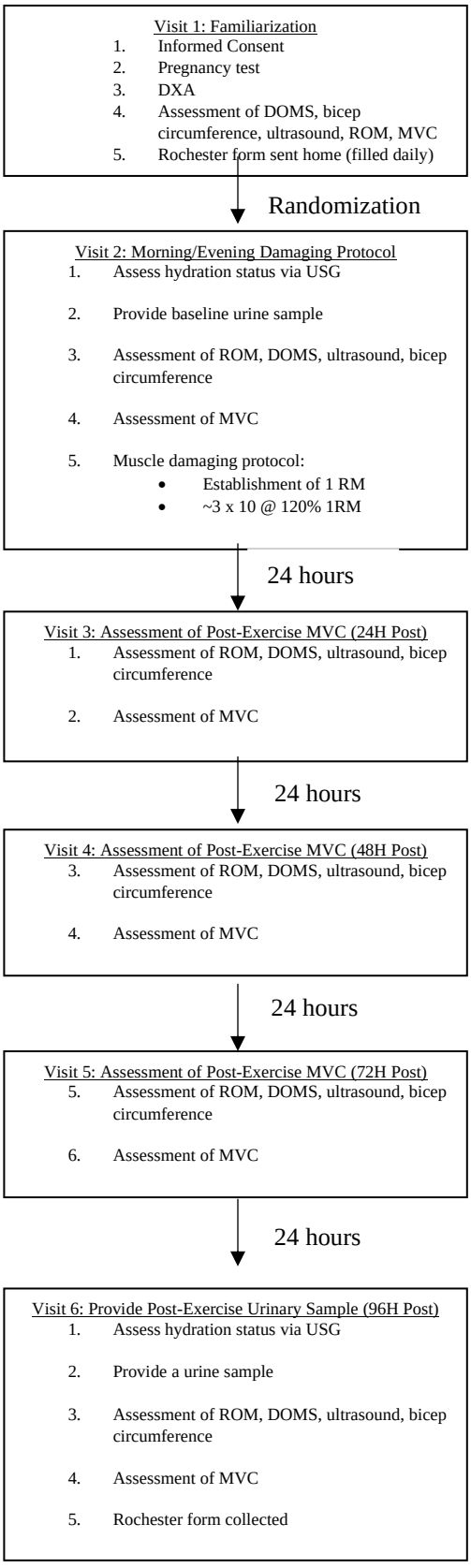
This study employed the use of between-group comparison design to examine changes in urinary titin concentration in response to morning versus evening muscle damaging eccentric exercise. A total of 6 visits to the laboratory were required to complete this study. A visual flow chart of the procedures is shown in Figure 1. During the first visit, participants were familiarized with experimental procedures including: maximal voluntary isometric contraction assessment on an isokinetic dynamometer (KinCom, Chattanooga, TN, USA), exercise technique and protocol, and instructions on proper urine sample provisions. Peak maximal voluntary contraction was assessed from three attempts. Participants baseline measures of DOMS, muscle swelling (assessed via tape measure), muscle thickness (assessed via ultrasound), range of motion, and MVC were assessed in that order. Average ROM was recorded from three assessments. All testing was performed in the non-dominant arm. Participants were required to provide clean catch urine samples for titin analysis. Finally, a body composition assessment using a dual-energy x-ray absorptiometry (DXA) was conducted to assess lean body mass. Female participants were required to fill out a menstrual history form and were required to perform a pregnancy test prior to DXA assessment. Participants were also screened for symptoms of long-COVID syndrome and were instructed to fill out the Rochester Fatigue Index for the duration of the study to assess fatigue and sleep pattern abnormalities. Following the first visit, participants were randomized by a digital randomizer app to determine if they were in the morning or evening group for visit 2. The morning protocol took place between 07:00 to 08:30h and the evening protocol between 17:00 to 18:30h. Upon arrival for visit 2, participants were asked to provide a urine sample for hydration status assessment via urine specific gravity refractometer

(USG). If USG values were within 1.004 and 1.029 A.U., participants were instructed to provide a clean catch urine sample for baseline titin measurements and then assessed on DOMS, muscle swelling, muscle thickness, range of motion and maximal isometric voluntary contraction of the biceps. If USG values were higher than the previously mentioned range, participants were asked to rehydrate for another assessment 10-15 minutes later. If USG values fell below the range, participants were rescheduled due to hyperhydration. Upon completion of baseline maximal voluntary isometric contraction assessment (MVC), participants were instructed to provide a one repetition maximum on the unilateral concentric bicep curl. Participants were given five minutes of rest between attempts until a maximum concentric repetition was established. The weight was taken at the top of the movement by the researcher to prevent extraneous eccentric muscle actions that could potentially damage the muscle or influence the subsequent performance. Following the establishment of the one-repetition maximum, the participants were instructed to perform 3 second eccentric tempo curls at 120% of their established concentric one repetition maximum. The eccentric exercise protocol consisted of a minimum of three sets of ten repetitions at 120% of their established one repetition maximum until their MVC fell to 60% of their baseline value. This protocol was successfully used to induced damage by Hight and colleagues [57]. MVC was assessed after each set and if MVC had not declined to 60% of baseline upon completion of the first three sets, an additional set was performed and MVC was reassessed. Upon completion of visit 2, participants were instructed to return to the laboratory within 24 hours to assess DOMS, arm circumference, and ultrasound assessment of muscle swelling, muscle thickness, ROM, and MVC of the biceps for visit 3. Visit 4 took place 24 hours following visit 3 and visit 5 took place 24 hours following visit 4 and the same assessments in the given order were conducted. Following completion of visit 5, participants were asked to

return to the laboratory after 24 to assess hydration status and to provide a urine sample and was assessed on DOMS, arm circumference, muscle thickness, ROM, and MVC.

To control confounding factors that might affect internal and external validity, participants were asked to maintain their normal physical activity, sleep and dietary schedule to avoid invoking the Hawthorne effect. The Hawthorne effect is a psychological phenomenon in which participants alter lifestyle behavior due to their awareness of being observed. Participants were asked to abstain from exercise and recovery interventions such as icing, heating, massage, until completion of the protocol. Participants were asked to replicate their diet each day throughout the protocol. All participants were tested using the same devices and protocols for both morning and evening visits and will receive similar degrees of verbal encouragement. Further, the morning and evening protocols were separated into two groups to account for the repeated bout effect. The repeated bout effect is a protective phenomenon that occurs in response to a single bout of eccentric exercise, where subsequent bouts of exercise result in attenuated exercise induced muscle damage. The repeated bout effect has been shown to last several weeks up to 6 months[14]. Previous work from Yamaguchi and colleagues demonstrated urinary titin fragments (UTF) to sensitively reflect the repeated bout effect, with significant reductions in UTF in the second bout of eccentric elbow flexor exercise compared to the first bout [15]. Both visits were separated by a 2-week period.

<u>Figure 1</u>: Overview of experimental procedures



3.04 Measurement Protocols

Urinary Titin

The assessment of urinary titin was conducted using the Human Titin N-Fragment (Urine) enzyme linked immunosorbent assay (ELISA) Kit (IBL, Fujioka-Shi, Gunma, JAPAN). Participants were instructed to provide a urine sample 96 hours after eccentric muscle damaging protocol to be analyzed. Reported assay sensitivity is 27.91 pmol/L and intra-assay precision coefficient of variance is 4.5% for humans[46,58]. Urinary titin (UT) values were normalized to urinary creatinine (CR) values to adjust for kidney function. The following equation was used to correct urinary titin values: UT/CR = Titin N Fragment (pmol/L) / Creatinine (mg/dL).

Maximal Isometric Voluntary Contraction

Another outcome variable that was assessed was maximal voluntary isometric contraction of the biceps. Participants were seated with hips at 90 degrees and their non-dominant arm placed in 90 degrees of elbow flexion on an isokinetic dynamometer (KinCom, Chattanooga, TN, USA). They were instructed to perform elbow flexion against the lever arm of the dynamometer. Force output from the KinCom will be interfaced with a Biopac MP150 (Biopac Systems, Inc., Goleta, CA) and sampled at 2000 Hz. Data was displayed to the participant using Acknowledge software (v4.4, Biopac Systems Inc., Goleta, CA) to provide visual feedback to aid maximal force production. Strong verbal encouragement will be provided during each effort. The use of the KinCom dynamometer for assessing maximal voluntary contraction is well-established and has been demonstrated to have high reliability in assessments of constant velocity resisted muscle shortening and muscle lengthening [59].

Muscle Swelling

Swelling of the elbow flexors will be assessed in two ways: 1) by measuring circumference of the biceps using a tape measure and 2) through the use of doppler ultrasound. To assess circumference of the elbow flexors, the midline of the humerus was identified and marked as half the distance between the lateral epicondyle of the elbow and the acromion process on the shoulder. A tape measure was placed around the upper arm perpendicular to the midline of the humerus and the circumference will be recorded in centimeters. Participants were told to stand with their arm hanging passively at their side during the measurement. Three measures were taken, and the two most similar measures were averaged for the criterion assessment of circumference.

Muscle Thickness

Assessment of muscle thickness was conducted by an ultrasound device (Fukuda Denshi UF-750XT, Redmond, WA, USA). Participants were instructed to be in a seated position, with their hands supinated and elbow placed in approximately 90 degrees of flexion while resting on a padded support. Ultrasound gel was applied to participants biceps and the linear probe was placed longitudinally on muscle belly of the biceps at the midline of the humerus as described above[60]. The "Freeze" button was selected and the "F1-DIST" prompt was selected. The first point was then set on the visual interface where the epidermis and adipocyte layer ends, and the final point was set just before the humerus, to obtain thickness in centimeters.

Elbow Range-of-Motion

A handheld goniometer was used to assess elbow ROM. The fulcrum/axis of rotation of the goniometer was placed at the lateral epicondyle, the stationary arm of the goniometer was placed along the line of the humerus. The traveling arm was then placed in line with the ulna and

was used to assess degrees of rotation (range of motion – ROM) after the participant had been asked to flex their elbow as much as possible. Starting from full extension of the arm, participants were instructed to perform elbow flexion and the peak elbow flexion was assessed via goniometer [61]. Range of motion was assessed by a single researcher and the average of three attempts was recorded.

Muscle Soreness

Delayed onset muscle soreness was assessed using a visual analogue scale (VAS) with values ranging from 0-100, with 100 being the highest level of perceived pain and soreness. Participants will perform elbow flexion with a light weight (5 lbs for female and 10 lbs for male participants) through a full range of motion. Upon completion they were asked to place a mark/line on the VAS scale that best represented the amount of pain experienced during flexion.

DXA Scan

Body composition/lean tissue mass was determined using a whole body DXA scan (Lunar Prodigy Advance; GE-Medical Systems, Madison, WI) and corresponding analysis software (enCore 2011, version 13.60, GE-Healthcare, Madison, WI) performed according to the manufacturer's instructions. Custom region of interest (ROI) boxes were drawn for upper arm specific analysis. The ROI area for the biceps were measured from the armpit to the antecubital fossa. Participants were asked to lie in a supine position with their arms resting against the sides of the body during each scan and the DXA equipment was calibrated according to the protocol provided by the manufacturer.

3.05 Data Management and Analysis

All statistical analysis of outcome variables was assessed using a digital statistical software package (SPSS v28, SPSS Inc., Chicago, IL, USA). A paired T-test with alpha set at

0.05 was conducted to compare differences in pre-exercise morning and evening urinary titin concentration to post-exercise morning and evening urinary titin concentration. Normality of distribution was assessed using the Shapiro-Wilk test. A mixed-model repeated measures ANOVA (2 groups—morning and evening exercise x 6 time points) was used to assess change in MVC, ROM, Circumference, Muscle Thickness, and DOMS from baseline, immediately post exercise, 24hr, 48hr, 72hr, and 96 hours post-exercise between the morning and evening exercise groups An independent T-test with alpha set at 0.05 was conducted to compare pre-exercise urinary titin values between evening and morning groups. To determine relationships between baseline urinary titin concentrations and changes in performance metrics of muscle damage, Pearson Product Moment correlations were conducted.

CHAPTER IV

RESULTS

4.01 Subject Characteristics

A total of 31 participants were consented and screened in visit 1, but only 28 were included in this data set. There were 14 in the morning group (07:00-08:30H) and 14 in the evening group (17:00-18:30H). Table 1 displays the mean ± SD of age, height, weight, 1RM, and lean body mass of their non-dominant arm (LBMbi).

Table 1. Descriptive Statistics (N = 28)

	Morning Group (N = 14, F = 7)	**Evening Group (N = 14, F = 9)**	**P-Value**
Age (years)	22.86 ± 3.00	23.14 ± 3.25	0.41
Height (cm)	169.92 ± 6.96	172.54 ± 8.81	0.18
Weight (kg)	70.07 ± 12.74	69.49 ± 13.18	0.46
1RM (kg)	11.90 ± 4.37	11.05 ± 4.81	0.47
LBMbi (kg)	2.83 ± 0.79	2.93 ± 1.05	0.39
BF%	31.66 ± 11.10	31.87 ± 10.80	0.48

Values displayed as mean ± SD. 1RM: One repetition maximum; LBMbi: Lean body mass of biceps; BF% = Body fat percentage

4.02 Maximal Voluntary Isometric Contraction

Absolute MVC

Figure 2 displays changes in maximal voluntary isometric contraction and expressed as absolute force (newtons). There was no significant time x group interaction ($p = 0.72$). There was no main effect for group between the morning and evening groups ($p = 0.55$) There was a significant main effect of time ($p < 0.001$), with differences observed when comparing pre-exercise MVC to immediately post-exercise, 24-hours post, 48 hours-post, 72-hours-post, 96-hours post ($p < 0.001$).

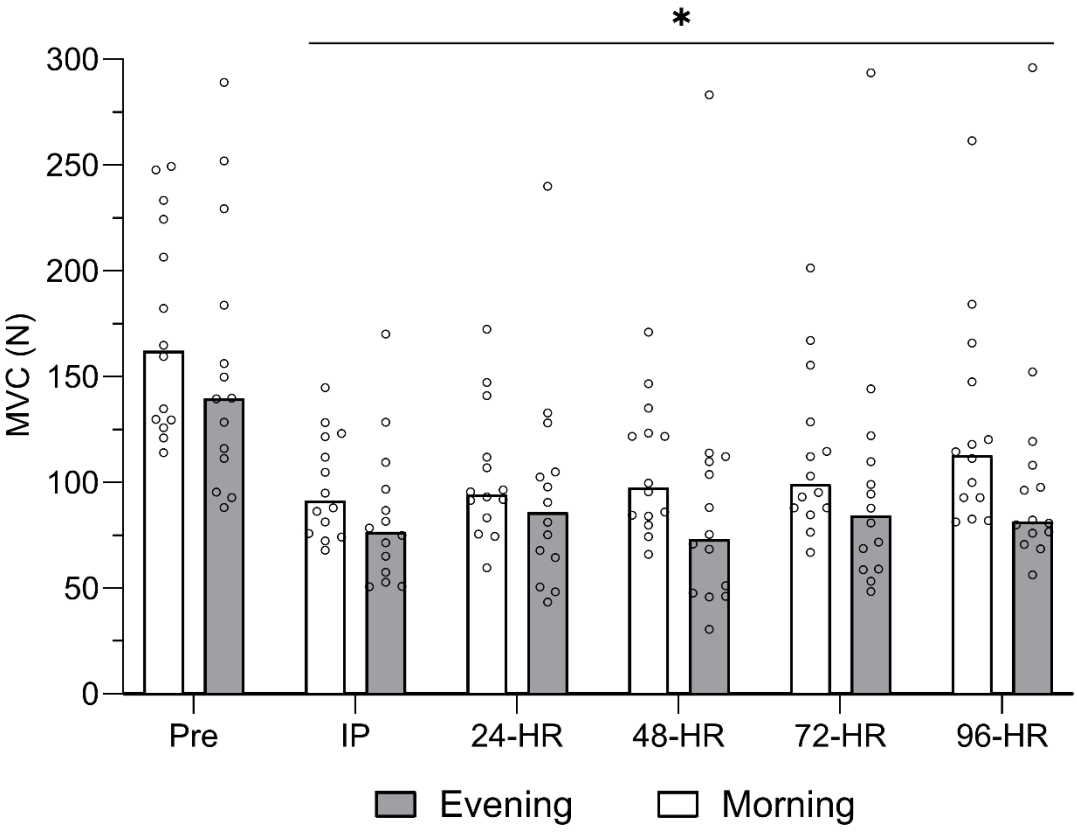


Figure 2. – Absolute maximal voluntary contraction force prior to (PRE) and over 4 days following eccentric exercise performed in the morning (white) and evening (gray). Values are displayed as means. * indicates a significant difference (main comparison) from pre-exercise values ($p < 0.05$).

%Δ Maximal Voluntary Isometric Contraction

Figure 3 displays relative changes in maximal voluntary isometric contraction and is expressed as percent change from PRE (%Δ). There was no significant time x group interaction ($p = 0.64$). There was no main effect for group ($p = 0.51$) There was a significant main effect of time ($p < 0.001$), with no differences when comparing immediately post-exercise to 24-hours post and 48-hours post ($p \geq 0.06$). Differences were observed when comparing immediately post-exercise MVC values to 72-hours post and 96-hours post ($p \geq 0.001$).

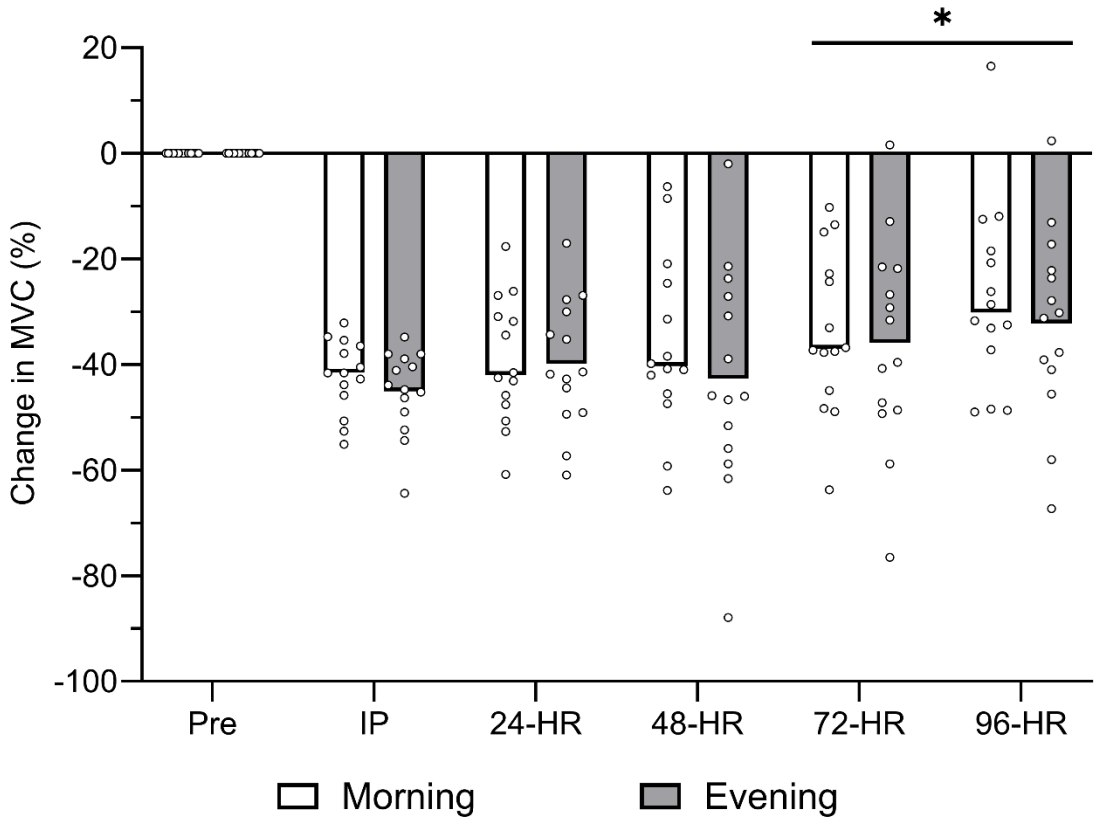


Figure 3. – Percent change maximal voluntary contraction force following eccentric exercise performed in the morning (white) and evening (gray). Values are displayed as means. * indicates a significant difference (main comparison) from immediately post (IP) values ($p < 0.05$).

4.03 Muscle Swelling

Absolute Muscle Swelling

Figure 4 displays changes in values of muscle swelling expressed as circumference values (cm). There was no significant time x group interaction ($p = 0.689$). There was no main effect of group ($p = 0.982$). There was a main effect of time ($p < 0.001$) with significant differences observed when comparing pre-exercise swelling to immediately post, 24-hours post, 48-hours post, 72-hours post, and 96-hours post ($p < 0.001$).

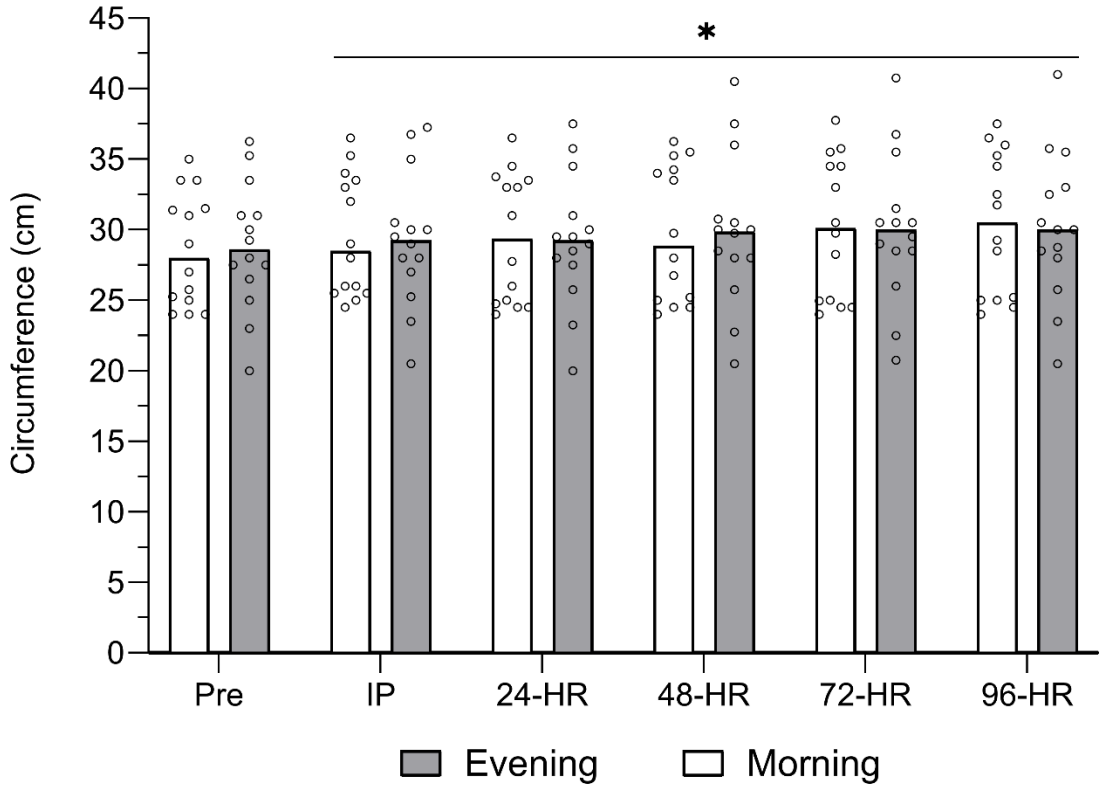


Figure 4. –Absolute muscle swelling values prior to (PRE) and over 4 days following eccentric exercise performed in the morning (white) and evening (gray). Values are displayed as means. * indicates a significant difference (main comparison) from pre-exercise values ($p < 0.05$).

%Δ Muscle Swelling

Figure 5 displays relative changes in muscle swelling and is expressed as percent change from PRE (%Δ). There was no significant time x group interaction ($p = 0.81$). There was no main effect for group ($p = 0.09$) There was a significant main effect of time ($p < 0.001$), with no differences when comparing immediately post-exercise to 24-hours post and 48-hours post ($p \geq 0.135$). Differences were observed when comparing immediately post-exercise MVC values to 72-hours post and 96-hours post ($p \geq 0.003$).

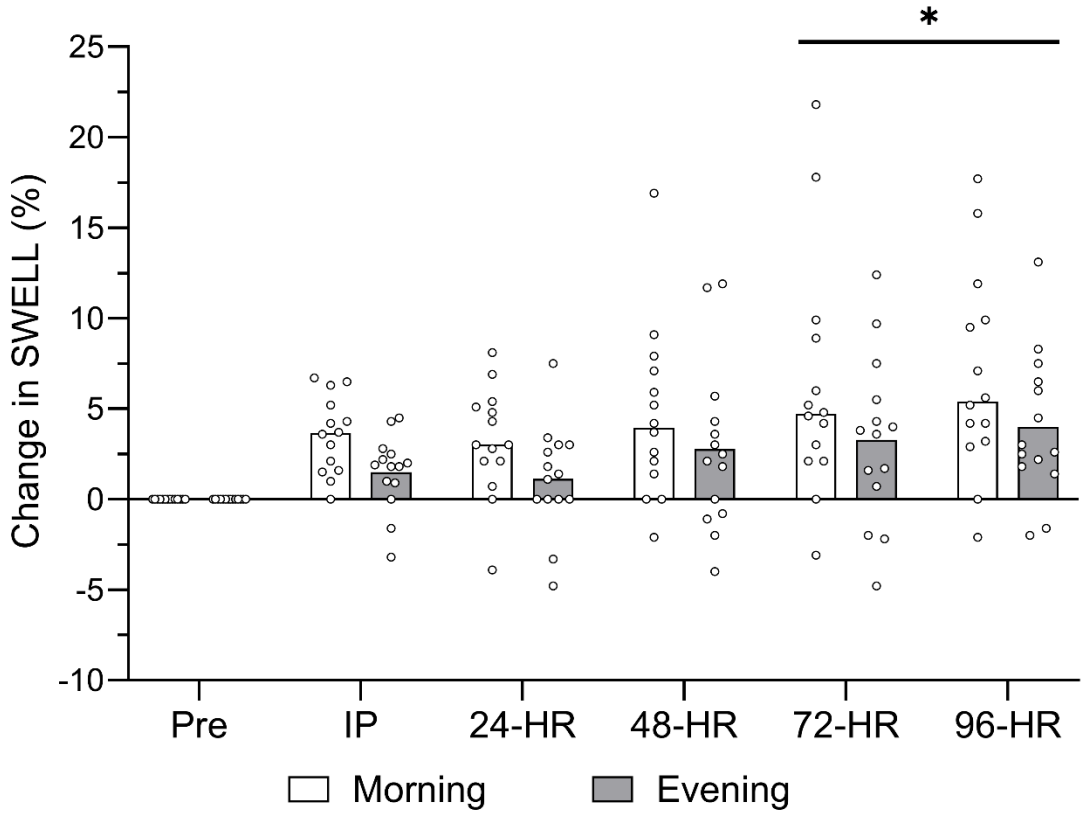


Figure 5. – Percent change muscle swelling following eccentric exercise performed in the morning (white) and evening (gray). Values are displayed as means. * indicates a significant difference (main comparison) from immediately post (IP) values ($p < 0.05$).

4.04 Muscle Thickness

Absolute Muscle Thickness

Figure 6 displays changes in values of muscle thickness expressed as thickness (cm). There was no significant time x group interaction ($p = 0.25$). There was no main effect of group ($p = 0.54$). There was a main effect of time ($p < 0.001$) with significant differences observed when comparing pre-exercise thickness to immediately post, 24-hours post, 48-hours post, 72-hours post, and 96-hours post ($p < 0.001$).

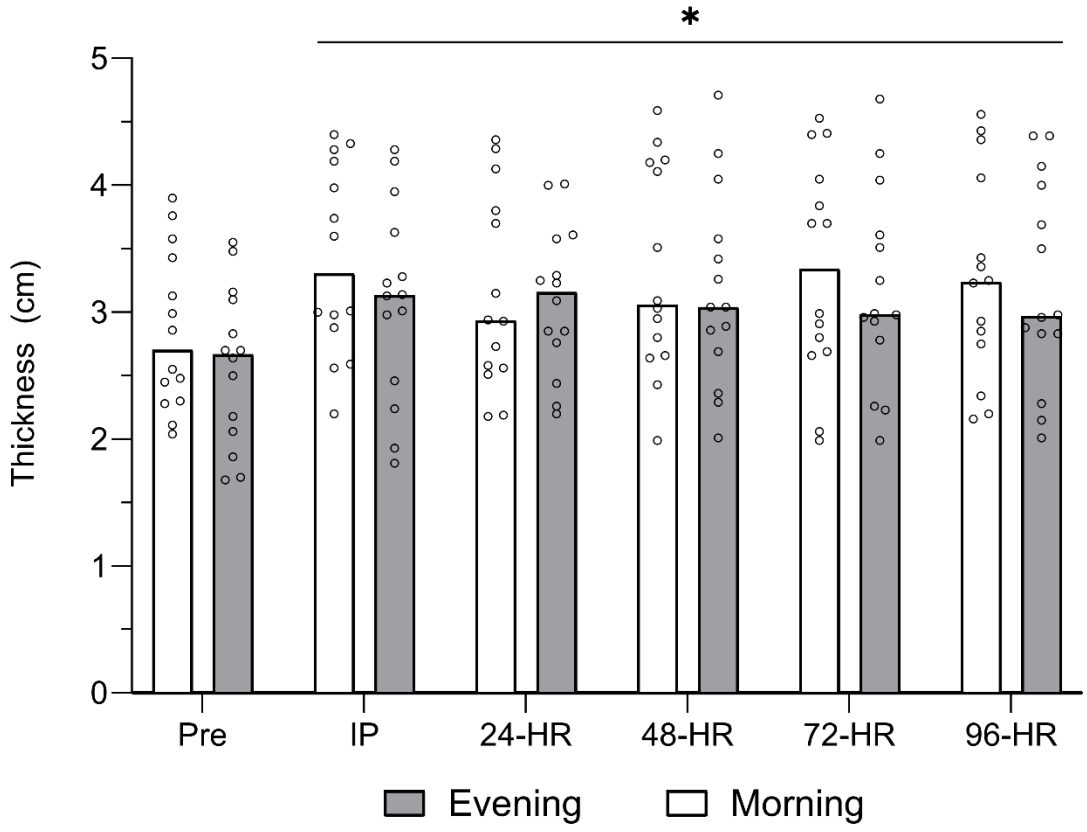


Figure 6. – Muscle thickness values prior to (PRE) and over 4 days following eccentric exercise performed in the morning (white) and evening (gray). Values are displayed as means. * indicates a significant difference (main comparison) from pre-exercise values ($p < 0.05$).

%Δ Muscle Thickness

Figure 7 displays relative changes in muscle thickness expressed as percent change (%Δ) from PRE. There was no significant time x group interaction ($p = 0.34$). There was no main effect of group ($p = 0.35$). There was no main effect of time ($p = 0.529$).

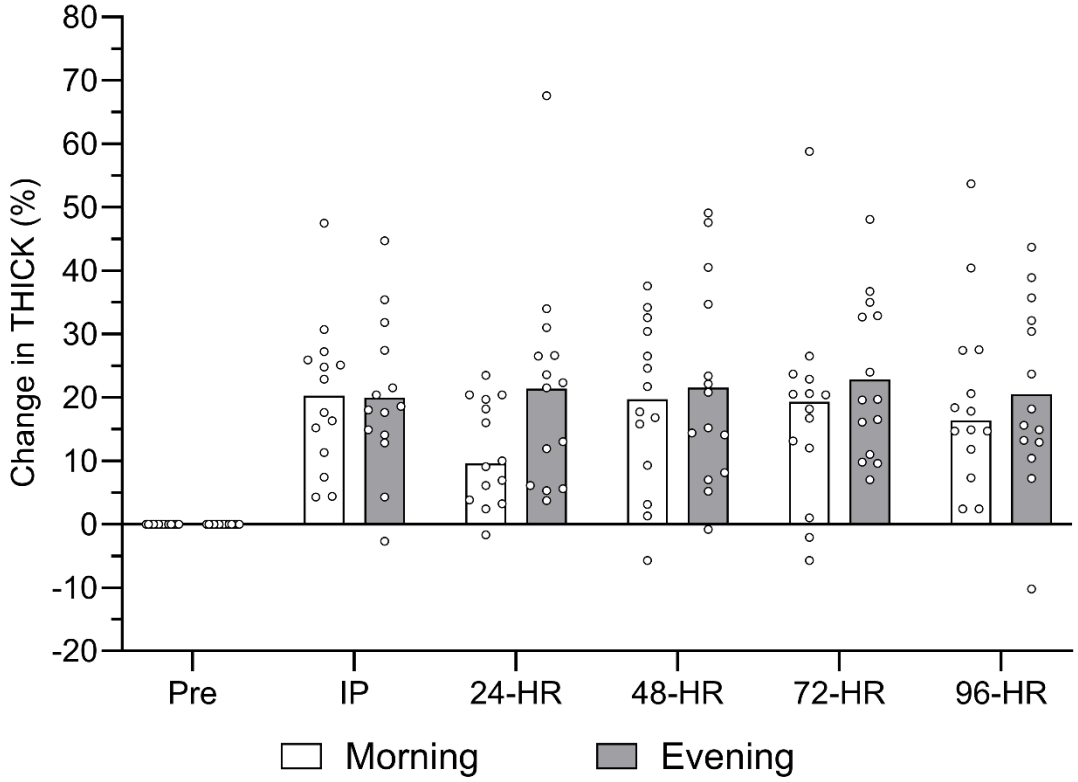


Figure 7. – Percent change muscle thickness following eccentric exercise performed in the morning (white) and evening (gray). Values are mean ± SD.

4.05 Elbow Range of Motion

Absolute Range of Motion

Figure 8 displays changes in values of absolute range of motion expressed as degrees of flexion. There was no significant time x group interaction ($p = 0.75$). There was no main effect of group ($p = 0.75$). There was a main effect of time ($p < 0.001$) with significant differences observed when comparing pre-exercise swelling to immediately post, 24-hours post, 48-hours post, 72-hours post, and 96-hours post ($p < 0.001$).

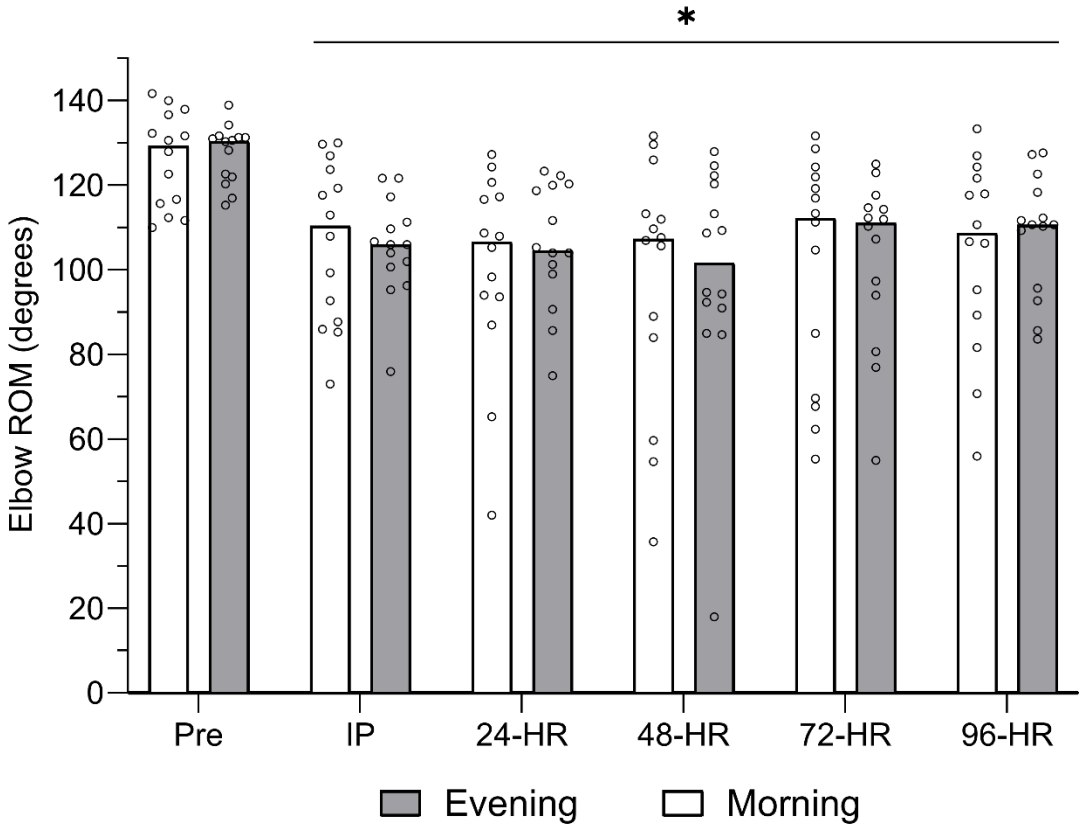


Figure 8. – Percent change range of motion following eccentric exercise performed in the morning (white) and evening (gray). Values are displayed as means. * indicates a significant difference (main comparison) from pre-exercise values ($p < 0.05$).

%Δ Range of Motion

Figure 9 displays relative changes in range of motion and is expressed as percent change from PRE (%Δ). There was no significant time x group interaction ($p = 0.35$). There was no main effect for group ($p = 0.37$) There was a significant main effect of time ($p = 0.03$), with no differences when comparing immediately post-exercise to 24-hours post, 72-hours post, and 96-hours post ($p \geq 0.10$). Differences were observed when comparing immediately post-exercise MVC values to 48-hours post ($p = 0.032$).

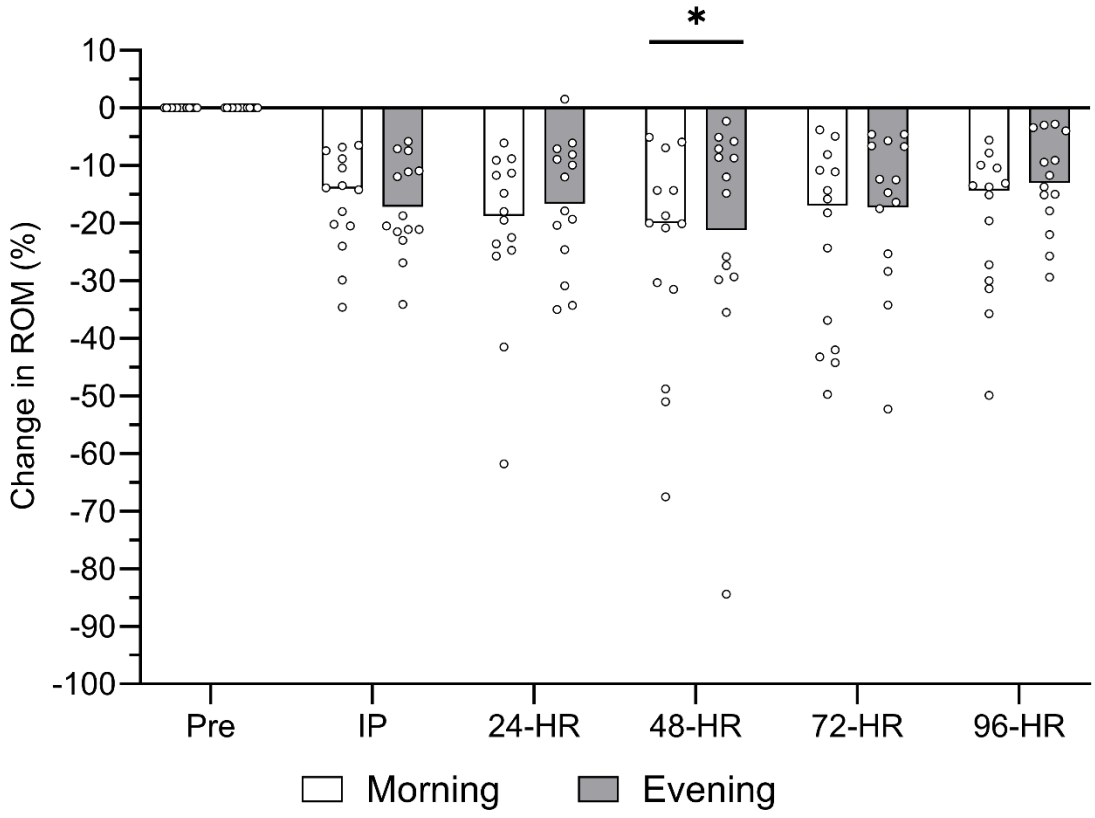


Figure 9. – Muscle soreness prior to (PRE) and over 4 days following eccentric exercise performed in the morning (white) and evening (gray). Values are displayed as means. * indicates a significant difference (main comparison) from immediately post (IP) values ($p < 0.05$).

4.06 Muscle Soreness

Absolute Soreness

Figure 10 displays changes in values of absolute muscle soreness expressed as soreness (0-100). There was no significant time x group interaction ($p = 0.14$). There was no main effect of group ($p = 0.17$). There was a main effect of time ($p < 0.001$) with significant differences observed when comparing pre-exercise swelling to immediately post, 24-hours post, 48-hours post, 72-hours post, and 96-hours post ($p < 0.001$).

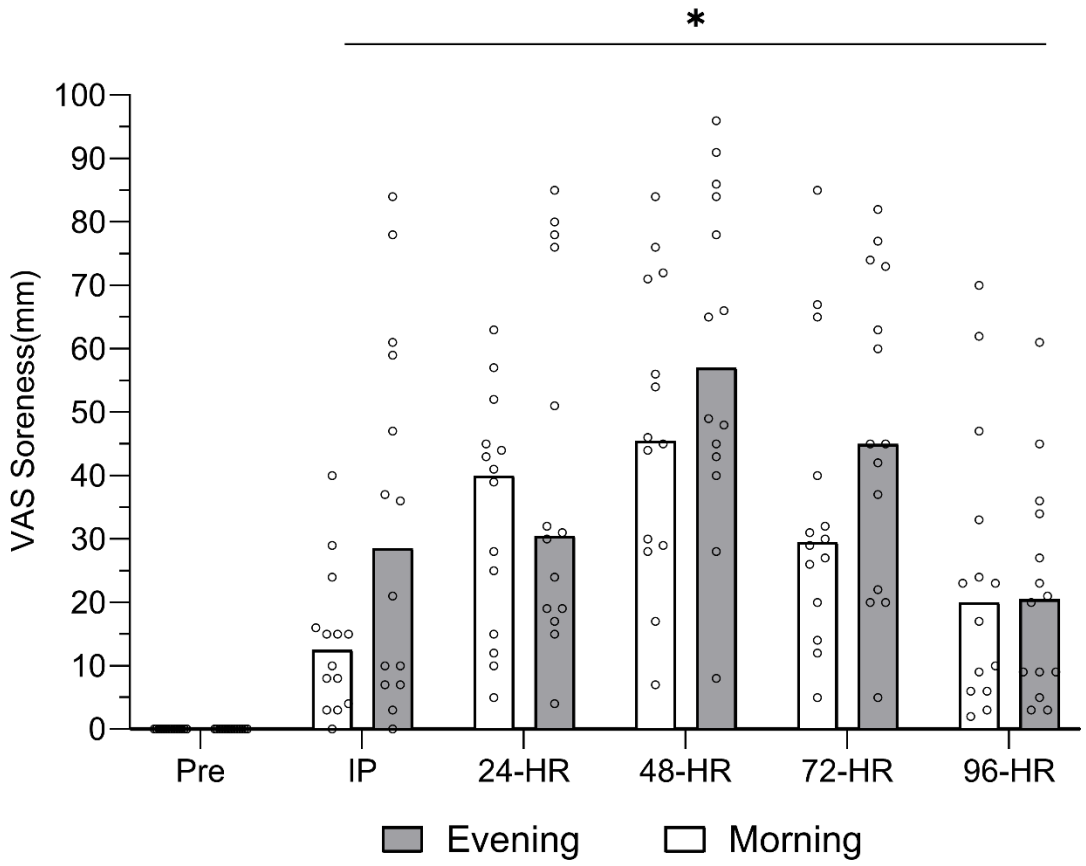


Figure 10. – Muscle soreness prior to (PRE) and over 4 days following eccentric exercise performed in the morning (white) and evening (gray). Values are displayed as means. * indicates a significant difference (main comparison) from pre-exercise values ($p < 0.05$).

4.07 Rochester Fatigue Index

Figure 11 displays changes in fatigue and energy state of participants expressed as values from 0 – 100. There was no significant time x group interaction ($p = 0.06$). There was no significant main effect of time ($p = 0.41$).

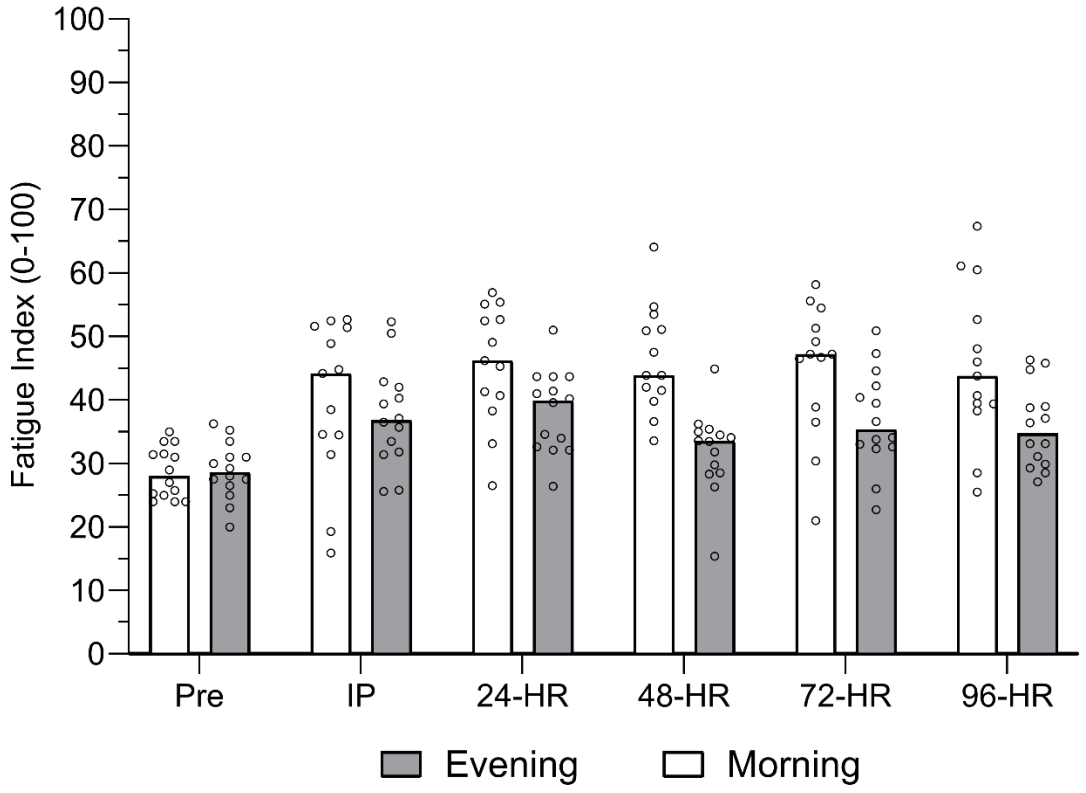


Figure 11. – Fatigue and energy state prior to visit 2 and over 4 days following eccentric exercise performed in the morning (white) and evening (gray). Values are displayed as means.

Table 2. Pearsons Product Moment Correlations of All Metrics of Damage

	MVC48h	DOMS48h	THICK48h	SWELL48h	ROM48h	TITINpre
MVC48h	---					
DOMS48h	-0.21	---				
THICK48h	0.39*	0.36	---			
SWELL48h	0.39*	0.29	0.86*	---		
ROM48h	0.18	-0.59*	-0.61*	-0.57*	---	
TITINpre	0.22	-0.23	0.07	0.29	0.14	---

**Represents significant correlation ($p < 0.05$) observed. MVC = Maximal Voluntary Contraction, DOMS = Delayed Onset Muscle Soreness, THICK = Muscle Thickness, SWELL = Muscle Swelling, ROM = Range of Motion, TITINpre = Pre-Exercise Urinary Titin*

4.08 Urinary Titin

Pre Exercise Urinary Titin

Post-exercise (96h post) urinary titin values were collected but upon analysis, exceeded the top detection limit of the assay kit (48.66-3000 pmol/L). All urinary titin values were corrected by creatinine values. Pre-exercise urinary titin values were not significantly different between groups (Morning Group = 46.46, Evening Group = 29.97, $p = 0.10$, Figure 12). Pre-exercise urinary titin values were not significantly correlated to any post-exercise metric of muscle damage ($p \geq 0.05$, Table 2). Pre-exercise urinary titin values were not significantly different between sexes in the entire cohort ($p = 0.42$) Table 3. displays the correlations between all metrics of muscle damage at 48 hours, as this ensured that changes were attributed mostly to muscle damage and not fatigue.

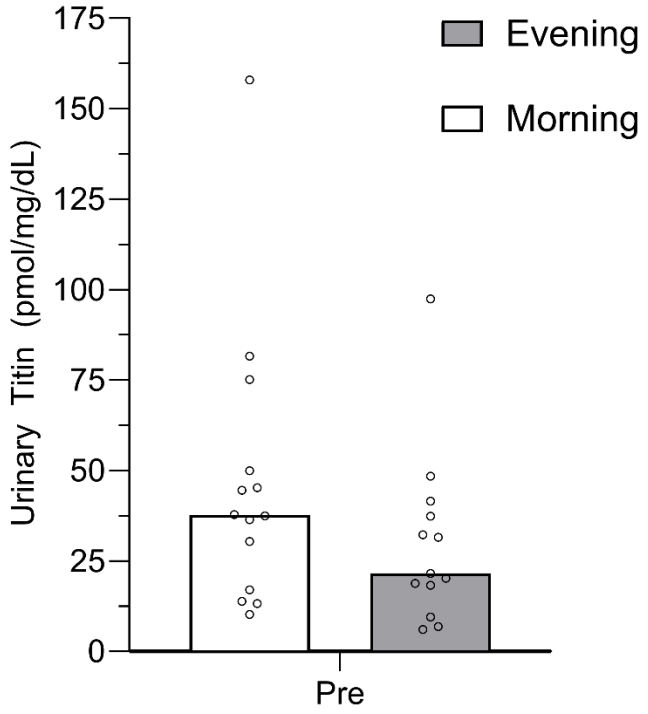


Figure 12. – Pre-exercise urinary titin values between morning (white) and evening (gray).

Table 3. Pearsons Product Moment Correlation of Urinary Titin (pmol/mg/dl)

	p Value, r Value		**p Value, r Value**
MVC (newtons)		**SWELL (cm)**	
0h	p = 0.76, r = 0.06	*0h*	p = 0.19, r = 0.28
24h	p = 0.64, r = 0.09	*24h*	p = 0.09, r = 0.33
48h	p = 0.27, r = 0.22	*48h*	p = 0.14, r = 0.29
72h	p = 0.34, r = 0.19	*72h*	p = 0.11, r = 0.32
96h	p = 0.29, r= 0.19	*96h*	p = 0.09, r = 0.32
DOMS (VAS 1-100)		**ROM (degrees of flexion)**	
0h	p = 0.69, r = 0.08	*0h*	p = 0.30, r = 0.31
24h	p = 0.75, r = 0.06	*24h*	p = 0.41, r = 0.17
48h	p = 0.24, r = -0.23	*48h*	p = 0.49, r = 0.14
72h	p = 0.08, r = -0.35	*72h*	p = 0.37, r = 0.18
96h	p = 0.13, r = -0.30	*96h*	p = 0.85, r = 0.04
THICK (cm)			
0h	p = 0.50, r = 0.05		
24h	p = 0.80, r = 0		
48h	p = 0.74, r = 0.07		
72h	p = 0.93, r = 0.02		
96h	p = 0.53, r = 0.13		

MVC: Maximal Voluntary Contraction; DOMS: Delayed Onset Muscle Soreness; THICK: Muscle Thickness; SWELL: Muscle Swelling; ROM: Range of Motion

Chapter V

Discussion

5.01 Discussion

Assessing diurnal variations in muscle damage may be important for clinicians and practitioners alike. Determining if evening exercise elicits the more muscle damage compared to morning exercise, or vice-versa, may be beneficial to strength and conditioning practitioners who wish to periodize a regimen in which resistance training interferes least with sport-specific training. Strength and physique athletes may want to prioritize a time-of-day in which more muscle damage occurs to capitalize on hypertrophic responses. However, the relationship between muscle damage and skeletal muscle hypertrophy remains contested. Clinicians may also want to schedule therapeutic resistance training during times-of-day in which less muscle damage may occur to minimize soreness as delayed onset muscle soreness is a common symptom of muscle damage. The resultant soreness may be a biopsychosocial deterrent for some.

Previous work in rodent models has demonstrated that titin splicing is regulated in a circadian manner through modulation of RNA Binding Motif Protein 20 (RBM20) expression[9]. This study by Riley and colleagues[9] utilized a chronic clock stop model to mechanistically understand circadian regulation of titin. When a core clock-controlled gene, BMAL1, was knocked out, downstream expression of RBM20 was reduced which reduced titin length and increased sarcomeric heterogeneity. BMAL1 in conjunction with CLOCK are important clock-controlled genes known to regulate the positive arm of the molecular clock, initiating the active phase. Should daily oscillations of these clock-controlled genes be profound enough, notable changes in sarcomeric integrity would be observed due to reductions in titin. Further, previous

work in rodent cardiomyocytes demonstrated a diurnal turnover of cardiac titin, with reductions in the inactive phase of the molecular clock[33]. Given titin plays an important structural role in the sarcomere, combined with the observed circadian regulation in rodent models, we wanted to examine this potential mechanism of muscle damage in a human study. We wanted to utilize the assessment of titin through urine samples as a non-invasive alternative to muscle biopsies and as a proxy of skeletal muscle titin. We reasonably assumed that urinary titin values would be reflective of skeletal muscle values (albeit from several days prior), as the assessment of urinary titin has been validated as a biomarker of muscle damage. Further, urinary titin concentrations have been demonstrated to be greater in that of patients suffering from muscle atrophy compared to healthy controls[42,47].

The primary findings of current study were that 1) pre-exercise values of urinary titin did not differ between the morning and evening groups, and 2) significant differences in muscle damage between morning (07:00-08:30H) and evening (17:00-18:30H) were not observed when looking at changes in maximal voluntary isometric contraction, delayed onset muscle soreness, muscle thickness, range of motion, and muscle swelling of the biceps. This may indicate that diurnal variation of titin in human skeletal muscle may not occur in a magnitude similar to that observed in animals.

Despite previous research from Hammouda and colleagues demonstrating an increase in muscle damage biomarkers occurring in the evening when compared to morning[12,13], we failed to replicate those findings in the present study. These previous studies utilized a one group, randomized, crossover design with a short latency period in elite rugby athletes. This design did not properly account for the repeated bout effect[15,57]. The degree of subsequent muscle damage with the repeated bout effect has been shown to be as low as 1/3rd of the initial bout of muscle

damage[63]. Yamaguchi and colleagues[15] also demonstrated that urinary titin fragments sensitively reflect the repeated bout effect in the elbow flexor with significant reductions observed in exercise bouts separated by one week. In the previous studies from Hammouda and colleagues[12,13] biomarkers of muscle damage such as creatine kinase were also immediately assessed post-exercise which calls into question whether these measures are attributed to metabolic perturbation, fatigue, or muscle damage. We took into consideration the methodological errors in previous studies and utilized a randomized two-group design and assessed performance metrics of muscle damage.

Previous work from Kanda and colleagues utilizing urinary titin as a biomarker of muscle damage has demonstrated that assessment of creatine kinase at 48 and 72 hours post exercise was significantly correlated to urinary titin at 48 and 72 hours post exercise[17]. In their damage protocol, they utilized a single-ankle plantar flexion exercise consisting of 10 sets of 40 repetitions with 3 minutes rest between sets. They also assessed MVC as a performance metric of muscle damage and only found significance when comparing immediately post, and 24 hours post to pre-exercise values. The latter finding is inconsistent with our findings, where we found significance at all time points (0h, 24h, 48h, 72h, 96h) compared to pre-exercise. This may be resultant of our protocol, where we standardized the degree of muscle damage each participant received instead of assigning an absolute workload. We performed 3 sets of 10 repetitions of eccentric bicep curls and ensured that participant MVC fell to 60% of their baseline MVC, ensuring an approximate 40% decline. Inami and colleagues[64] induced muscle damage of the elbow flexors utilizing an absolute workload and instructed participants to perform 5 sets of 10 eccentric repetitions with a dumbbell weighing 50% of elbow joint MVC. Inami and colleagues reported a 51% decline in MVC observed. Urinary titin fragments were significantly increased at

96h post-exercise. Range of motion and soreness of the elbow flexor at immediately post, 24h, 48h, 72h, and 96h post exercise[64]. These findings were consistent with our findings.

Previous work from Lee and colleagues[65] looked at different urinary titin fragment responses to two separate dynamic eccentric exercises of the lower limbs. They utilized repeated drop jumps and eccentric ergometer exercise of the quadriceps. Although not significant, they found changes in plasma myomesin-3 fragments, creatine kinase, myoglobin, and urinary titin fragments. However, the changes in urinary titin fragments were significantly correlated with those of creatine kinase and myoglobin. This provides reasonable evidence that significant muscle damage did not occur, but urinary titin fragments sensitively reflect muscle damage[65]. Post-exercise titin values which were collected at 96h post-exercise, exceeded the top detection limit of the assay kit. We were not able to quantify the degree of change in urinary titin fragments but can reasonably confirm its use as a non-invasive biomarker of muscle damage.

An interesting observation from the present study, was the lack of percent change in muscle thickness observed by an ultrasound, despite meaningful changes in muscle swelling when measuring circumference of the elbow flexor. This may be due to a combination of our sample consisting of untrained individuals and poor detection of the ultrasound device. Reductions in antagonist co-activation is a neuromuscular adaptation to training, with little to or lack thereof in untrained populations. This increase in circumference may be resultant of increases in damage of the elbow extensors and perfusion of intramuscular fluid from the biceps to the triceps. We cannot confirm this as metrics of muscle damage were not assessed on the elbow extensors, but it is a reasonable speculation.

5.02 Limitations

In this present study, we set forth criteria to determine the training status of individuals and we aimed to recruit untrained individuals for a more homogenous muscle damage response. Our criteria set forth for untrained status was ~6 months of no upper body resistance training. Previously well-trained individuals who have not resistance trained in the past 6 months met this criterion and were recruited. There is currently no distinct literature on the differences in muscle damage response between untrained and detrained individuals. Further, we cannot say with confidence that post-exercise urinary titin was different between groups, as these obtained values exceeded the detection limit of the ELISA kit.

5.03 Conclusion

We conclude that in untrained healthy college-aged men and women, that there is no significant difference in muscle damage between morning and evening. We also support previous work utilizing the current eccentric damaging protocol of the biceps as we identified a significant main effect of time, indicating significant muscle damage occurred. Further, we conclude that there is no diurnal variation in basal urinary titin values in healthy college-aged men and women. The assessment of diurnal variations in muscle damage can serve as an important tool for clinicians and practitioners and so future research should further investigate diurnal variations in skeletal muscle damage and urinary titin. Should a strong relationship be determined in a larger sample, it is important to determine basal circadian fluctuations in urinary titin to understand circadian related skeletal muscle integrity. Further investigation is needed to compare various time points in the day on its effect on skeletal muscle damage and should be investigated in trained athletes.

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APPENDIX A: IRB APPROVAL LETTER

The UNIVERSITY of OKLAHOMA

Institutional Review Board for the Protection of Human Subjects

Initial Submission – Board Approval

Date:	September 21, 2023	**IRB #:**	16226
To:	Christopher D Black, PhD	**Meeting Date:**	09/11/2023
		Approval Date:	09/21/2023
		Expiration Date:	08/31/2024

Study Title: Effect of Time-of-Day of Resistance Exercise on Muscle Damage and Urinary Titin Concentrations

Study Status: Active - Open - Expedited | Check-In Req

The University of Oklahoma Health Sciences Center's Institutional Review Board (IRB) reviewed and approved the above-referenced research study at its regularly scheduled meeting. It is the IRB's judgement that the proposed research, including the process of obtaining informed consent, will be conducted in a manner consistent with the requirements of 45 CFR 46 or 21 CFR 50 & 56.

Approval for this research is limited to the activities described in the approved protocol and application. In accordance with this approval, specific conditions for the conduct of this research are listed below, and informed consent from participants must be obtained as indicated.

Risk/Benefit Assessment: Research not involving greater than minimal risk.

Informed Consent Determination:
Informed consent and research privacy authorization must be obtained using the currently approved, stamped forms. You must retain all original, signed forms.

Continuing Review Determination:
As part of this approval, annual continuing review is not required. However, this research is still subject to annual monitoring. You must promptly submit an Annual Check-In Form to the IRB upon notification, approximately 30 days prior to the check-in due date indicated above.

Principal Investigator Responsibilities:

- Conduct the research study in a manner consistent with the requirements of the IRB and federal regulations at 45 CFR 46 and/or 21 CFR 50 and 56.
- Request approval from the IRB prior to implementing any/all modifications.
- Promptly report to the IRB any harm experienced by a participant that is both unanticipated and related per IRB Policy.
- Maintain accurate and complete study records for evaluation by the HRPP quality improvement program and if applicable, inspection by regulatory agencies and/or the study sponsor.

The following are also required if applicable to this research study:

- You may *not begin your study* until the contract through Office of Research Administration (ORA) is finalized and signed as per OUHSC Institutional policy.
- If this study involves external sites requiring a reliance agreement for OUHSC to serve as IRB of record, submit a modification to add each non-OU site and non-OU collaborator to the application after a reliance agreement has been finalized.

865 Research Parkway, Suite 400, Oklahoma City, OK 73104 (FWA00007961)

APPENDIX B: INFORMED CONSENT FORM

Consent Form to Participate in a Research Study
University of Oklahoma Health Sciences Center (OUHSC)

Study Title: Effect of Time-of-Day of Resistance Exercise on Muscle Damage and Urinary Titin Concentrations

Sponsor: University of Oklahoma Health and Exercise Science
Principal Investigator: Christopher Black, PhD
Phone Number: (706) 255-3750

KEY INFORMATION ABOUT THE RESEARCH STUDY

You are being asked to participate in a research study. Research studies are voluntary and include only people who choose to take part. This consent form begins with a 'Key Information' section to provide important information to help you decide whether or not to participate in this study. More detailed information is provided after the key information. Please take your time, discuss this with family and friends, and ask the investigator and study team any questions you may have.

WHY HAVE I BEEN ASKED TO PARTICIPATE IN THIS STUDY?

You are being asked to participate in this research study because you are a healthy college-age individual between the ages of 18-30 with no known contraindications to exercise.

WHY IS THIS STUDY BEING DONE AND HOW LONG WILL IT LAST?

The purpose of this study is to compare the effects of diurnal resistance exercise on muscle damage on healthy college aged individuals by assessment of urinary titin concentrations, decline in maximal voluntary isometric contraction, delayed onset muscle soreness, muscle swelling and muscle thickness.

We think that you will be in the study for six days, with a total time commitment of 2.5 hours.

WHAT WILL I BE ASKED TO DO IN THIS STUDY?

If you decide to participate in this study, you will be asked to perform high intensity eccentric exercise (exercise where you lower a very heavy weight down) in either the morning or early evening. You will be tracked over the course of several days to assess any changes in strength of your leg muscles and to assess your levels of muscle swelling, muscle thickness, muscle soreness, elbow range-of-motion, and maximal isometric strength (amount of strength you can exert against an immovable object). You will provide a urine sample prior to exercising and 96 hr (3 days) after the bout of exercise to examine the levels of a protein called titin in your urine.

WHY MIGHT I WANT TO PARTICIPATE IN THIS STUDY?

If you agree to take part in this study, there will not be direct medical benefit to you. We hope that the information learned from this study will benefit other patients with this disease in the future.

WHY MIGHT I NOT WANT TO PARTICIPATE IN THIS STUDY?

You may decide you may not want to participate as you will experience moderate to severe soreness of the lower extremities upon completion of experimental trials. There is also an unlikely chance of the occurrence of rhabdomyolysis, a condition where you experience severe muscle breakdown which is accompanied by large amounts of swelling in the damaged muscle as well as large amounts of protein in your blood and urine (making your urine appear red or brown). This condition may lead to kidney damage.

The researchers do not know all of the side effects that could happen. For a complete description of known risks, refer to the Detailed Information section of the consent form.

HOW WILL PARTICIPATING IN THE STUDY AFFECT ME FINANCIALLY?

There is no additional cost to you if you participate in this study.

You will be financially compensated with a gift card valued at $10. Termination from the study will deem you ineligible for financial compensation.

DETAILED INFORMATION ABOUT THE RESEARCH STUDY

The following pages of the consent form will provide you with more information about this study. Please take your time in reviewing this information and ask the investigator and study team any questions you may have.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About 40 people will take part in this study at this location.

WHAT IS INVOLVED IN THE STUDY?

If you decide to participate in this study, you will be asked to arrive to the Sensory and Muscle Function Lab for six visits. The first visit will encompass familiarization and paperwork. You will then be asked to perform a body composition analysis using dual energy x-ray absorptiometry (DEXA). For female participants, you will be screened for pregnancy beforehand, followed up by a menstrual history questionnaire. You will then be randomized to either a morning or evening group (07:00-08:30 h or 17:00-18:30 h respectively). You will be asked to provide a urine sample for assessment of hydration. For visit 2, upon successful completion of hydration assessment, you will be asked to provide a urine sample for baseline titin measurements. You will then be asked to perform a maximal voluntary isometric contraction (MVC) of the biceps muscle, and have range of motion (ROM), soreness (DOMS), circumference, and thickness of the biceps assessed. You will then be asked to work up to a 1 repetition maximum on the bicep curl with your non-dominant arm. You will then be asked to perform 3 sets of 10 repetitions of eccentric bicep curl at 120% of your one repetition maximum until your post-exercise MVC declines by 40% from baseline. You will then be asked to return 24 hours later for visit 3 to perform an MVC, and be assessed on ROM, DOMS, thickness, and circumference. You will then be asked to return 24 and 48 hours later to repeat the previous visit for visits 4 and 5. Finally, you will then be asked to return 24 hours

after visit 5 to provide urine for post-exercise titin assessment and perform another MVC, and be assessed on ROM, DOMS, thickness and circumference.

WHAT ARE THE RISKS OF THE STUDY?

As with any physical activity, there is minimal risk of musculoskeletal injury by participating in this study. In addition, there is minimal risk of developing a condition called exertional rhabdomyolysis. This typically occurs with overexertion during strenuous, novel physical activity, especially in certain populations (liver disease, sickle cell trait). To minimize this risk (<1%), you will be screened for factors that may predispose you to developing rhabdomyolysis and be excluded from participation should you exhibit any factor(s) predisposing you to greater than minimal risk. You will likely experience muscle soreness and discomfort after each eccentric exercise protocol. If at any point you experience severe pain in the exercised arm, greater than expected swelling of your arm, and/or notice your urine is dark red/brown in color contact the researchers immediately. You are encouraged to drink more water than usual while you are in the study and to avoid strenuous exercise with your arms (swimming, lifting weights, carrying heavy objects, boxing, etc.) for the duration of the study.

If you participate in this research, you will be exposed to radiation from a DXA scan (a type of xray). The amount of radiation to which you will be exposed from one DXA scan is approximately less than 1% of the amount of radiation that we are exposed to each year from natural background sources of radiation. The risk of radiation exposure is cumulative over your lifetime.

In addition to the risks described in the Key Information section, you may also be at risk for these side effects. You should discuss these with the researcher and/or your regular doctor. Other drugs may be given to make side effects less serious and uncomfortable. Many side effects go away shortly after the exercise protocol are stopped, but in some cases side effects can be serious or long lasting and permanent.

Risks and side effects related to the exercise protocol we are studying include:
1. Dizziness or nausea
2. Discomfort and/or soreness during and in the days following the exercise protocol which should subside within a week

For more information about risks and side effects, ask the researcher

TO WHAT EXTENT WILL MY INFORMATION BE KEPT CONFIDENTIAL?

Efforts will be made to keep your personal information confidential. You will not be identifiable by name or description in any reports or publications about this study. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. You will be asked to sign a separate authorization form for use or sharing of your protected health information.

There are organizations outside the OUHSC that may inspect and/or copy your research records for quality assurance and data analysis. These organizations may include the US Food & Drug Administration and other regulatory agencies. The OUHSC Human Research Participant Program office, the OUHSC Institutional Review Board, OUHSC Office of Compliance, and other University administrative offices may also inspect and/or copy your research records for these purposes.

Storing and Sharing Your Information:

Your data/measurements may be used for future studies without your additional consent. We will remove direct identifiers from your data/measurements and assign a code. The key to this code will be kept separately and only the researcher for this study will have access to the code. If your data/measurements is shared with another investigator for research purposes, they will not have access to the key code and will not be able to re-identify you.

CAN I WITHDRAW FROM THE STUDY?

You can stop participating in this study at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher first.
There may be circumstances under which your participation may be terminated by the investigator without your consent.

Examples:

- [He/She] feels that it is in your medical best interest.
- Your condition worsens.
- New information becomes available.
- You fail to follow study requirements.

WHAT IF I AM INJURED OR BECOME ILL WHILE PARTICIPATING IN THIS STUDY?

In the case of injury or illness results from this study, emergency medical treatment is available. If symptoms are not resolved in what the research team deems a reasonable time period then medical advice or assistance may be sought from the Goddard Health Center (405-325-4611) or by calling 911 if the research team deems it necessary.

The sponsor will reimburse your reasonable and necessary medical costs for diagnosis and treatment for a research-related illness or injury if the illness or injury:

- is a result of device being studied;
- is not the sole result of the investigator's failure to follow the study plan;
- is not the sole result of negligence of the investigator, the University, or a University employee.

Complications arising as a result of the natural progression of an underlying or pre-existing condition may be billed to you or your insurance. Please check with the investigator or with your insurance company if you have questions.

No other funds have been set aside by the University of Oklahoma Health Sciences Center, you or your insurance may be charged to compensate you in the event of injury, illness, or for other damages related to your event of injury or illness.

Complications arising as a result of the natural progression of an underlying or pre-existing condition will be billed to you or your insurance. Please check with the investigator or with your insurance company if you have questions.

WHAT ARE MY RIGHTS AS A PARTICIPANT?
Taking part in this study is voluntary. You may choose not to participate. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled.

If you agree to participate and then decide against it, you can withdraw for any reason and leave the study at any time. However, at certain times during the treatment, it may be harmful for you to withdraw, so please be sure to discuss leaving the study with the principal investigator or your regular doctor. You may discontinue your participation at any time without penalty or loss of benefits to which you are otherwise entitled.

We will provide you with any significant new findings developed during the course of the research that may affect your health, welfare, or willingness to continue your participation in this study.

You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study has completely finished. You consent to this temporary restriction.

Results of research testing on your sample will be returned to you if asked.

DO I HAVE ANY OTHER RIGHTS OVER MY DATA?
Depending on where the sponsor for your study is located and other factors, you may have additional rights over your personal data collected in this study. For example, the European Union General Data Protection Regulation (GDPR) and some state privacy laws might apply. If the GDPR applies, generally you may have the following rights:

1. The right to request the information collected to be corrected.
2. The right to withdraw your consent for the use of your personal information at any time.
3. The right, in some circumstances, to receive your personal information in a structured, commonly used and machine-readable format and the right to provide your information to a third party.
4. The right to strict confidentiality of your personal data when it is used/shared.
5. The right to limit the use/sharing of your personal information in certain circumstances.
6. The right under some circumstances to request the erasure of your personal data.
7. The right to file a complaint with a privacy protection regulator if you believe any of the rights above have been violated.

You can receive more information regarding these rights in the Privacy Notice for Research Participants, located on the OUHSC Office of Human Research Participant Protection (HRPP) website at *https://compliance.ouhsc.edu/HRPP/Participant/Privacy-Notice*.

If you have any questions and requests, please contact the HRPP Office at 405-271-2045.

WHOM DO I CALL IF I HAVE QUESTIONS, SUGGESTIONS, OR CONCERNS?
If you have questions, concerns, or complaints about the study or have a research-related injury, contact Christopher Black, PhD at (706) 255-3750

If you cannot reach the Investigator or wish to speak to someone other than the investigator and for questions about your rights as a research participant, contact the OUHSC Director, Office of Human Research Participant Protection, at 405-271-2045.

SIGNATURE:

By signing this form, you are agreeing to participate in this research study under the conditions described. You have not given up any of your legal rights or released any individual or entity from liability for negligence. You have been given an opportunity to ask questions. You will be given a copy of this consent document.

I agree to participate in this study:

SIGNATURE OF PERSON OBTAINING CONSENT	**Printed Name**	**Date**
SIGNATURE OF WITNESS	**Printed Name**	**Date**

APPENDIX C: HIPAA AUTHORIZATION FORM

University of Oklahoma Health Sciences CenterResearch Privacy Form 1
PHI Research Authorization

AUTHORIZATION TO USE or SHARE
HEALTH INFORMATION[1] THAT IDENTIFIES YOU FOR RESEARCH
An Informed Consent Document for Research Participation may also be required.
Form 2 must be used for research involving psychotherapy notes.

Title of Research Project: **Effect of Time-of-Day of Resistance Exercise on Muscle Damage and Urinary Titin Concentrations**

Leader of Research Team: **Christopher D. Black, PhD**

Address: **1401 Asp Avenue, #110 SFC, Norman, OK, 73019**

Phone Number**: 303-506-2562 (cell); 405-325-7668 (office)**

If you decide to sign this document, University of Oklahoma Health Sciences Center (OUHSC) researchers may use or share information that identifies you (protected health information) for their research. Protected health information will be called PHI in this document.

PHI To Be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your PHI. If you give permission, the researchers may use or share with the people identified in this Authorization any PHI related to this research from your medical records and from any test results. Information used or shared may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form; medical records and charts; name, address, telephone number, date of birth, race, government-issued identification numbers, and nothing else.

Purposes for Using or Sharing PHI. If you give permission, the researchers may use your PHI to determine if it is safe for your to participate in the exercise used in this study.

Other Use and Sharing of PHI. If you give permission, the researchers may also use your PHI to develop new procedures or commercial products. They may share your PHI with other researchers, the research sponsor and its agents, the OUHSC Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Food and Drug Administration (FDA) and the Department of Health and Human Services (HHS), and when required by law. The researchers may also share your PHI with with your physician and/or a University of Oklahoma physician in the event of a serious health risk or adverse event that occurs during the study.

Confidentiality. Although the researchers may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information

[1] **Protected Health Information includes all identifiable information relating to any aspect of an individual's health whether past, present or future, created or maintained by a Covered Entity.**

confidential, but confidentiality is not guaranteed. The law does not require everyone receiving the information covered by this document to keep it confidential, so they could release it to others, and federal law may no longer protect it.

YOU UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING A COMMUNICABLE OR NONCOMMUNICABLE DISEASE.

Voluntary Choice. The choice to give OUHSC researchers permission to use or share your PHI for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OUHSC researchers to use or share your PHI if you want to participate in the research and, if you cancel your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care unrelated to this study from OUHSC.

Canceling Permission. If you give the OUHSC researchers permission to use or share your PHI, you have a right to cancel your permission whenever you want. However, canceling your permission will not apply to information that the researchers have already used, relied on, or shared or to information necessary to maintain the reliability or integrity of this research.

End of Permission. Unless you cancel it, permission for OUHSC researchers to use or share your PHI for their research will never end.

Contacting OUHSC: You may find out if your PHI has been shared, get a copy of your PHI, or cancel your permission at any time by writing to:

Privacy Official University of Oklahoma Health Sciences Center PO Box 26901 Oklahoma City, OK 73190	or	Privacy Board University of Oklahoma Health Sciences Center PO Box 26901 Oklahoma City, OK 73190

If you have questions, call: (405) 271-2511 or (405) 271-2045.

Access to Information. You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study is completely finished. You consent to this temporary restriction.

Giving Permission. By signing this form, you give OUHSC and OUHSC's researchers led by the Research Team Leader permission to share your PHI for the research project listed at the top of this form.

University of Oklahoma Health Sciences CenterResearch Privacy Form 1
PHI Research Authorization

Patient/Participant Name (Print): _____

_____ _____
Signature of Patient-Participant Date
or Parent if Participant is a minor

Or

_____ _____
Signature of Legal Representative** Date

**If signed by a Legal Representative of the Patient-Participant, provide a description of the relationship to the Patient-Participant and the authority to act as Legal Representative:

_____
OUHSC may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Patient-Participant or the Legal Representative at the time this signed form is provided to the researcher or his representative.

APPENDIX D: THE PHYSICAL ACTIVITY READINESS QUESTIONNAIRE

2022 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1) Has your doctor ever said that you have a heart condition ☐ **OR** high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** _____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it **does not limit your current ability** to be physically active. **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

☑ **If you answered NO to all of the questions above, you are cleared for physical activity.**
Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow Global Physical Activity Guidelines for your age (https://www.who.int/publications/i/item/9789240015128).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

PARTICIPANT DECLARATION
If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for its records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

 1 / 4
01-11-2021

2022 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?

If the above condition(s) is/are present, answer questions 1a-1c — If **NO** ☐ go to question 2

1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES ☐ NO ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES ☐ NO ☐

2. Do you currently have Cancer of any kind?

If the above condition(s) is/are present, answer questions 2a-2b — If **NO** ☐ go to question 3

2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES ☐ NO ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES ☐ NO ☐

3. Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm

If the above condition(s) is/are present, answer questions 3a-3d — If **NO** ☐ go to question 4

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES ☐ NO ☐
3c.	Do you have chronic heart failure?	YES ☐ NO ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES ☐ NO ☐

4. Do you currently have High Blood Pressure?

If the above condition(s) is/are present, answer questions 4a-4b — If **NO** ☐ go to question 5

4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES ☐ NO ☐

5. Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes

If the above condition(s) is/are present, answer questions 5a-5e — If **NO** ☐ go to question 6

5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES ☐ NO ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES ☐ NO ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES ☐ NO ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES ☐ NO ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES ☐ NO ☐

2022 PAR-Q+

6. **Do you have any Mental Health Problems or Learning Difficulties?** This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome

If the above condition(s) is/are present, answer questions 6a-6b If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. **Do you have a Respiratory Disease?** *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*

If the above condition(s) is/are present, answer questions 7a-7d If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. **Do you have a Spinal Cord Injury?** *This includes Tetraplegia and Paraplegia*

If the above condition(s) is/are present, answer questions 8a-8c If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. **Have you had a Stroke?** *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*

If the above condition(s) is/are present, answer questions 9a-9c If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. **Do you have any other medical condition not listed above or do you have two or more medical conditions?**

If you have other medical conditions, answer questions 10a-10c If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: _____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

01-11-2021

2022 PAR-Q+

If you answered NO to all of the FOLLOW-UP questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact

www.eparmedx.com

Email: eparmedx@gmail.com

Citation for PAR-Q+

Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration. The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada 4(2):3-23, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

Key References

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2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance: Consensus Document. APNM 36(S1):S266-s298, 2011.
3. Chisholm DM, Collis ML, Kulak LL, Davenport W, and Gruber N. Physical activity readiness. British Columbia Medical Journal. 1975;17:375-378.
4. Thomas S, Reading J, and Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). Canadian Journal of Sport Science 1992;17:4 338-345.

APPENDIX E: MENSTRUAL HISTORY QUESTIONNAIRE

Department of Health and Exercise Science
University of Oklahoma

MENSTRUAL HISTORY QUESTIONNAIRE

Participant ID:_____Date:_____

We are asking you to give us as complete a menstrual history as possible. All information is strictly confidential.

Are you pregnant (circle your response)

YES- Do not complete the rest of this form
NO- Continue to section A.

SECTION A: CURRENT MENSTRUAL STATUS

1. Approximately how many menstrual periods have you had during the past 12 months?
   (please circle what months you have had a period. This means from this time last year to the present month)

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

2. What is the usual length of your menstrual cycle (first day of your period to the next onset of your period)?

   _____days. Today is day _____ of your present menstrual cycle.

3. When was the date of the onset of your last period?

4. When do you expect your next period?

5. What is the average length (number of days) of your menstrual flow? _____ days

   How many of these days do you consider "heavy"?_____days

6. Do you take oral contraceptives or any other medication that includes estrogen and/or progesterone?

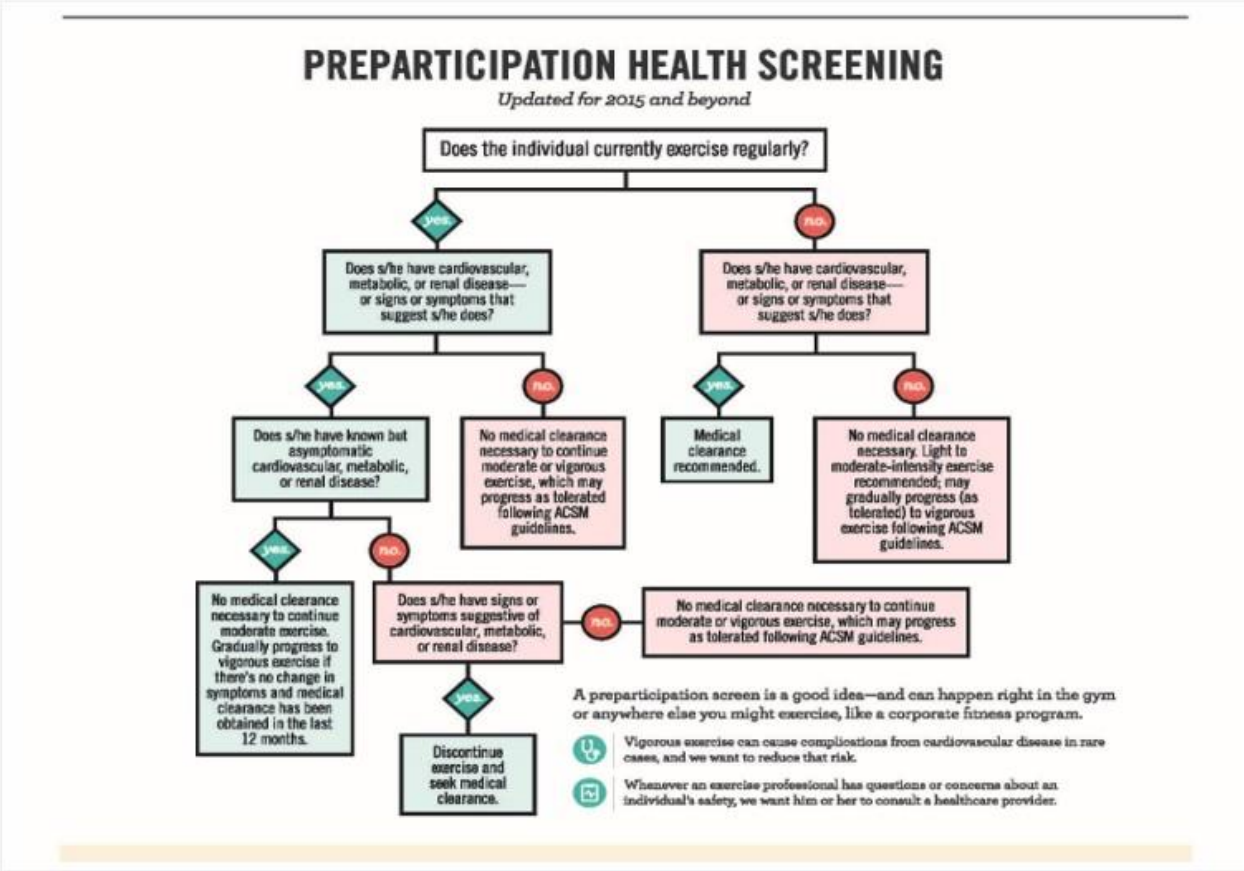
   If yes, how long have you been taking this medication?_____

   What is the brand name and dosage of this mediation?_____

Has this medication affected your menstrual cycle (regularity, length and amount of flow)? If yes, indicate changes.

IRB NUMBER: 15311
IRB APPROVAL DATE: 01/18/2023

APPENDIX F: HEALTH SCREENING QUESTIONNAIRE



APPENDIX G: RHABDOMYALSIS SCREENING

Screening questionnaire

Participant ID:_____
Date: _____

1. Do you participate in some form of physical activity at least 3 days per week? Yes or No
2. If you answered "Yes" to #1, please list and describe the type and frequency of activity in which you typically engage
   _____
   _____
   _____
3. Have you had any shoulder, elbow, and/or wrist injuries in the previous 6 months? Yes or No
4. Have you taken any type of pain relievers within the previous 7 days? Yes or No
5. Are you taking any medications, prescription or over-the-counter including birth control? Yes or No
6. If you answered "Yes" to #4 or 5, please list the medications, the reasons for taking them, the prescribed dosage, and how long you have been taking them on a consistent basis.
   _____
   _____
   _____
7. Have you consumed any alcohol, tranquilizers, sleeping pills, antidepressants, opiates, cocaine, amphetamines, PCP, or barbiturates within the previous 7 days days? Yes or No
8. Have you consumed any antibiotics, laxatives, diuretics, neuroleptics, or theophyline within the previous 7 days? Yes or No
9. Are you consuming any performance enhancing drugs? Yes or No
10. Are you consuming any vitamins or dietary supplements? Yes or No
11. If you answered "Yes" to #7 to 10, please list what you have been taking?
    _____
    _____
    _____
12. Have you been ill within the previous week or are you currently ill (cold, flu, etc.)? Yes or No
13. Have you made in changes in your diet in the last month? Yes or No
14. Do you have to maintain a specific type of diet for any reason? Yes or No
15. If so, why are you having to maintain the diet?
    _____
    _____
16. Have you been diagnosed with diabetes or high blood pressure? Yes or No
17. Do you have any history of kidney or liver dysfunction? Yes or No
18. Do you have any history of heat illness? Yes or No
19. Do you have any history of swelling after exercise? Yes or No
20. Do you have any history of bruising easily? Yes or No
21. Do you have a family history of muscle disease? Yes or No
22. Are you currently undergoing statin or thyroid replacement therapy? Yes or No

APPENDIX H: RECRUITMENT FLYER

Interested in the Circadian Cycle and Muscle Damage?

Research Participants Needed

The Sensory and Muscle Function Lab is conducting a study titled:

Diurnal Effects of Exercise on Markers of Muscle Damage in College-Aged Individuals

To participate

- Males and females between 18-30 years of age.
- Healthy participants with no renal disorders or past ICU hospitalization and free from any musculoskeletal injuries.

6 visits required

- Total time commitment is approximately 2.5 hours.
- Testing will take place in the Sensory and Neuromuscular Functions lab at the University of Oklahoma Norman Campus.

Compensation

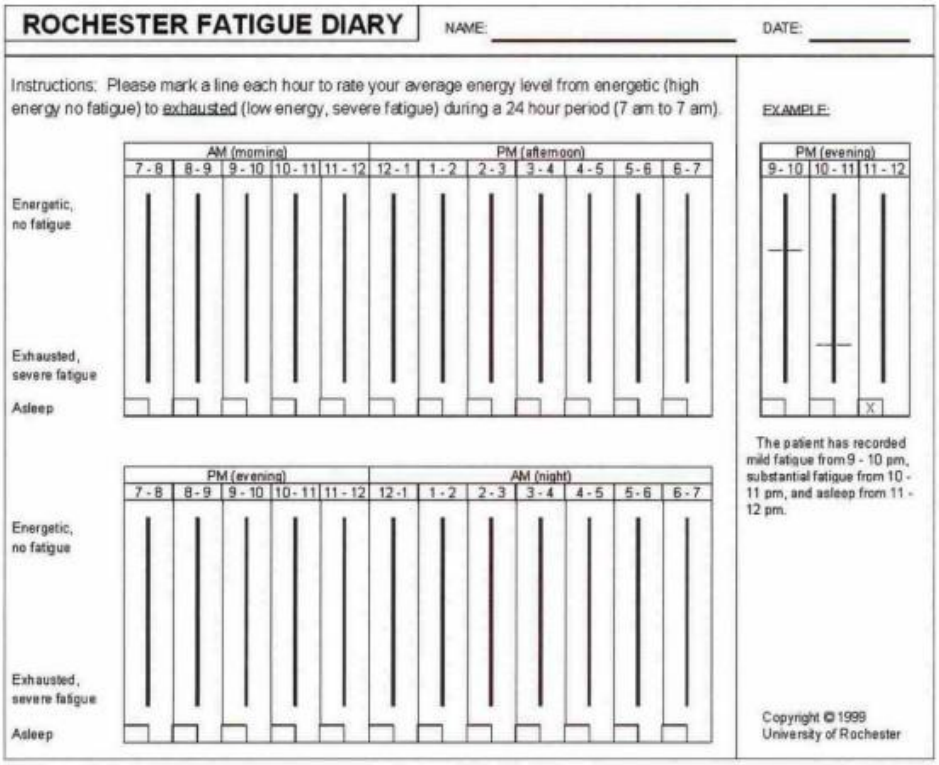
- You will be compensated for your time in the form of a gift card and a DXA scan.

If you are eligible and interested please contact Chinguun Khurelbaatar , chinguun.khurelbaatar-1@ou.edu or Dr. Chris Black (Primary Investigator), cblack@ou.edu

The University of Oklahoma is an equal opportunity institution.

Chinguun Khurelbaatar chinguun.khurelbaatar-1@ou.edu (715) 252-6049	Chinguun Khurelbaatar chinguun.khurelbaatar-1@ou.edu (715) 252-6049	Chinguun Khurelbaatar chinguun.khurelbaatar-1@ou.edu (715) 252-6049	Chinguun Khurelbaatar chinguun.khurelbaatar-1@ou.edu (715) 252-6049	Chinguun Khurelbaatar chinguun.khurelbaatar-1@ou.edu (715) 252-6049	Chinguun Khurelbaatar chinguun.khurelbaatar-1@ou.edu (715) 252-6049	Chinguun Khurelbaatar chinguun.khurelbaatar-1@ou.edu (715) 252-6049	Chinguun Khurelbaatar chinguun.khurelbaatar-1@ou.edu (715) 252-6049	Chinguun Khurelbaatar chinguun.khurelbaatar-1@ou.edu (715) 252-6049	Chinguun Khurelbaatar chinguun.khurelbaatar-1@ou.edu (715) 252-6049

APPENDIX I: ROCHESTER FATIGUE



ROCHESTER FATIGUE DIARY NAME: _____ DATE: _____

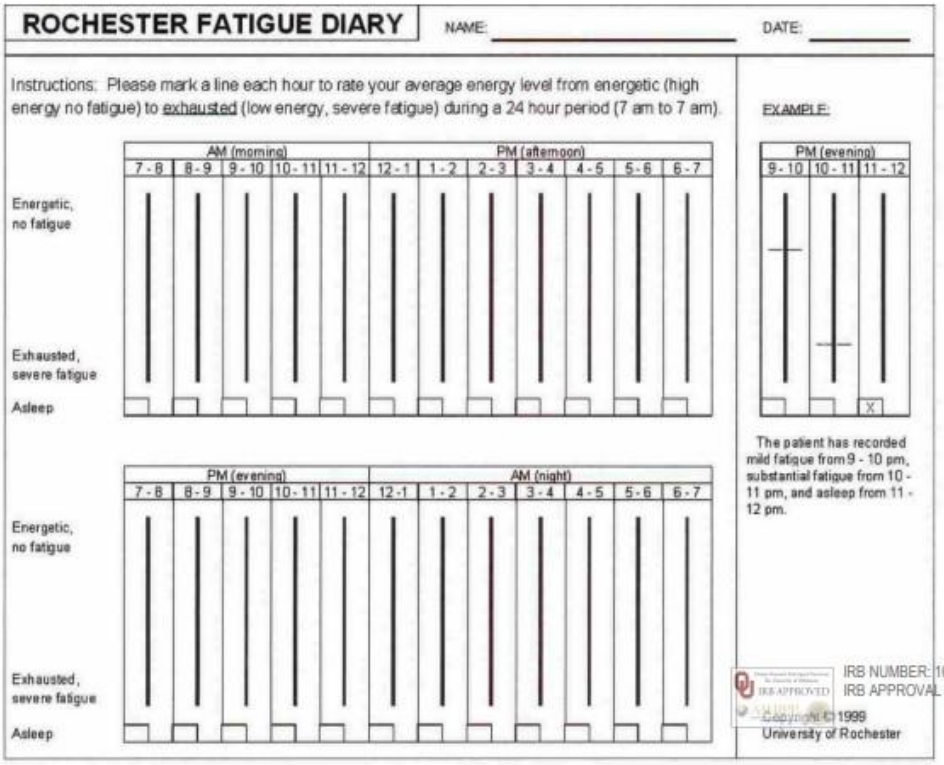
Instructions: Please mark a line each hour to rate your average energy level from energetic (high energy no fatigue) to exhausted (low energy, severe fatigue) during a 24 hour period (7 am to 7 am).

EXAMPLE:

AM (morning): 7 - 8 | 8 - 9 | 9 - 10 | 10 - 11 | 11 - 12
PM (afternoon): 12 - 1 | 1 - 2 | 2 - 3 | 3 - 4 | 4 - 5 | 5 - 6 | 6 - 7
PM (evening): 9 - 10 | 10 - 11 | 11 - 12
AM (night): 12 - 1 | 1 - 2 | 2 - 3 | 3 - 4 | 4 - 5 | 5 - 6 | 6 - 7

Energetic, no fatigue
Exhausted, severe fatigue
Asleep

The patient has recorded mild fatigue from 9 - 10 pm, substantial fatigue from 10 - 11 pm, and asleep from 11 - 12 pm.



ROCHESTER FATIGUE DIARY NAME: _____ DATE: _____

Instructions: Please mark a line each hour to rate your average energy level from energetic (high energy no fatigue) to exhausted (low energy, severe fatigue) during a 24 hour period (7 am to 7 am).

EXAMPLE:

AM (morning): 7 - 8 | 8 - 9 | 9 - 10 | 10 - 11 | 11 - 12
PM (afternoon): 12 - 1 | 1 - 2 | 2 - 3 | 3 - 4 | 4 - 5 | 5 - 6 | 6 - 7
PM (evening): 9 - 10 | 10 - 11 | 11 - 12
AM (night): 12 - 1 | 1 - 2 | 2 - 3 | 3 - 4 | 4 - 5 | 5 - 6 | 6 - 7

Energetic, no fatigue
Exhausted, severe fatigue
Asleep

The patient has recorded mild fatigue from 9 - 10 pm, substantial fatigue from 10 - 11 pm, and asleep from 11 - 12 pm.

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APPENDIX J: VISUAL ANALOGUE SCALE

Visual analog pain intensity scale

No pain at all _____ Most intense pain imaginable

APPENDIX K: COVID SCREENING

Q1.
What year are you in college?

A. Freshman
B. Sophomore
C. Junior
D. Senior
E. Graduate Student
F. Other

Q2.
What is your age in years? _____

Q3.
What is your height in centimeters? _____

Q4.
What is your weight in kilograms? _____

Q5.
What is your biological sex?

A. Man
B. Woman
C. Other

Q6.
Are you Hispanic/Latino?

A. Yes
B. No

Q7.
What race do you identify as?

A. White
B. Black or African American
C. American Indian or Alaska Native
D. Asian
E. Native Hawaiian or Pacific Islander
F. Other

Q8.
Smoking Status

A. Non-smoker
B. Former smoker
C. Current smoker
D. Prefer not to say

Q9.

Do you have any of the following conditions (circle all that apply)?

- COPD
- Hypertension
- Asthma
- Fibromyalgia
- Anxiety
- Depression
- Migraine
- Erectile Dysfunction
- Multiple Sclerosis
- Celiac Disease
- Irritable Bowl Syndrome
- Endometriosis
- Anemia / Low Hemoglobin Content
- Polycystic Ovarian Syndrome
- Back Pain
- Arthritis
- Cancer
- Chronic Sinusitis
- Disordered Eating
- Substance Misuse/Abuse
- Deafness
- Blindness

Q10.

Are you vaccinated for COVID-19?

A. No
B. Partially (one shot of Moderna or Pfizer but not both)
C. Fully (two shots of Moderna or Pfizer, or one shot of Johnson & Johnson)
D. Boosted Once (fully vaccinated and another shot received)
E. Boosted Twice (fully vaccinated and 2 more shots received)

Q11.

How many times have you had COVID-19 (confirmed by COVID-19 PCR test and/or a rapid at-home test)?

A. 0
B. 1
C. 2
D. 3
E. 4+

If you answered B.

When did you get COVID-19 the first time?

Month: _____

Year: _____

What was your vaccination status at the time of your first infection?

A. Unvaccinated
B. Partially vaccinated (one shot of Moderna or Pfizer but not both)
C. Fully vaccinated (both shots of Moderna or Pfizer, or one Johnson & Johnson)
D. Fully vaccinated and boosted (both shots of Moderna or Pfizer, or the J&J AND a booster shot)
E. Fully vaccinated and boosted twice (both shots of Moderna or Pfizer, or the J&J, and two booster shots)

If you answered C.

When did you get COVID-19 the second time?

Month: _____

Year: _____

What was your vaccination status at the time of your second infection?

A. Unvaccinated
B. Partially vaccinated (one shot of Moderna or Pfizer but not both)
C. Fully vaccinated (both shots of Moderna or Pfizer, or one Johnson & Johnson)
D. Fully vaccinated and boosted (both shots of Moderna or Pfizer, or the J&J AND a booster shot)
E. Fully vaccinated and boosted twice (both shots of Moderna or Pfizer, or the J&J, and two booster shots)

If you answered D.

When did you get COVID-19 the third time?

Month: _____

Year: _____

What was your vaccination status at the time of your third infection?

A. Unvaccinated
B. Partially vaccinated (one shot of Moderna or Pfizer but not both)
C. Fully vaccinated (both shots of Moderna or Pfizer, or one Johnson & Johnson)
D. Fully vaccinated and boosted (both shots of Moderna or Pfizer, or the J&J AND a booster shot)
E. Fully vaccinated and boosted twice (both shots of Moderna or Pfizer, or the J&J, and two booster shots)

If you answered E.

When did you get COVID-19 the fourth time?

Month: _____

Year: _____

What was your vaccination status at the time of your fourth infection?

A. Unvaccinated
B. Partially vaccinated (one shot of Moderna or Pfizer but not both)

C. Fully vaccinated (both shots of Moderna or Pfizer, or one Johnson & Johnson)
D. Fully vaccinated and boosted (both shots of Moderna or Pfizer, or the J&J AND a booster shot)
E. Fully vaccinated and boosted twice (both shots of Moderna or Pfizer, or the J&J, and two booster shots)

Q12.

Were you hospitalized for any of your infection(s)?

A. Yes (if yes, circle all that apply)
    a. First time
    b. Second time
    c. Third time
    d. Fourth time
B. No

Q13.

Did you have symptoms with your first infection?

A. Yes
B. No

If yes, how severe were the following symptoms? (0-10, 0 = no symptoms, 10 = very severe)

Fever: _____

Cough: _____

Headache: _____

Shortness of Breath: _____

Change in Smell/Taste: _____

Fatigue or Tiredness That Interferes with Daily Life: _____

Difficulty Thinking or Concentrating: _____

Chest Pain: _____

Body/Joint Aches: _____

Sleep Problems: _____

Heart Palpitations: _____

Dizziness When Standing Up: _____

Diarrhea: _____

Stomach Pain: _____

Rash: _____

Paresthesia: _____

Anxiety: _____

Depression: _____

Change in Menstrual Cycle: _____

Change in Sexual Function: _____

Q14.

Did you have symptoms with your second infection?

A. Yes
B. No

If yes, how severe were the following symptoms? (0-10, 0 = no symptoms, 10 = very severe)

Fever: _____

Cough: _____

Headache: _____

Shortness of Breath: _____

Change in Smell/Taste: _____

Fatigue or Tiredness That Interferes with Daily Life: _____

Difficulty Thinking or Concentrating: _____

Chest Pain: _____

Body/Joint Aches: _____

Sleep Problems: _____

Heart Palpitations: _____

Dizziness When Standing Up: _____

Diarrhea: _____

Stomach Pain: _____

Rash: _____

Paresthesia: _____

Anxiety: _____

Depression: _____

Change in Menstrual Cycle: _____

Change in Sexual Function: _____

Q15.

Did you have symptoms with your third infection?

A. Yes
B. No

If yes, how severe were the following symptoms? (0-10, 0 = no symptoms, 10 = very severe)

Fever: _____

Cough: _____

Headache: _____

Shortness of Breath: _____

Change in Smell/Taste: _____

Fatigue or Tiredness That Interferes with Daily Life: _____

Difficulty Thinking or Concentrating: _____

Chest Pain: _____

Body/Joint Aches: _____

Sleep Problems: _____

Heart Palpitations: _____

Dizziness When Standing Up: _____

Diarrhea: _____

Stomach Pain: _____

Rash: _____

Paresthesia: _____

Anxiety: _____

Depression: _____

Change in Menstrual Cycle: _____

Change in Sexual Function: _____

Q16.

Did you have symptoms with your fourth infection?

A. Yes
B. No

If yes, how severe were the following symptoms? (0-10, 0 = no symptoms, 10 = very severe)

Fever: _____

Cough: _____

Headache: _____

Shortness of Breath: _____

Change in Smell/Taste: _____

Fatigue or Tiredness That Interferes with Daily Life: _____

Difficulty Thinking or Concentrating: _____

Chest Pain: _____

Body/Joint Aches: _____

Sleep Problems: _____

Heart Palpitations: _____

Dizziness When Standing Up: _____

Diarrhea: _____

Stomach Pain: _____

Rash: _____

Paresthesia: _____

Anxiety: _____

Depression: _____

Change in Menstrual Cycle: _____

Change in Sexual Function: _____

Q17.

Are you currently experiencing any post-COVID symptoms that have lasted longer than 3 months?

A. Yes
B. No

APPENDIX L: DATA COLLECTION FORM

Visit 1 – Familiarization
Time:_____ AM / PM
Subject ID:_____ Name:_____ Date:_____
Sex: M | F

Informed Consent: Y | N
Rhabdomyolysis Screening: Y | N
Health Screening Questionnaire: Y | N
PAR-Q: Y | N
(If Female)
Pregnancy Test: P | F
Menstrual History Questionnaire: Y | N

DEXA ASSESSMENT

LBM (KG): _____
BMC (KG): _____
FM (KG): _____

RANDOMIZATION (CIRCLE ONE)
Morning (07:00 – 08:30 am)
Evening (05:00 – 06:30 pm)

Visit 2 – Experimental Protocol
SUBJECT ARRIVAL
Time:_____ AM/PM

PRE-EXERCISE PROTOCOL
Diet Log: Y | N
Sleep Questionnaire: Y | N
Hydration Status (1.004 – 1.029): _____ USG
Baseline Urine Sample: Y | N
Baseline Bicep ROM: _____
Ultrasound Thickness: _____ mm
Bicep Circumference: _____ cm

BASELINE MAXIMAL VOLUNTARY CONTRACTION
Attempt 1: _____ (Nm)
Attempt 2: _____ (Nm)
Attempt 3: _____ (Nm)

Peak MVC Value _____ (Nm)

1RM ESTABLISHMENT

Warm Ups:

Load_____Reps_____
Load_____Reps_____
Load_____Reps_____
Load_____Reps_____
Load_____Reps_____
Load_____Reps_____
Load_____Reps_____
Load_____Reps_____

1 RM = _____ lbs

EXERCISE PROTOCOL (3 x 10 @ 120% 1 RM)

Load_____Reps_____
Load_____Reps_____
Load_____Reps_____

(Additional Sets – If Needed)

Load_____Reps_____
Load_____Reps_____
Load_____Reps_____
Load_____Reps_____
Load_____Reps_____
Load_____Reps_____

Post-Exercise Maximal Voluntary Contraction

Time _____ AM / PM

Attempt 1: _____ (Nm)
Attempt 2: _____ (Nm)
Attempt 3: _____ (Nm)
Peak MVC Value _____ (Nm)
% Decline _____

POST-EXERCISE PROTOCOLS

Post-Exercise Bicep ROM: _____
Ultrasound Thickness: _____ mm
Bicep Circumference: _____ cm

Visit 3 (24h post visit 2)

SUBJECT ARRIVAL

Time:_____ AM/PM

Diet Log: Y | N
Sleep Questionnaire: Y | N

Bicep ROM: _____
Ultrasound Thickness: _____ mm
Bicep Circumference: _____ cm
Delayed Onset Muscle Soreness: _____ VAS

MAXIMAL VOLUNTARY CONTRACTION
Attempt 1: _____ (Nm)
Attempt 2: _____ (Nm)
Attempt 3: _____ (Nm)
Peak MVC Value _____ (Nm)
% Decline from Baseline: _____

Visit 4 (24h post visit 3)
SUBJECT ARRIVAL
Time:_____ AM/PM

Diet Log: Y | N
Sleep Questionnaire: Y | N
Bicep ROM: _____
Ultrasound Thickness: _____ mm
Bicep Circumference: _____ cm
Delayed Onset Muscle Soreness: _____ VAS

MAXIMAL VOLUNTARY CONTRACTION
Attempt 1: _____ (Nm)
Attempt 2: _____ (Nm)
Attempt 3: _____ (Nm)
Peak MVC Value _____ (Nm)
% Decline from Baseline: _____

Visit 5 (24h post visit 4)
SUBJECT ARRIVAL
Time:_____ AM/PM

Diet Log: Y | N
Sleep Questionnaire: Y | N
Bicep ROM: _____
Ultrasound Thickness: _____ mm
Bicep Circumference: _____ cm
Delayed Onset Muscle Soreness: _____ VAS

MAXIMAL VOLUNTARY CONTRACTION
Attempt 1: _____ (Nm)
Attempt 2: _____ (Nm)
Attempt 3: _____ (Nm)
Peak MVC Value _____ (Nm)
% Decline from Baseline: _____

Visit 6 (24h post visit 5)
SUBJECT ARRIVAL
Time:_____ AM/PM

Diet Log: Y | N
Sleep Questionnaire: Y | N
Hydration Status (1.004 – 1.029): _____ USG
Post-Exercise Urine Sample: Y | N
Bicep ROM: _____
Ultrasound Thickness: _____ mm
Bicep Circumference: _____ cm
Delayed Onset Muscle Soreness: _____ VAS

MAXIMAL VOLUNTARY CONTRACTION
Attempt 1: _____ (Nm)
Attempt 2: _____ (Nm)
Attempt 3: _____ (Nm)
Peak MVC Value _____ (Nm)
% Decline from Baseline: _____
