Effects of an Acute High-Volume Isokinetic Intervention on Inflammatory and Strength Changes: Influence of Age

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EFFECTS OF AN ACUTE HIGH VOLUME ISOKINETIC INTERVENTION ON
INFLAMMATORY AND STRENGTH CHANGES: INFLUENCE OF AGE

by

JOSEPH A. GORDON III
B.S. Sport & Exercise Science, University of Central Florida, 2014

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ABSTRACT

PURPOSE: The purpose of this study was to compare the effects of a high volume isokinetic intervention on lower body strength and inflammation, as well as markers of muscle damage in the subsequent 48 hours between younger and middle-aged men. METHODS: 19 healthy, recreationally trained men were randomly assigned to two groups, younger adults (YA: 21.8 ± 2.0 y; 90.7 ± 11.6 kg; 21.5 ± 4.1 % body fat), or middle-aged adults (MA: 47.0 ± 4.4 y; 96.0 ± 21.5; 24.8 ± 6.3 % body fat). Both groups reported to the human performance laboratory (HPL) on four separate occasions. On the first visit (D1), anthropometric assessment, as well as a familiarization session with the isokinetic dynamometer, was performed. A muscle damaging protocol (HVP) was performed on the second visit (D2) consisting of 8 sets of 10 repetitions at 60°·sec⁻¹ on the isokinetic dynamometer. An assessment protocol (AP) was performed to assess performance decrements between the YA and MA groups. For this protocol, a maximal voluntary isometric contraction (MVIC) was performed, as well as 3 isokinetic kicks at 2 different speeds (240°·sec⁻¹ and 60°·sec⁻¹). For the MVIC, values for peak torque (PKT), average torque (AVGT), rate of torque development at 100 ms (RTD100), and 200 ms (RTD200) were recorded. For the isokinetic kicks at 240°·sec⁻¹ (ISK240) and 60°·sec⁻¹ (ISK60), values were also recorded for peak torque (PKT), average torque (AVGT), as well as peak power (PP), and average power (AVGP). The AP was performed before the HVP (BL), immediately after the HVP (IP), 120 minutes after the HVP (120P), as well as one (24H) and two (48H) days following the HVP. Blood draws were also taken at BL, IP, 24H, and 48H, as well as 30 minutes (30P), and 60 minutes (60P) following the HVP to assess circulating levels of creatine kinase (CK), myoglobin (Mb), c-reactive protein (CRP), and interleukin 6 (IL-6). Ultrasound
assessment was also performed at BL and IP as well to assess changes in muscle morphology as a result of the intervention. Performance, blood, and ultrasound markers were analyzed using a repeated measures ANOVA to observe between group comparisons for all of the outcome variables. **RESULTS:** There were no group differences observed for isometric or isokinetic peak torque or average torque, nor were there differences in isokinetic peak power or average power between the two groups as a result of the intervention. There were, however, differences in the pattern for rate of torque development at 100 ms and 200 ms between the two groups. RTD 100 was decreased at IP and 48H in YA, with MA showing decreases at IP, but also 120P and 24H unlike YA. RTD200 was decreased at all time points in YA, while MA was decreased at IP, 24H, and 48H, but not 120P. For markers of muscle damage and inflammation, there were no differences in the response of Mb, CK, CRP, or IL-6 between groups. **CONCLUSIONS:** Age does not appear to be a driving factor in the inflammatory or muscle damage response from a high volume isokinetic intervention. Though changes in peak torque and average torque from a high volume isokinetic intervention do not seem to differ between younger and middle-aged adults, the rate of torque production at 100ms and 200ms is different between groups. This suggests that while recovery to average or maximal strength after an exercise bout may not be affected greatly by age, the rate of neuromuscular recovery from exercise may be primarily affected by other factors such as training status.
To my parents, Mary and Joseph Gordon. Without the commitment to excellence that you have represented throughout my life, I know with confidence that I would not be the man I am today. Your example has shown me to take pride in my work, hold high expectations, and always strive to reach my highest potential.
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LIST OF ACRONYMS/ABBREVIATIONS

AVGT- Average torque

CK- Creatine kinase

CRP- C-reactive protein

CSA- Cross-sectional area

EI- Echo intensity

HPL- Human performance laboratory

HVP- High-Volume Isokinetic Protocol

IL-1RA- Interleukin-1 receptor antagonist

IL-6- Interleukin 6

IL-10- Interleukin 10

MA- Middle age

Mb- Myoglobin

MT- Muscle thickness

MVIC- Maximal voluntary isometric contraction

PA- Pennation angle

PKT- Peak torque
RF- Rectus femoris

RTD100- Rate of torque development at 100 ms

RTD200- Rate of torque development at 200 ms

TNF-α- Tumor necrosis factor alpha

TWD- Total work done

US- Ultrasound assessment

VAS- Visual analog scale

VL- Vastus lateralis

Y- Years

YA- Younger age
CHAPTER I: INTRODUCTION

Sarcopenia, or the age-related decrease in muscle mass and function, as well as issues with neuromuscular activation, are significant factors in the decline of quality of life in middle-aged and older adults (Janssen et al., 2000; Macaluso et al., 2004). Incorporation of regular resistance exercise can attenuate age-associated losses in muscle strength and bone mineral density, as well as enhance immune function, functional capacity, and even increase cognitive function (Chang et al., 2012; Johnston et al., 2008). While the topics of cognitive function and bone health are beyond the scope of this work, it is important to note the many beneficial effects seen from regular resistance exercise throughout life. With roughly only one quarter (~27%) of U.S. adults estimated in engaging in recreational resistance exercise, and even less (~10%) for adults over the age of 50, it can be posited that an increase in this type of training can mitigate or even eliminate the effects of many debilitating and chronic conditions (Peterson et al., 2011).

The positive effects of resistance exercise are well known and well catalogued, with there being many benefits, including increased muscle mass and strength, increased bone mineral density, and even increased insulin sensitivity (Castaneda et al., 2002; Conroy et al., 1993; Ishii et al., 1998; Skerry et al., 1997; Staron et al., 1994). Though increasing age is a risk factor for many conditions including osteoporosis, cardiovascular disease, cancer, etc., an individual’s chronological age and their biological age can differ greatly. Previous research has reported that age-related decreases in muscle mass, maximal strength and diminished exercise capacity may be associated with an increase in recovery time from physical activity in older adults (Frontera et al., 2000; Hunter et al., 2004; Toft et al., 2002). As a result, aged-associated adjustments may be necessary for the exercise prescription of older adults (>60 y) to allow for appropriate recovery.
and maximize physiological adaptation from the training program (Candow et al., 2011). Research has shown enhanced recovery from muscle damage can augment both muscular and immune function (Frontera et al., 1988; Hunter et al., 2004). In younger adults, performance following muscle damaging exercise may significantly decrease in comparison to both middle-aged and older adults due to higher levels of muscle strength, which may contribute to enhanced recovery. These greater strength levels in younger adults does not provide much insight into potential mechanisms for greater recovery patterns in these age groups. In regards to the immune response between younger and older adults (e.g. > 65 y), a decrease in the magnitude of the immune response is seen with advanced age (Ceddia et al., 1999; Mazzeo et al., 1998). Only a limited number of studies have examined recovery from resistance exercise in older adults, but none that we are aware of in middle-aged (40 – 59 y) individuals. Previously, it has been demonstrated that the recovery of recreationally trained (3-6 hours of exercise per week) older adults (~ 69 y) is impaired compared to younger adults (Toft et al., 2002). However, the mechanism of how these differences occur was not elucidated. Therefore, the purpose of this study was to examine differences in the recovery and inflammatory response from an acute resistance exercise session between young (18 – 30 y) and middle-aged (40 – 60) men.

**Purposes**

1. To compare the effects of a high-volume isokinetic intervention on lower body strength and inflammatory markers between young and middle-aged men.

2. To compare the effect of a high-volume isokinetic intervention on markers of muscle damage in 48-hour post-recovery period between young and middle-aged men.
Hypotheses

It was hypothesized that:

1. An attenuation in the inflammatory and muscle damage response would be shown in young compared to middle-aged men following a high volume isokinetic training protocol.

2. An accelerated recovery in strength would be observed in young versus middle-aged men following the exercise protocol.
CHAPTER II: REVIEW OF LITERATURE

General Response of the Body from Damaging Exercise

The role of exercise in health is known to be a potent modulator of cardiovascular, respiratory, immune, and muscle function (Calle & Fernandez, 2010; Kelley & Kelley, 2000; McArdle et al., 2015; Peterson et al., 2011). The aging process leads to decreased levels of health and wellness, characterized by decreases in muscle mass, strength, fat free mass, and increases in time to recovery and chronic disease risk (Fulop et al., 2010; McArdle et al., 2015). Age-related decreases in muscle mass are referred to as sarcopenia, while age-related decreases in muscle strength are known as dynapenia (Clark & Manini, 2008). Though there are many potential mechanisms for the diminished recovery response occurring with age, it is likely due in part to the role of muscle in the recovery process (Fell et al., 2008). It is posited that both sarcopenia and dynapenia significantly affect the ability to recover from muscle damage, and while this has been demonstrated in animal studies, whether or not this relationship occurs in humans is still unclear (Brooks et al., 1994; Mcbride et al., 1994). Appropriately designed training programs can improve human performance, improve the quality of life, and attenuate physiological deficits associated with aging (Baechle et al., 2008; Cartee et al., 2016; Peterson 2010). The mechanisms associated with these physiological benefits include increasing bone mineral density, increasing lean body mass, and enhancing immune function, all of which can decrease the risk of disease and physiological dysfunction (DeSalles et al., 2010; Johnston et al., 2008; McArdle et al., 2015). The exercise stimulus may result in a degree of acute muscular damage that results in physiological adaptation (Ebbeling & Clarkson, 1989). The extent of muscle damage can be quantified by assessing muscle soreness, measuring muscle damage markers in the peripheral
circulation, and examining changes in muscle performance. Adaptation is often reported following an acute protocol as “recovery”, which can be quantified by the duration of time following exercise-induced muscle damage where performance returns to baseline levels. During this recovery period following exercise, an individual can experience decreased neuromuscular and immune function, so reducing time to full recovery can attenuate many of the potential adverse effects of exercise-induced muscle damage (Byrne & Easton, 2002; Gleeson 2007; Hakinnen et al., 1993). The importance of the differences within these responses will be further explicated in the scope of this section. Much of the research focusing on recovery from resistance exercise has primarily been directed at the endocrine response, or is quantified in conjunction with ingestion of a nutrient or supplement before or following exercise (Børsheim et al., 2002; Kraemer et al., 2005; Pasiakos et al., 2014; Rawson et al., 2007). This review of literature will examine the differences in recovery with respect to neuromuscular and immune changes between younger and middle-aged populations.

Muscle Damage Markers

After an acute bout of exercise, changes occur in the body’s physiological response, including increases in inflammatory markers, increases in blood pressure, increases in markers of muscle damage (i.e. creatine kinase), and increases in circulating myoglobin (Kraemer et al., 1999; Kraemer et al., 2005; Shaner et al., 2014). These are all dependent upon the amount of muscle mass utilized, as well as exercise mode, intensity, and volume. Creatine Kinase (CK) is an intracellular protein found in muscle cells that appears in the blood following disruption of these cells (Baird et al., 2012; Magal et al., 2010). CK is a generally well-accepted marker of muscular damage, with increased levels being reported following exercise (Brancaccio et al.,
Basal levels of CK vary between individuals, however the relative change in CK following an acute exercise bout can be used as a marker quantifying the magnitude of muscle damage resulting from an exercise session (Clarkson et al., 2006). Although the use of CK as a tool in assessing muscular damage is well acknowledged (Baird et al., 2011; Sayers & Clarkson, 2003), myoglobin (Mb) is also often used to assess muscle damage resulting from exercise (Brancaccio et al., 2010; Clarkson & Hubal, 2002; Magal et al., 2010). Myoglobin is an oxygen transporting protein in muscle that allows oxygen to be stored within the muscle, and following exercise may be released from damaged skeletal tissue into circulation (Clarkson et al., 2006; Sayers & Clarkson, 2003). Myoglobin is a much smaller protein than CK, and thus is leaked into the circulation much earlier than CK, providing a first indication of acute muscle damage (Sayers & Clarkson, 2003). Because CK is a larger protein requiring the assistance of the lymphatic system to enter circulation, its increase in circulation is delayed. For this reason, Mb is often used as a marker for the acute-phase muscle damage response, while CK provides a better measure of the response 24 – 48 hours following exercise (Sayers & Clarkson, 2003). Changes in CK concentrations typically parallel increases in Mb (Clarkson et al., 2006; Sayers & Clarkson, 2003).

Immune Response

Changes in the immune response to exercise are dependent upon the metabolic and mechanical stress of the workout, as well as the training experience of the individual (Gordon et al., 2012). Increases in inflammatory markers in the circulation following acute exercise initiates the immune response to the site of muscle damage (Calle & Fernandez, 2010; Gleeson 2007). Inflammatory markers not only represent the level of muscular damage following exercise, but
also have many different roles in the breakdown, repair, and overall recovery process. Interleukin 6 (IL-6) is an inflammatory cytokine that facilitates communication among cells for the mobilization, proliferation, and differentiation of immune cells to the site of tissue damage (Calle & Fernandez, 2010). IL-6 has a role as an adipokine (cytokine released from a fat cell) and as a myokine (cytokine released from a muscle cell) (Pedersen & Febbraio, 2005; Trayhurn et al., 2010). Its role as an adipokine has been shown to be pro-inflammatory, potentially contributing to insulin resistance and potentially impeding recovery by modulation of other inflammatory cytokines (Pedersen & Febbraio, 2005; Trayhurn et al., 2004, 2010). Conversely, its role as a myokine is considered to be anti-inflammatory, potentially aiding in the recovery process by inhibiting pro-inflammatory cytokines and stimulating other anti-inflammatory cytokines (Mathur et al., 2009; Pedersen & Febbraio, 2005). There is a marked increase in IL-6 following an exercise bout due to the contraction of muscle, and its appearance in the circulation precedes the appearance of other cytokines in the blood (Febbraio & Pedersen, 2002; Mathur et al., 2009). Because of this, IL-6 is a common biomarker used to show the degree of inflammation caused by exercise (Febbraio & Pedersen, 2002).

C-Reactive Protein (CRP) is a protein present in the blood that is associated with the acute-phase response of inflammation (Du Clos & Mold, 2004). Like IL-6, it is also a marker of systemic inflammation, and many other cytokines (including IL-6) are thought to play an integral role in inducing CRP production (Gleeson 2007; Stewart et al., 2007). CRP levels reflect the circulating levels of IL-6, however CRP increases and decreases more rapidly and dramatically compared to other inflammatory markers (Du Clos & Mold, 2004). Understanding this, circulating concentrations of IL-6 and CRP provide an indication of the immune and
inflammatory processes resulting from exercise (Gleeson, 2007; Stewart et al., 2007). Increases in both of these biomarkers have been reported following acute exercise, and a faster return to baseline concentrations may be indicative of an enhanced rate of recovery from damaging exercise (Clarkson & Hubal, 2002; Gordon et al., 2012). Additionally, elevated basal concentrations of both CRP and IL-6 have been shown to increase with advancing age, and high concentrations have also been reported in individuals with lower levels of fitness (decreasing rate of recovery and increasing disease risk) (Aronson et al., 2004; Bruunsgaard 2002; Kasapis et al., 2005; Rohde et al., 1999; Toft et al., 2002).

Acute resistance exercise can cause short-term decreases in strength in both untrained and trained populations, regardless of age (Byrne & Easton, 2002; Clarkson & Hubal, 2002). Though the time to recovery (i.e. return to baseline strength) is dependent upon many factors (i.e. training status, age, activity level, etc.), greater degrees of exercise-induced muscle damage incurred during an acute bout of resistance exercise may lead to significant reductions in strength and power (Clarkson & Hubal, 2002). As the extent of muscle damage becomes magnified, recovery processes may be delayed (Byrne et al., 2004).

**Effect of Age on the Recovery Response**

Previous investigations have illustrated many of the strength and inflammatory changes that occur following exercise in younger and older populations, with younger populations showing higher maximal strength and circulating markers of muscle damage during the recovery period (Kraemer et al., 1999; Smilios et al., 2007; Walker et al., 2014; 2015). Understanding the recovery response between different age groups may provide a more effective, or appropriate, exercise prescription. Increasing age is associated with increased disease risk and functional
limitations, and decreased muscle mass and strength; these changes together or in part, can impair the recovery process following exercise (Candow et al., 2005; Miszko et al., 2003; Peterson et al., 2011). Increased muscle mass and strength changes observed with regular resistance exercise can enhance recovery from exercise-induced mechanical damage (Brandt & Pedersen, 2010; Johnston et al., 2007; Kelley & Kelley, 2000; Powers et al., 2014).

Importance of Preserving Muscle Mass

Sarcopenia and dynapenia not only decrease quality of life, but also increase health care costs, risk of falls, and many other negative consequences (Hunter et al., 2004). Strength decreases that are associated with age are primarily due to decreases in muscle mass (Frontera et al., 2000; Volpi et al., 2004). Maximal muscle strength is achieved before the third decade of life, with rapid declines in muscle strength and function after the fifth decade of life (Hakkinen et al., 1998; Hunter et al., 2004). Decreases in neuromuscular function seen after this third decade of life are associated with increases in joint stiffness, as well as a reduction in bone mineral density (Volpi et al., 2004). Whether this is a result of a detraining effect or early signs of functional performance loss related to aging is not clear. In any event, decreases in physical function observed with advancing age can be attenuated or reversed by maintaining or increasing muscle size and strength (Frontera et al., 1988; Volpi et al., 2004). Because lower body musculature appears to have greater strength decreases than upper body musculature (Candow et al., 2011), maintenance of these muscle groups in particular (i.e. legs) becomes essential in preserving muscle mass, muscle strength, and physical function throughout the course of life (Candow et al., 2011; Janssen et al., 2000). Twelve weeks of resistance exercise has been shown to eliminate age-related deficits in elbow flexor, and lower limb strength (Candow et al., 2011).
These findings are significant, because they demonstrate that muscle quality, or the capacity of skeletal tissue to perform is maintained or even increased, with regular low to moderate intensity resistance exercise, in older adults (Peterson et al., 2011; Silva et al., 2014). The interaction between these age-related decreases, and the attenuation of said decreases through training may be the key to understanding the potential changes in recovery from muscle damage throughout life.

**Role of Exercise in Enhancing Muscular and Immune Function**

Acute resistance exercise has been shown to substantially increase IL-6 concentrations post-exercise, while changes in CRP levels appear to be dependent on different factors (i.e. intensity, duration, and mode of exercise); though reductions of these inflammatory markers have been reported following chronic training (Kasapis et al., 2005; Mathur et al., 2009; Petersen et al., 2005). Changes in the concentrations of these immune factors may reflect overall training stress and may potentially serve as immune markers for training or metabolic disorders (Brandt & Pedersen, 2010; Gleeson 2007). The risk of overtraining increases with advancing age, with increased resting concentrations of these immune markers, and an impaired regeneration response to muscle damage reported in comparison to younger individuals (Mathur et al., 2009; Stewart et al., 2007; Toft et al., 2002). Though circulating CRP concentrations can be elevated up to 48-hours post-exercise, regular exercise training can attenuate resting CRP concentrations and the CRP response post-exercise (Kasapis et al., 2005). In addition, resistance training has previously been shown to attenuate resting CRP concentration to a greater degree than aerobic exercise training in sedentary individuals (Donges et al., 2010). Aside from stimulating the release of CRP, the role of IL-6 is multifactorial, while IL-6 is generally thought of as a pro-
inflammatory cytokine, it has also been reported to act as an anti-inflammatory cytokine when it is secreted from muscle tissue (Donges et al., 2010; Mathur et al., 2009; Pedersen & Febbraio, 2005). Therefore, increases in IL-6 concentrations and the subsequent increase in other anti-inflammatory cytokines such as Interleukin-10 (IL-10) and Interleukin-1 receptor antagonist (IL-1RA) following exercise suggests that IL-6 may have a direct role in the recovery process when secretion is induced by resistance exercise (Brandt & Pedersen, 2010; Mathur et al., 2009; Petersen et al., 2005). Additionally, lower basal levels of IL-6 and CRP can be achieved through resistance exercise in both younger and middle-aged populations, increasing their health and potentially enhancing immune function and recovery (Aronson et al., 2004; Petersen et al., 2005; Stewart et al., 2007).
CHAPTER III: METHODOLOGY

Participants

Nineteen recreationally active males were recruited to participate in this parallel designed study. Study participants were recruited into two groups based on age. The younger-age group (YA) consisted of men between the ages of 18 – 30 while the middle-age group (MA) consisted of men between the ages of 40 – 59 years. Inclusion criteria required participants to meet the age requirements of one of the groups and be recreationally active including resistance training for the previous 6 months prior to enrollment as defined by the American College of Sports Medicine (150 minutes of exercise/week). All participants were free of any physical limitations that may have affected performance. Additionally, all participants were free of any medications, performance-enhancing drugs, nor were they using any dietary supplements as determined by the health and activity questionnaire. Following an explanation of all procedures, risks and benefits, each participant provided his informed consent prior to participation in this study. The research protocol was approved by the Institutional Review Board at UCF prior to participant enrollment.

Study Design

Both groups reported to the Human Performance Laboratory (HPL) on four separate occasions (Figure 1). On the first visit (D1), participants reported to the HPL following a 2-hour fast. Anthropometric assessments were performed and included height, weight, and body composition. Following anthropometric assessment, participants performed a standardized warm-up. The standardized warm-up consisted of 5 minutes of pedaling on a cycle ergometer at 50 watts. Following the warm-up, participants completed a familiarization protocol on the isokinetic device. On the second visit (D2), participants arrived 10 hours post-prandial and
recorded their subjective levels of pain and soreness on a visual analog scale (VAS). Following the VAS, participants then provided a baseline blood sample followed by an ultrasound assessment (US) of their lower body musculature. After the blood sample and US were obtained, the participants performed the first lower body performance assessment (BL). After a brief five-minute rest period, participants then completed the high-volume isokinetic protocol (HVP). Immediately after the HVP (IP), participants again completed the lower body performance assessment to determine performance decrements resulting from the HVP. Following the IP measure, VAS and US assessments were performed. Blood samples were collected at 30- (30P), 60- (60P), and 120-minutes (120P) following the HVP. Participants reported to the HPL 24- (D3) and 48-hours (D4) following D2 for blood, ultrasound, and VAS measures. All blood draws during D3 and D4 were obtained following a 10-hour fast. The order of assessment, VAS, blood draw and US measures were consistent for all testing sessions. Participants also performed lower body performance assessments at D3 and D4.
On D1, participants were assessed for anthropometric measurement and completed a familiarization protocol prior to D2. On D2, participants provided blood samples at BL, IP, 30P, 60P, and 120P, ultrasound assessment at BL, IP, and 120P, as well as performance assessment at BL, IP, and 120P. The HVP was also performed on this day following the performance assessment at BL. On D3 and D4, a blood sample, ultrasound assessment, and performance assessment were collected at 24H and 48H, respectively.

**Dietary Recall**

All participants provided a 3-day dietary recall beginning the day before D2 testing until the morning of D4 testing. Participants were asked to maintain their regular diet for the duration of the investigation. FoodWorks nutrient analysis software (McGraw-Hill, New York, NY, USA) was used to analyze the self-reported dietary recalls for total kilocalorie intake and macronutrient distributions (carbohydrate, protein, and fat).
**Anthropometric Measurements**

Body mass (±0.1 kg), and height (±0.1 cm) was measured using a Health-o-meter Professional scale (Patient Weighing Scale, Model 500 KL, Pelstar, Alsip, IL, USA). Body composition was assessed using a direct segmental multi-frequency bioelectrical impedance analyzer (BIA) (InBody 770, Cerritos, CA, USA) according to the manufacturer’s guidelines. BIA estimates body composition using the conductivity differences of the various tissues due to its tissue characteristics (water and electrolyte content). This analyzer processes 30 impedance measurements by using six different frequencies (1, 5, 50, 250, 500, 1000 kHz) at each of five segments of the body (right arm, left arm, trunk, right leg, left leg) using tetrapolar 8-point tactile electrodes (Kurinami et al., 2016). Values for total and segmental body fat percentage were then recorded.

**Visual Analog Scales**

Participants were instructed to assess their subjective feelings of pain and soreness using a 100-mm visual analog scale (VAS) (Lee et al. 1991). Participants were asked to rate pain and soreness intensity by placing a mark on a horizontal 100-mm VAS (Bijur et al. 2001; Nosaka et al. 2002). No pain or soreness was recorded as 0 and the worst possible soreness or pain as 100. Evaluations were performed upon arrival to the HPL on D2, D3, D4, and following the HVP on D2.

**Ultrasound Assessment**

Non-invasive skeletal muscle ultrasound (US) images were collected from the dominant thigh, of all participants during all assessment time points. Participants were asked to lay supine on an examination table with both legs fully extended for a minimum of 5 minutes in order to
allow fluid shifts to occur (Arroyo et al., 2016). Prior to image collection, all anatomical
locations of interest were identified using standardized landmarks for the rectus femoris (RF),
and vastus lateralis (VL) muscles. The landmarks for the RF and VL were determined along the
longitudinal distance over the femur at 50% of the length of each muscle, respectively. The
length of the RF was defined as the length between the anterior, inferior suprailiac crest and the
proximal border of the patella, while the length of the VL encompassed the distance from the
lateral condyle of the tibia to the most prominent point of the greater trochanter of the femur. The
VL measurement required the participant to lay on their side. Pennation angle (PA), muscle
thickness (MT), cross-sectional area (CSA), and echo-intensity (EI) were all measurements
obtained from the US. All measures were obtained by passing a 12MHz probe (General Electric
LOGIQ P5, Wauwatosa, WI, USA) coated with water-soluble transmission gel (Aquasonic®
100, Parker Laboratories, Inc., Fairfield, NJ) over the surface of the thigh at the predetermined
anatomical locations outlined above. Measures of CSA, PA, and MT were captured using B-
mode ultrasonography with gain set at 50 and dynamic range set to 72 to optimize spatial
resolution. Image depth was fixed at 5 cm. Further analysis of all ultrasound images was
performed via ImageJ (National Institutes of Health, USA, version 1.45s) to quantify CSA, PA,
MT, and EI. Fascicle length (FL) was estimated using the following equation:

$$FL = \frac{MT}{\sin(\text{PA})} \quad (\text{Kawakami, 1995})$$

Echo-intensity (EI) was quantified through grayscale analysis using the standard histogram
function in ImageJ. The same investigator performed all ultrasound measurements. Intraclass
correlation coefficients and minimal differences (MD) for the VL were as follows: cross-
sectional area ($R = 0.99; \text{MD} = 0.85 \text{ cm}^2$), muscle thickness ($R = 0.97; \text{MD} = 0.09 \text{ cm}$), fascicle length ($R = 0.96; \text{MD} = 0.45 \text{ cm}$), pennation angle ($R = 0.98; \text{MD} = 0.68^\circ$), and echo-intensity ($R = 0.99; \text{MD} = 1.46 \text{ au}$). Intraclass correlation coefficients and minimal differences (MD) for the RF were as follows: cross-sectional area ($R = 0.96; \text{MD} = 0.78 \text{ cm}^2$), muscle thickness ($R = 0.94; \text{MD} = 0.21 \text{ cm}$), fascicle length ($R = 0.95; \text{MD} = 0.38 \text{ cm}$), pennation angle ($R = 0.91; \text{MD} = 1.32^\circ$), and echo-intensity ($R = 0.94; \text{MD} = 3.88 \text{ au}$).

**Isokinetic Assessment Protocol**

On D2 following the warm-up, participants were seated in the isokinetic dynamometer (S4, Biodex Medical System, Inc., New York, NY, USA), positioned with a hip angle of 110° and strapped into the chair at the waist, shoulders, and across the thigh. Chair and dynamometer settings were adjusted for each participant to correctly align the axis of rotation with the lateral condyle of the femur. All participants were tested on their right leg, which was secured to the dynamometer arm just above the medial and lateral malleoli. Isokinetic dynamometer settings for each individual were recorded and remained consistent throughout the study. The isokinetic assessment protocol was performed ~10 minutes following the initial US of the participant. Following the initial isokinetic assessment protocol, the participant remained seated, and a HVP was performed. For the HVP, the lever arm of the dynamometer was programmed to extend the participant’s leg to 155 degrees of knee flexion (where 180 degrees is full extension), and flex the participant’s leg to 95 degrees of flexion. This protocol consisted of 8 sets of 10 repetitions with concentric knee extension, and eccentric knee flexion at 60 degrees per second. Participants were instructed to give maximal effort throughout the HVP; verbal encouragement was provided. For all participants, the right leg was used for the HVP. Following the HVP, another isokinetic
assessment protocol was performed (IP) in order to quantify the performance decrements of the participant. Isokinetic assessment protocols were also performed 24 hours (24H), and 48 hours (48H) after the HVP. The lever arm of the dynamometer was programmed to extend the participant’s leg to 155 degrees of knee flexion (where 180 degrees is full extension), and flex the participant’s leg to 95 degrees of flexion. The isokinetic assessment protocol at each time point consisted of: (i) two maximal voluntary isometric contractions (MVIC’s) at a 70 degree angle, (ii) one set of 3 repetitions of concentric knee extension at 240 degrees per second and concentric knee flexion at 60 degrees per second, (iii) one set of 3 repetitions of concentric knee extension at 180 degrees per second and concentric knee flexion at 60 degrees per second, (iv) one set of 3 repetitions of concentric knee extension at 60 degrees per second and concentric knee flexion at 60 degrees per second, and (v) one set of 10 repetitions of concentric knee extension at 60 degrees per second and eccentric knee flexion at 60 degrees per second. A familiarization of this protocol was performed following anthropometric measurement on D1 in order to acquaint the participant with the isokinetic dynamometer. Work performed in each set was calculated as the product of the mean power of each kick over the time to complete the kick. Total work done (TWD) was calculated as the sum of the work performed in each of the 8 sets of ten repetitions during the HVP.

**Blood Measurements**

Blood samples were obtained at seven time points throughout the study (BL, IP, 30P, 60P, 120P, 24H, and 48H). The BL, IP, 30P, 60P and 120P blood samples were obtained using a Teflon cannula placed in a superficial forearm vein using a three-way stopcock with a male luer lock adapter and a plastic syringe. The cannula was maintained patent using a non-heparinized
isotonic saline solution (Becton Dickinson, Franklin Lakes, NJ, USA). All blood samples were obtained following a 15-minute equilibration period. The remaining time points (24H and 48H) were obtained by a single-use disposable needle with the subject in a supine position also for at least 15 minutes prior to sampling.

Whole blood was collected at each time-point in two 10 mL Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ) for a total of 20 mL. One containing K₂EDTA, which is an anticoagulant to prevent the blood from clotting, and a serum tube containing no anti-clotting agents. An aliquot of whole blood from the EDTA tube was immediately utilized to assess hematocrit, hemoglobin, and red blood cell count using a complete blood cell counter (Coulter® AC-T diff 2™ hematology analyzer). Blood in the serum tube was allowed to clot at room temperature and subsequently centrifuged at 4,000g for 15 minutes. The resulting serum was placed into separate 1.8-mL microcentrifuge tubes and frozen at -80° C for later analysis.

**Biochemical Analyses**

Serum Concentrations of Creatine Kinase (CK) were analyzed with the use of a commercially available kinetic assay kit (Sekisui Diagnostics, Charlottetown, PE, Canada), per manufacturer’s instructions. CRP, IL-6, and myoglobin concentrations were obtained via enzyme-linked immunosorbent assays (ELISA) (Calbiotech, Spring Valley, CA, USA). To eliminate inter-assay variability, all samples for a particular assay were thawed once, and analyzed by the same technician using a BioTek Eon spectrophotometer (BioTek, Winooski, VT). All samples were analyzed in duplicate with a mean coefficient of variation of 4.50% for CK, 7.38% for CRP, 3.55% for IL-6, and 5.03 % for myoglobin. All biochemical assays were run per the manufacturer’s instructions.
Statistical Procedures

Changes in subjective levels of pain and soreness, markers of inflammation and muscle damage, as well as performance measures, were analyzed via repeated measures analysis of variance (ANOVA). In addition, changes (Δ) in isometric and isokinetic performance from baseline were analyzed via repeated measures ANOVA. In the event of a significant F value, LSD post-hoc tests were used for pairwise comparison. Baseline performance comparisons of both groups were determined by independent t-tests. Outliers were identified when values exceeded 1.5 times the interquartile range (Barbato et al. 2011). For all analyses, a criterion alpha level of α ≤ 0.05 was used to determine statistical significance, and statistical software (SPSS V.21.0, Chicago, IL, USA) was used. All data are reported as mean ± standard deviation.
CHAPTER IV: RESULTS

Nineteen men volunteered to participate in this investigation. Nine men were in the younger group (YA), while the other ten were in the middle-aged group (MA). Groups were significantly different in age ($F = 7.969; p < 0.001$); however, there were no significant differences observed in height ($F = 3.112; p = 0.390$), body mass ($F = 1.111; p = 0.559$), or body fat percentage ($F = 0.255; p = 0.242$). Descriptive data for each group are depicted in Table 1. There were also no differences in TWD ($F = 0.882; p = 0.832$) during the isokinetic exercise protocol between the two groups.

Table 1. Participant Anthropometrics

<table>
<thead>
<tr>
<th></th>
<th>Younger (n = 9)</th>
<th>Middle-Aged (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>21.8 ± 2.2</td>
<td>47.0 ± 4.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179.5 ± 4.9</td>
<td>176.8 ± 7.6</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>91.2 ± 12.2</td>
<td>96.0 ± 21.5</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>21.8 ± 4.3</td>
<td>24.8 ± 6.3</td>
</tr>
</tbody>
</table>

No differences in average daily caloric ($F = 0.137; p = 0.685$), carbohydrate ($F = 0.009; p = 0.890$), protein ($F = 2.481; p = 0.337$), or fat intakes ($F = 0.154; p = 0.857$) were observed between YA and MA during the three-day study period. Details of nutrient intake for both groups can be observed in Table 2.
Table 2. Average Daily Nutrient Intake

<table>
<thead>
<tr>
<th></th>
<th>Younger</th>
<th>Middle-Aged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>2076.2 ± 545.2</td>
<td>1975.3 ± 520.4</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>220.3 ± 70.2</td>
<td>224.8 ± 69.1</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>110.2 ± 43.8</td>
<td>94.8 ± 21.6</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>76.0 ± 27.4</td>
<td>78.6 ± 32.9</td>
</tr>
</tbody>
</table>

Performance Measures

Isometric Assessment

Isometric performance measurements can be observed in Table 3, and changes in PKT are depicted in Figure 2. No significant group x time interactions were observed for PKT (F = 1.928; p = 0.116) or AVGT (F = 1.712; p = 0.158). Significant main effects for time were observed for both PKT (F = 17.574; p < 0.001) and AVGT (F = 15.345; p < 0.001). PKT and AVGT at IP, 120P, 24H, and 48H were all significantly lower (p < 0.001) than BL. Baseline differences were observed for AVGT (p = 0.043), and a trend was observed in PKT (F = 0.692; p = 0.057). However, there were no group x time interactions in Δ change observed for PKT (F = 2.033; p = 0.146), or AVGT (F = 1.096; p = 0.359).
Table 3. Isometric Performance Measures.

<table>
<thead>
<tr>
<th></th>
<th>Time Points</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>IP</td>
<td>120P</td>
<td>24H</td>
<td>48H</td>
</tr>
<tr>
<td>Peak Torque (Nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YA</td>
<td>296 ± 44</td>
<td>193 ± 41</td>
<td>240 ± 50</td>
<td>260 ± 48</td>
<td>260 ± 49</td>
</tr>
<tr>
<td>MA</td>
<td>248 ± 57</td>
<td>190 ± 48</td>
<td>214 ± 39</td>
<td>218 ± 43</td>
<td>214 ± 52</td>
</tr>
<tr>
<td>Average Torque (Nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YA</td>
<td>229 ± 36</td>
<td>147 ± 32</td>
<td>180 ± 35</td>
<td>188 ± 37</td>
<td>190 ± 34</td>
</tr>
<tr>
<td>MA</td>
<td>188 ± 46</td>
<td>146 ± 35</td>
<td>160 ± 30</td>
<td>169 ± 36</td>
<td>161 ± 43</td>
</tr>
<tr>
<td>Rate of Torque Development at 100 ms (Nm/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YA</td>
<td>1320 ± 370</td>
<td>599 ± 225</td>
<td>1117 ± 446</td>
<td>1063 ± 459</td>
<td>790 ± 324</td>
</tr>
<tr>
<td>MA</td>
<td>1170 ± 421</td>
<td>770 ± 325</td>
<td>953 ± 336</td>
<td>908 ± 374</td>
<td>964 ± 413</td>
</tr>
<tr>
<td>Rate of Torque Development at 200 ms (Nm/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YA</td>
<td>980 ± 214</td>
<td>465 ± 111</td>
<td>777 ± 185</td>
<td>668 ± 207</td>
<td>579 ± 93</td>
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<tr>
<td>MA</td>
<td>726 ± 260</td>
<td>573 ± 197</td>
<td>622 ± 144</td>
<td>532 ± 165</td>
<td>549 ± 266</td>
</tr>
</tbody>
</table>
Figure 2. Comparisons of PKT response from Isometric Performance Assessment.

All data are reported as mean ± SD.
Figure 3. Comparisons of AVGT response from Isometric Performance Assessment.

All data are reported as mean ± SD.

A significant interaction was observed for rate of torque development at both 100 ms (RTD100) (F = 3.408; p = 0.013), and 200 ms (RTD200) (F = 5.772; p < .001). RTD100 values at IP and 48H were significantly reduced (p < 0.001) compared to BL in YA, while significantly lower from BL at IP (p = 0.001), 120P (p = 0.023), and 24H (p = 0.011) in MA. For RTD200, values at IP (p < 0.001), 120P (p = 0.001), 24H (p = 0.004), and 48H (p = 0.001) were significantly lower than BL in the YA group, while RTD200 was significantly lower from BL at IP (p = 0.015), 24H (p = 0.002), and 48H (p = 0.021) in the MA group. No between group differences were observed at any time point for RTD100. However, RTD200 was significantly greater (p = 0.033) for YA than MA at BL. A group x time interaction in ∆ change was observed for both RTD100 (F = 4.249; p = 0.009) and RTD200 (F = 4.859; p = 0.005). The ∆ change from BL was significantly
greater for YA than MA in RTD100 at IP ($p = 0.033$) and 48H ($p = 0.044$), while the $\Delta$ from BL at RTD200 was significantly greater for YA than MA change at IP ($p = 0.001$) and 48H ($p = 0.037$) as well. Changes in RTD100 are depicted in Figure 4, while changes in RTD200 are depicted in Figure 5.

Figure 4. Differences in RTD100 between YA group and MA group. All data are reported as mean ± SD
Figure 5. Differences in RTD200 between YA group and MA group

* = Asterisk represents a significant difference in RTD200 between groups. All data are reported as mean ± SD.

Isokinetic Assessment

No significant group x time interactions were observed for PKT at either $240^\circ \cdot \text{sec}^{-1}$ ($F = 1.756; p = 0.174$) or $60^\circ \cdot \text{sec}^{-1}$ ($F = 1.928; p = 0.137$). Significant main effects for time were observed for PKT at both $240^\circ \cdot \text{sec}^{-1}$ ($F = 10.444; p < 0.001$) and $60^\circ \cdot \text{sec}^{-1}$ ($F = 15.629; p < 0.001$). PKT was significantly lower at IP ($p < 0.001$), 120P ($p = 0.007$), and 24H ($p = 0.032$) compared to BL at $240^\circ \cdot \text{sec}^{-1}$, and significantly lower than BL at IP ($p < 0.001$), 120P ($p < 0.001$), 24H ($p < 0.001$), and 48H ($p = 0.002$) at $60^\circ \cdot \text{sec}^{-1}$. Though no baseline differences were
found for PKT at either 240°·sec⁻¹ (F = 0.081; p = 0.061) or 60°·sec⁻¹ (F = 1.562; p = 0.083), trends were observed for both measures.

No significant group x time interactions were observed for AVGT at 240°·sec⁻¹ (F = 1.837; p = 0.160) or 60°·sec⁻¹ (F = 2.371; p = 0.086). Significant main effects for time were noted in AVGT at both 240°·sec⁻¹ (F = 12.494; p < 0.001) and 60°·sec⁻¹ (F = 16.425; p < 0.001). AVGT values at IP (p < 0.001), 120P (p = 0.002), 24H (p = 0.013), and 48H (p = 0.036) were all significantly lower than BL at 240°·sec⁻¹, and significantly lower at IP (p < 0.001), 120P (p < 0.001), 24H (p < 0.001), and 48H (p = 0.001) compared to BL at 60°·sec⁻¹. There were no baseline differences found for AVGT at either 240°·sec⁻¹ (F = 0.008; p = 0.940; p = 0.148), though a trend was observed for AVGT at 240°·sec⁻¹. There were no group x time interactions of Δ change observed for PKT at 240°·sec⁻¹ (F = 2.937; p = 0.070), PKT at 60°·sec⁻¹ (F = 2.410; p = 0.101), AVGT at 240°·sec⁻¹ (F = 2.665; p = 0.092), or AVGT at 60°·sec⁻¹ (F = 2.796; p = 0.069). All isokinetic performance measurements are depicted in Table 4.
### Table 4. Isokinetic Performance Measures.

<table>
<thead>
<tr>
<th>Time Points</th>
<th>BL</th>
<th>IP</th>
<th>120P</th>
<th>24H</th>
<th>48H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Torque at 240°·sec⁻¹ (Nm/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YA</td>
<td>151 ± 23</td>
<td>112 ± 36</td>
<td>134 ± 33</td>
<td>136 ± 39</td>
<td>145 ± 39</td>
</tr>
<tr>
<td>MA</td>
<td>129 ± 24</td>
<td>103 ± 20</td>
<td>117 ± 23</td>
<td>118 ± 18</td>
<td>110 ± 23</td>
</tr>
<tr>
<td>Average Torque at 240°·sec⁻¹ (Nm/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YA</td>
<td>136 ± 26</td>
<td>92 ± 32</td>
<td>115 ± 30</td>
<td>119 ± 35</td>
<td>125 ± 36</td>
</tr>
<tr>
<td>MA</td>
<td>115 ± 26</td>
<td>90 ± 20</td>
<td>101 ± 25</td>
<td>104 ± 20</td>
<td>98 ± 24</td>
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<tr>
<td>Peak Torque at 60°·sec⁻¹ (Nm/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YA</td>
<td>234 ± 28</td>
<td>157 ± 46</td>
<td>196 ± 40</td>
<td>197 ± 36</td>
<td>207 ± 33</td>
</tr>
<tr>
<td>MA</td>
<td>205 ± 39</td>
<td>161 ± 25</td>
<td>172 ± 29</td>
<td>169 ± 32</td>
<td>171 ± 52</td>
</tr>
<tr>
<td>Average Torque at 60°·sec⁻¹ (Nm/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>YA</td>
<td>187 ± 30</td>
<td>113 ± 41</td>
<td>154 ± 32</td>
<td>159 ± 32</td>
<td>166 ± 24</td>
</tr>
<tr>
<td>MA</td>
<td>164 ± 36</td>
<td>121 ± 27</td>
<td>138 ± 33</td>
<td>130 ± 32</td>
<td>133 ± 47</td>
</tr>
</tbody>
</table>

* = significantly different than BL.

### Blood Analyses

No significant group x time interactions were observed for myoglobin (Mb) \((F = 0.307; p = 0.640)\), creatine kinase (CK) \((F = 0.607; p = 0.551)\), C-reactive protein (CRP) \((F = 0.320; p = 0.602)\), or IL-6 \((F = 0.466; p = 0.589)\). However, significant main effects for time were observed for Mb \((F = 8.708; p = 0.005)\) and CK \((F = 8.127; p = 0.001)\). Mb was significantly higher at 30P
(p = 0.002), 60P (p = 0.001), and 120P (p = 0.007) compared to BL, while CK concentrations at 24H (p = 0.002) and 48H (p = 0.006) were significantly higher than BL. Although no significant main effect for time was observed for CRP (F = 3.042; p = 0.097), a trend was noted for IL-6 (F = 3.689; p = 0.052). Changes in Mb concentrations are pictured in Figure 4, while changes in CK concentrations, CRP concentrations, and IL-6 concentrations are pictured in Figures 5, 6, and 7, respectively.

![Figure 6. Changes in Mb concentration.](image)

* = significant difference compared to BL for both YA and MA combined. All data are reported mean ± SD.
Figure 7. Changes in CK concentration.
* = significant difference compared to BL for both YA and MA combined. All data are reported mean ± SD.
Figure 8. Changes in CRP concentration.

All data are reported mean ± SD.
Visual Analog Scales

No significant group x time interactions were observed for subjective measures of pain ($F = 0.102; p = 0.959$) or soreness ($F = 0.886; p = 0.455$). No significant main effect for time was observed for subjective levels of pain ($F = 1.085; p = 0.351$), however a significant main effect for time was observed for subjective levels of muscle soreness ($F = 7.319; p < 0.001$). Muscle soreness was significantly higher at 30P ($p = 0.001$), 24H ($p = .001$) and 48H ($p = .002$) compared to BL. VAS values for each group are depicted in Table 5.
Table 5. Average Levels of Subjective Pain and Soreness

<table>
<thead>
<tr>
<th></th>
<th>Time Points</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>30P</td>
<td>24H</td>
<td>48H</td>
</tr>
<tr>
<td>VAS for Pain (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YA</td>
<td>0.06 ± 0.19</td>
<td>0.072 ± 1.53</td>
<td>0.070 ± 1.16</td>
<td>0.076 ± 1.97</td>
</tr>
<tr>
<td>MA</td>
<td>0.17 ± 0.52</td>
<td>0.54 ± 1.54</td>
<td>0.50 ± .68</td>
<td>0.64 ± 1.05</td>
</tr>
<tr>
<td>VAS for Soreness (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YA</td>
<td>0.25 ± 0.53</td>
<td>3.31 ± 2.10 *</td>
<td>2.78 ± 2.55 *</td>
<td>2.67 ± 2.75 *</td>
</tr>
<tr>
<td>MA</td>
<td>0.08 ± 0.25</td>
<td>1.76 ± 2.64 *</td>
<td>1.97 ± 1.83 *</td>
<td>3.23 ± 3.23 *</td>
</tr>
</tbody>
</table>

* = significant difference compared to BL for both YA and MA combined. All data are reported mean ± SD.

Ultrasound Assessment

No significant group x time interactions were observed for muscle CSA (F= 0.246; p 0.747), or MT (F= 0.687; p = 0.530). However, a main effect for time was observed for both CSA (F= 13.460; p < 0.001) and MT (F= 3.685; p 0.028). Muscle CSA and MT were significantly greater at IP (p < 0.001) compared to BL. CSA and MT are depicted for each group in Table 6.
Table 6. Muscle Morphology Characteristics

<table>
<thead>
<tr>
<th>Time Points</th>
<th>BL</th>
<th>IP</th>
<th>120P</th>
<th>24H</th>
<th>48H</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSA (cm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YA</td>
<td>34.2 ± 6.0</td>
<td>37.5 ± 5.2</td>
<td>34.1 ± 5.6</td>
<td>34.8 ± 6.2</td>
<td>34.4 ± 5.9</td>
</tr>
<tr>
<td>MA</td>
<td>32.1 ± 6.5</td>
<td>34.7 ± 7.4</td>
<td>31.8 ± 5.6</td>
<td>31.9 ± 5.7</td>
<td>31.5 ± 5.5</td>
</tr>
<tr>
<td>MT (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YA</td>
<td>1.8 ± 0.4</td>
<td>2.0 ± 0.4</td>
<td>1.8 ± 0.3</td>
<td>1.9 ± 0.5</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>MA</td>
<td>1.5 ± 0.3</td>
<td>1.8 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.6 ± 0.4</td>
<td>1.6 ± 0.4</td>
</tr>
</tbody>
</table>

*=significant different from BL. All data are reported as mean ±SD.
CHAPTER V: DISCUSSION

This investigation sought to compare changes in neuromuscular performance, muscle damage, and inflammation between young and middle-aged adults on recovery from a fatiguing isokinetic exercise protocol. In this investigation, there were no baseline differences in the height, body mass, body fat percentage, average daily caloric intake, or average daily nutrient intake between the two groups. There was also no difference in muscle size or thickness between the groups. In regards to muscular performance, there were no baseline difference in isometric or isokinetic peak torque, isokinetic average torque, or total work between the groups. There were baseline differences in isometric average torque, and the rate of torque development at 200 ms. Also at baseline, a trend towards a difference was observed for isometric peak torque, isokinetic peak torque (at both 240°·sec⁻¹ and 60°·sec⁻¹), and isokinetic average torque at 240°·sec⁻¹.

Results of the study indicated that although both groups experienced significant performance decrements, no between-group differences were observed between the younger and middle-aged participants for these measures. Furthermore, no differences between the groups were observed in the inflammatory or muscle damage response.

Decreases in muscle mass, function, and neuromuscular activation are major contributors to the decrease in quality of life for adults (Janssen et al., 2000; Kamen et al., 1995). The amount of force a muscle can generate, how long it can generate optimal force, and how quickly it can generate this force, are all vital in assessing the quality of muscle, and its effect on health and activity. The benefits of resistance training (e.g. increased muscle size and function) can be observed in both younger adults, as well as older adults (Hakkinen et al., 1998; Walker et al., 2014).
Peak torque is a measure of the maximal performance capability of an individual. Previous investigations examining the effect of age on isokinetic performance have reported significantly greater peak torque in younger adults (means ranging from 23 - 29 y) compared to older adults (mean ranging from 60 – 65 y), Candow et al., 2005; Hakkinen et al., 1998; Lynch et al., 1999). Similar differences in muscular performance have also been observed when comparing middle-aged (41 – 42 y) and older adults (70 – 72 y) (Hakkinen et al., 1998; 2000). Evidence from these investigations indicate that there are age-related decreases in muscle performance (e.g. power, and rate of force development). In contrast to these studies, baseline differences were found in average torque, and in the rate of torque development at 200 ms, with only a trend observed in peak torque. As previously discussed, trends were also found in isokinetic peak torque (at 240°·sec⁻¹ and 60°·sec⁻¹), and average torque at 240°·sec⁻¹ between the two groups at baseline. It should also be noted that all of the aforementioned studies included participants who were untrained or novice to resistance exercise. In addition, the population comparison performed in this investigation was unique, in which this appears to be the first study to compare young to middle-aged adults with recreational resistance training experience. The results of this study also indicate that recovery of peak torque from fatiguing exercise appears to be similar between middle-aged and young adults with recreational resistance training experience. Whether this is a function of this age group or training status is not clear, but the data does appear to suggest that recreational training does maintain recovery capability from high volume isokinetic resistance exercise.

There were no differences in peak or mean torque between groups during the recovery period. There were also no differences between these groups in Δ change from BL for these
variables. Differences in the pattern of recovery between the groups were noted in the rate of torque development. YA experienced a significantly greater Δ change between BL and IP, and between BL and 48H for both RTD100 and RTD200. YA experienced a 52.6% decrease from BL to IP during RTD100 and a 50.6% decrease in RTD200. In contrast, MA experienced a 31.4% and an 18.3% reduction for RTD100 and RTD200, respectively. The results of this investigation are in agreement with other studies that have reported decreases in force and the rate of force development with increasing age (Hakkinen et al., 1998; Izquierdo et al., 1999; Thompson et al., 2014). It appears that the rate of force development declines at a greater rate than muscle performance with increasing age. This provides a potential explanation for the differences observed in the rate force development between the groups at baseline, as well as differences in the rate of torque development at 100 ms and 200 ms during the recovery period.

Both the younger and middle-aged adults showed similar patterns of muscle damage, as reflected by the comparable myoglobin and CK responses from the exercise protocol. Elevations in myoglobin and creatine kinase concentrations in both groups are consistent with the expected physiological response from high volume exercise (Clarkson et al., 2006; Sayers & Clarkson, 2003). Considering the similar training background (e.g. recreational) of these subjects, these results are not surprising. These results also suggest that middle-aged recreationally active men who regularly engage in resistance training are not at greater risk for either muscle soreness or muscle damage in comparison to younger recreationally trained adults.

No significant elevations from BL were observed in either C-reactive protein or IL-6 concentrations for either group. This again is likely related to the recreational training status of both groups. Previous research has reported that regular exercise can attenuate the C-reactive
protein response (Kasapis et al., 2005; Toft et al., 2002). IL-6 has generally been reported to increase following acute exercise (Kasapis et al., 2005; Pedersen & Febbraio, 2005). Although no significant elevations were observed in the IL-6 response, a trend towards an increase was detected. Considering the participants were at least 10 hours fasted before the initial exercise protocol, although speculative, a reduction of muscle glycogen may have influenced the IL-6 response. Muscle glycogen depletion has been reported to upregulate IL-6 transcription (Steensberg et al., 2001).

No differences in subjective levels of pain or soreness were observed between the groups. The results regarding subjective feelings of pain are consistent with the inflammatory response observed, while the elevations observed in subjective feelings of soreness is supportive of the pattern of response observed in the muscle damage markers. The significant increase in muscle soreness through 48H, accompanied by elevated markers of muscle damage (i.e. myoglobin, creatine kinase) is consistent with other studies assessing muscle damage from an acute exercise bout (Jajtner et al., 2015; Kanda et al., 2013). The CRP concentrations in the current investigation remained unchanged, which is consistent with other investigations of recreationally experienced subjects (Croisier et al., 1999; Jajtner et al., 2015). However, in contrast to the current findings, Jajtner and colleagues (2015) reported significant elevations in IL-6; these differences may be related to differences in the exercise intervention or muscle mass utilized.

Conclusions

This investigation sought to compare the changes in the recovery response from a high-volume intervention among younger and middle-aged men. Although participants from each group displayed baseline differences in isometric average torque and rapid force production, age
did not influence the ability to recover from a damaging high-volume isokinetic protocol
between younger and middle-aged men. There was not a pronounced physiological difference
observed in the recovery response in the groups studied in this investigation, however previous
research has shown differences in recovery with age groups different from the current research
(e.g. younger to older adults; middle-aged to older adults). Furthermore, age may not
significantly affect the recovery response from exercise in mid-life, but may become a primary
factor in older age. Age also may be more of a factor in recovery depending on the muscle mass
utilized, as well as the intensity and volume of exercise. Results of this study seem to suggest
that there may be other primary contributing factors to the rate of recovery from exercise beyond
an individual’s chronological age (e.g. training status) during mid-life.
APPENDIX A: UCF IRB LETTER
Notice that UCF will Rely Upon Other IRB for Review and Approval

From: UCF Institutional Review Board
FWA00000051, IRB00001118
To: Adam J. Wells
Date: December 06, 2016
IRB Number: SBE-16-1294
Study Title: Effects of an Acute High-Volume Isokinetic Intervention on Inflammatory and Strength Changes: Influence of Age

Dear Researcher:

The research protocol noted above was reviewed by the University of Central Florida IRB Designated Reviewer on December 05, 2016. The UCF IRB accepts the New England’s Institutional Review Board review and approval of this study for the protection of human subjects in research. The expiration date will be the date assigned by the New England’s Institutional Review Board and the consent process will be the process approved by that IRB.

This project may move forward as described in the protocol. It is understood that the New England’s IRB is the IRB of Record for this study, but local issues involving the UCF population should be brought to the attention of the UCF IRB as well for local oversight, if needed.

All data, including signed consent forms if applicable, must be retained and secured per protocol for a minimum of five years (six if HIPAA applies) past the completion of this research. Any links to the identification of participants should be maintained and secured per protocol. Additional requirements may be imposed by your funding agency, your department, or other entities. Access to data is limited to authorized individuals listed as key study personnel.

Failure to provide a continuing review report for renewal of the study to the New England’s IRB could lead to study suspension, a loss of funding and/or publication possibilities, or a report of noncompliance to sponsors or funding agencies. If this study is funded by any branch of the Department of Health and Human Services (DHHS), as Office for Human Research Protections (OHRP) IRB Authorization form must be signed by thesignatory officials of both institutions and a copy of the form must be kept on file at the IRB office of both institutions.

On behalf of Sophie Czapsiakowski, Ph.D., L.C.S.W., UCF IRB Chair, this letter is signed by:

[Signature]

Signature applied by Paris Davis on 12/06/2016 09:11:38 AM EST

IRB Coordinator
APPENDIX B: NEIRB APPROVAL LETTER
January 16, 2017

Adam Wells, PhD
University of Central Florida
12494 University Boulevard
Orlando, Fl. 32816, United States

NEIRB: 120160966
Study Title: Effects of an Acute High-Volume Isokinetic Intervention on Inflammatory and Strength Changes: Influence of Age

Notification of Approval

This is to inform you that New England Independent Review Board (NEIRB) has approved the following for the above referenced research.

Date of Review: 01/16/2017

Approval Includes:
Protocol (Submitted on 01-13-2017)
Main Consent (01-16-2017), NEIRB Version 4.0
Dietary Recall Instructions #67160.0 - As Submitted

NEIRB has approved the following locations to be used in the research:
• University of Central Florida Human Performance Lab, 12494 University Boulevard, Orlando, Florida 32816

Continued approval is conditional upon your compliance with the following requirements:
• Compliance with all aspects of NEIRB’s Investigator Responsibilities (available at www.neirb.com).
• The study cannot continue after the End Approval Date unless re-approved by NEIRB. A Study Continuing Review Report must be completed and returned to NEIRB prior to the study end date of the approval period.
• Compliance with all federal, state and local laws pertaining to this research.
• Any and all necessary FDA approvals must be received prior to your initiation of the trial.

Please contact NEIRB with any questions about this determination.
Thank you.

Copy: NEIRB study file
Enclosures
APPENDIX C: APPROVED INFORMED CONSENT
Effects of an Acute High-Volume Isokinetic Intervention on Inflammatory
and Strength Changes: Influence of Age

Informed Consent

Principal Investigator(s): Dr. Adam J. Wells, Ph.D.
Sub-Investigators: Dr. Jeffrey R. Stout, Ph.D.
Dr. Jay R. Hoffman, Ph.D.
Joseph A. Gordon III, B.S.
Eliott Arroyo, B.S.
Alyssa Varnoske, M.S.

Phone number: (407) 823-3167

Sponsor: UCF Institute of Exercise Physiology and Wellness

Investigational Site(s): University of Central Florida
College of Education and Human Performance
Institute of Exercise Physiology and Wellness

Introduction:
You are being invited to take part in a research study which will recruit about 30 people at UCF and its surrounding areas. You have been asked to take part in this research study because you are a recreationally active male between the ages of 18 and 30 years or between the ages of 40 and 60 years.

The investigators conducting the research are Dr. Jay R. Hoffman, Dr. Jeffrey R. Stout, Professors of Sport and Exercise Science in the College of Education and Human Performance, Dr. Adam J. Wells (Assistant Professor of Sport and Exercise Science in the College of Education and Human Performance), Mr. Joseph A. Gordon III, Mr. Eliott Arroyo, and Ms. Alyssa N. Varnoske (Graduate Students of Sport and Exercise Science in the College of Education and Human Performance).

What you should know about a research study:
- Someone will explain this research study to you.
- A research study is something you volunteer for.
- Whether or not you take part is up to you.
- You should take part in this study only because you want to.

1 of 10
Approved by NEEB on 01/20/2017
NEEB Version 3.0

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• You can choose not to take part in the research study.
• You can agree to take part now and later change your mind.
• Whatever you decide it will not be held against you.
• Feel free to ask all the questions you want before you decide.

Purpose of the research study:

The purpose of this study is to compare the effects of an acute, high-volume isokinetic exercise intervention on the rate of recovery in younger versus older recreationally-trained males. Recovery parameters include lower-body performance and markers of muscle damage and inflammation, which will be assessed in the subsequent 48 hours following a muscle-damaging protocol.

What you will be asked to do in the study:

Screening Visit:

You will be asked to read and sign this consent form before any study-related procedures are performed. During the screening visit, the following will be done:
- Physical activity readiness questionnaire (PAR-Q)
- Your age, race and gender will be collected
- Self-reported confidential medical and activity history questionnaire

During the screening visit, we will review the inclusion/exclusion criteria with you. We will also inform you of the requirements of the study and determine whether you have any intolerance to the exercise.

Study Protocol:

Investigator expectations are the same for all participants. All procedures are being done solely for research purposes. Following enrollment into the study, you will report to the Human Performance Lab for assessment on four separate occasions:

• Day 1: On the first visit, you will report to the Human Performance Laboratory (HPL) following a 10-hour fast. Upon arrival, your weight and height will be measured using a standard scale and measuring tape, respectively. Additionally, your body composition will be assessed using bioelectrical impedance analysis. Following a brief warmup, you will be familiarized with the lower body performance assessments (isokinetic device and isometric mid-thigh pull).
• Day 2: On the second visit, again following a 10-hour fast, you will be outfitted with a near-infrared spectroscopy (NIRS) optode. Following this, you will undergo baseline (BL) measurements including an ultrasound assessment, a blood sample collection, a lower-body performance assessments protocol, and a visual analog scale (VAS) to indicate your current level of pain and soreness. After a brief rest period following the
lower-body performance assessment, you will undergo the acute muscle-damaging protocol (MDP).
- Immediately upon completion of the MDP (2P), you will complete a blood sample collection, a second lower-body assessment protocol followed by an ultrasound assessment.
- Thirty minutes post MDP (30P), you will complete another ultrasound assessment, a blood sample collection, and a VAS.
- At sixty minutes (60P) and 120 minutes (120P) post MDP, additional blood samples will be collected.
- A third lower-body performance assessment protocol (BioDex S4 isokinetic dynamometer, and mid-thigh pull) and an additional ultrasound assessment will be completed at 120P.
- Day 3: On the third visit, you will report to the HPL again following a 10-hour fast and perform the 24-hour post (24H) ultrasound assessment, a blood sample collection, and lower-body performance assessment protocol. Additionally, you will indicate your level of pain and soreness using the VAS.
- Day 4: On the fourth and final visit, you will again report to the HPL following a 10-hour fast. You will then complete the 48-hour post (48H) assessment, which includes an ultrasound assessment, a blood sample collection, and the lower-body performance assessment protocol. Additionally, you will indicate your level of pain and soreness using the VAS.

The figure below outlines the daily procedures throughout the investigation:
Anthropometric Measurements

Body mass and height will be measured using a standard professional scale. Body composition will be assessed using a bioelectrical impedance analysis device. You will be asked to remove your footwear and socks before performing the test. This assessment requires you to wash your hands and feet with a wet towel prior to analysis. Next, you will stand on the platform while holding the two handles out to the side. You will hold this position for one minute as the device conducts an electrical current through your body to determine body composition. Values for total and segmental body fat percentage will be recorded. The test will be completed in approximately 5 minutes. There are no risks or discomforts associated with the use of bioelectrical impedance analysis.

Lower-Body Performance Assessment Protocol

The lower-body performance assessment protocol will consist of three assessments. Two assessments will be performed on an isokinetic dynamometer, and one on a force platform (mid-thigh pull).

Mid-thigh pull

Following a general warm-up, you will complete a two mid-thigh pulls to evaluate isometric strength of the lower body. Two isometric mid-thigh pulls will be assessed. You will be instructed to stand on a force platform, bent slightly at the knees and hip. A barbell will be adjusted so that it is fixed at mid-thigh position. You will be instructed to pull upwards on the barbell as hard and forcefully as you can for 6 seconds. Between isometric mid-thigh pull assessment sets, you will be provided with three minutes of rest. Potential risks and/or discomforts associated with the isometric mid-thigh pull assessment may include muscle pain and/or soreness, similar to those experienced when exercising.

Isokinetic Dynamometer

Following the mid-thigh pull, you will be seated in an isokinetic device positioned with a hip angle of 110° and strapped into the chair at the waist, shoulders, and across the thighs to complete two maximal voluntary isometric contractions (MVIC) where you will be required to kick maximally against a fixed resistance for 5-seconds as your leg stays in one position. Between MVIC sets, you will be provided with three minutes of rest. Potential risks and/or discomforts associated with the lower-body isokinetic and isometric assessment may include muscle pain and/or soreness, similar to those experienced when exercising.

After completing the MVIC test, you will then complete a series of isokinetic contractions on the BioDex 54 isokinetic dynamometer. The seat and leg setup will be the same as it was for the MVIC test. You will complete 5 sets of isokinetic leg extension at 60, 180, and 240 degrees per second, respectively. The order of the sets will be randomized to account for any fatigue from each set. The starting point for a repetition will be when the knee is at 90 degrees, with 180 degrees representing full extension. You will be given two practice attempts at each speed before recording begins. You will be instructed to give maximal effort as you extend the knee joint to 180 degrees. Then you will relax the muscles to allow the leg to return to the starting position. This process of starting at 90 degrees, extending to 180 degrees and then returning
back to 90 degrees will be considered 1 repetition. You will be given 3 minutes of rest
between each set. This test should last approximately 10 minutes.

Acute Lower-Body Muscle-Damaging Protocol

After completing the lower-body assessment protocol, you will remain seated in the isokinetic
dynamometer chair. Following a five-minute rest, you will perform the MDP. The MDP is composed
of eight sets of 10 repetitions. You will be instructed to give maximal effort during this protocol. You
will be provided with one minute of rest between each set of the MDP. Potential risks and/or
discomforts associated with the lower-body MDP are the same as associated with the lower-body
isokinetic and isometric assessment.

Visual Analog Soreness Questionnaire

You will be asked to quantify your degree of lower-body muscle soreness and pain using a 15-cm
visual analog scale (VAS). You will provide your levels of pain and soreness by making a mark on a
horizontal line with words anchored at each end of the VAS.

Blood Measurements

You will report to the HPL at the same time on each day following a 10-hour fast. The blood samples
collected at BL, IP, 30P, 60P, and 120P will be drawn from a forearm vein using a Teflon™
cannula by personnel trained in phlebotomy with extensive experience in both research and clinical settings.
A cannula is a hollow tube, which can be inserted into the opening of a vein and serve as a channel for
the transport of fluid. The cannula prevents the need for multiple needle pricks from being performed.
The cannula will be kept open following each blood draw with an infusion of a saline solution. This
solution contains salt that is similar to the osmolarity of the blood and acts to minimize potential blood
clogging within the cannula that may occur with prolonged use. The cannula placement will not
interfere with your ability to perform the exercise routine. The blood draws at 14 and 48H will be
made using a 21 gauge, 1 ¼ inch Vacutainer® blood collection needle. The total amount of blood that
will be taken during the study as a whole will not exceed 149 mL (20 mL per blood draw). This is
approximately 9.5 tablespoons. To put the total volume of blood being drawn in proper perspective,
one pint (475 mL) of blood is typically drawn when donating blood. All blood draws will be conducted
under sterile conditions. As an additional safeguard in preventing contamination, new disposable
gloves will be used for all blood draws. The discomforts associated with the blood drawing procedures
are minimal, but sometimes bruising and infection may occur, as well as soreness in the arm.

Muscle Ultrasound

To evaluate the muscle architecture and morphology of your upper thigh, non-invasive ultrasound
measurements will be made using a linear probe ultrasound. The probe will be coated with a water-
based conduction gel. You will be positioned on your non-dominant leg side, with your hips
perpendicular to the examination table. You will be instructed to keep you legs, knees, and ankles
stacked together. A mat will be made at on your thigh at a specified location to guide location of the
image capture. Ultrasound images will be later analyzed for muscle morphological characteristics.
There are no potential risks associated with ultrasonography.
Near Infrared Spectroscopy

To assess tissue oxygenation during the acute muscle damage protocol, a near infrared spectroscopy (NIRS) optode (PortisLine, Armino Medical Systems, Gelderland, the Netherlands) will be placed over the vastus lateralis muscle of your right leg. The optode will be secured using a self-adhering bandage. There are no risks or discomforts associated with the use of near infrared spectroscopy.

Dietary Recall

You will be instructed to remember as accurately as possible everything they consumed during the 24-hours preceding BL assessments. You will also be required to provide a dietary recall upon arrival to the HPL on Day 1 (4th) and Day 2 (5th), be required to you will be interviewed at each visit, and asked to recall all food consumed on each of these days.

Location:
All testing will be performed in the Institute of Exercise Physiology and Wellness at the Human Performance and Strength and Conditioning Laboratories at the University of Central Florida.

Time required:
We expect that the time requirements for participation in this study will be 4 days, which will consist of a total of 4 visits to the HPL. The familiarization visit will last approximately 60 minutes, the intervention visit will last approximately 240 minutes, and the subsequent two consecutive days following the intervention visit will be approximately 60 minutes each. The total time that you will be in the laboratory will be approximately 7 hours over a period of 4 days.

Funding for this study:
There will be no funding provided for the completion of this study.

Risks:

- The intervention protocol consists of both concentric and eccentric muscle contractions at a high volume. It is expected that you will experience the normal soreness that accompanies a bout of resistance exercise.

- The risks associated with the blood draw include some momentary pain at the time the needle is inserted into the vein, but other discomfort should be minimal. It is also possible for a bruise to develop at the needle site or for individuals to report dizziness and faint after the blood is drawn. It is also rare, but possible to develop minor infections and pain after the blood draw. To minimize the risks, the skin area where the needle or catheter is to be inserted will be cleansed and prepared with a disinfectant wipe before the needle or catheter is inserted. In addition, the catheter will be inserted while the participant is lying supine and all blood draws will be performed with the subject in a supine position.
• There are no risks or discomforts associated with any of the ultrasound or anthropometric measures.

• All testing will be overseen by individuals certified in CPR and AED. An AED is located in the Wellness Research Center within the Building (approximately 200 feet from the Human Performance Laboratory).

• If at any time during the study, you feel discomfort or do not wish to continue, you are encouraged to inform the researcher. Discontinuation of participation may occur at any time. Any discomfort should be immediately reported to Joseph Gordon III (407-821-3367), Alyssa Vanonoke (407-823-2367) or to the professional performing the test and later to the principal investigator (Dr. Adam Wells, 407-823-3909).

**Benefit:**
There are no expected benefits to you for taking part in this study.

**Compensation or payment:**
There is no compensation or other payment to you for taking part in this study.

**Medical care and compensation for injury:**
This is a minimal-risk study and it is unlikely that you will experience adverse effects. However, in the event that an adverse effect occurs, you will be instructed to immediately report any discomforts or adverse effects to the principal investigator. An adverse effect is defined as an intolerable response, perceived to be a direct consequence of participation in this study. If immediate assistance is needed it will be provided via the emergency medical system. For non-emergency injuries, you must seek treatment from your own physician. If you suffer a physical injury as a result of participation in this study, you may be reimbursed for medical expenses to treat the injury, to the extent not paid by your insurance. You should receive medical care in the same way as you would normally. No funds have been set aside for payments or other forms of compensation (such as for lost wages, lost time, or discomfort). You do not give up any of your legal rights by signing this consent form. Adverse events/side effects will be reported to the New England Independent Review Board (NEIRB) immediately upon notification.

**Alternative:**
The alternative is to not participate in the study.

**Cost:**
There is no cost to you to be in the study.

**Confidentiality:**
Records of your participation in this study will be held confidential to the extent permitted by law. The study investigator or it’s designee, and, under certain circumstances, the Institutional Review Board will be able to inspect and have access to confidential data that identifies you by name. Any
publication or presentation of the data will not identify you. By signing this consent form, you authorize the study investigator to release your medical records to the IRB.

The results of this study will be published as a group as part of a scientific publication. No individual results will be published or shared with any person or party. All information learned from the medical and activity questionnaires or performance tests will be held in strict confidence. Individual results will remain confidential. Individual test results will be shared with the participant only at their request. Test results will not be shared with participants. All medical and activity questionnaires, as well as data collection sheets will be kept in a locked cabinet during and following the study. Your names will be kept separately from the study results as a separate electronic file under password protection. This file will be stored on a computer in the Education and Human Performance building. All information will be destroyed 5 years from the end of the study. Your folders and blood storage tubes will be marked with an ID number to protect against a breach of confidentiality and the ID number will be removed upon disposal. Your names will be stored apart from the blood samples. The identifiers will be removed from the samples and destroyed when the samples are disposed of. All the medical information taken during the study will not be useful for you or cannot be used to supplement/replace medical care.

Study contact for questions about the study or to report a problem:
If you have questions, concerns, or complaints, or think the research has hurt you, contact Dr. Adam Wells, Human Performance Laboratory, Sport and Exercise Science (407) 823-3906 or by email at adam.wells@ucf.edu.

IRB contact about your rights in the study or to report a complaint:
For information about the rights of people who take part in research or if you have questions, complaints or concerns, please contact New England Independent Review Board at (800) 255-9570 or email at info@NERB.com.

Withdrawing from the study:
Your participation in the study may also be terminated at any time by the researchers in charge of the project for the following reasons:
- Inability to adhere to the study protocol. This includes:
  - Failure to adhere to requirements
  - Failure to complete all visits to the human performance lab
  - Failure to provide a fasting blood draw at the start of the control or exercise trial
- Cancellation of the study.

The investigator or the New England IRB can also stop your participation in this study at any time.

For Students and Employees of UCF:
Your participation in this study is voluntary. You are free to withdraw your consent at any time without prejudice or penalty. Your decision to participate or not participate in this study will in no way affect your continued employment or your relationship with individuals who may have an interest in this study. Employees of UCF cannot participate to the study.
during work hours. In addition, if you are a student you have the right to withdraw at any time and without any negative affect on your grade or on your ability to continue your education at the University.

Results of the research:

Biologic specimens obtained from this research are not expected to be part of or lead to the development of a commercial product. As previously stated, individual results will remain confidential, and you will be informed of only your results upon request.
VOLUNTEER'S STATEMENT

I have been given a chance to ask questions about this research study. These questions have been answered to my satisfaction. I may contact Joseph Gordon or Elliott Arroyo (student investigators), or Adam Wells, Ph.D., if I have any more questions about taking part in the study.

I understand that my participation in this research project is voluntary. I know that I may quit the study at any time without harming my future medical care or losing any benefits to which I might be entitled. I also understand that the investigator in charge of this study may decide at any time that I should no longer participate in the study.

If I have any questions about my rights as a research subject in this study I may contact:

New England Independent Review Board, 187 First Avenue, Suite 250, Needham, MA, 02464
E-Mail: info@neirb.com
Telephone 1300.232.3570

By signing this form, I have not waived my on my legal rights.

I have read and understand the above information. I agree to participate in this study. I understand that I will be given a copy of this signed and dated form for my own records.

Name of participant

Signature of participant

Date

Signature of person obtaining consent

Date

Printed name of person obtaining consent

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Approved by NEIRB on 01/20/2017

NEIRB Version 3.0
APPENDIX D: MEDICAL AND ACTIVITY HISTORY QUESTIONNAIRE
Confidential Medical and Activity History Questionnaire

Participant #__________

When was your last physical examination? ________________________________

1. List any medications (prescription or over-the-counter), herbs, or supplements you currently take or have taken the last month:

<table>
<thead>
<tr>
<th>Medication</th>
<th>Reason for medication</th>
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<td></td>
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</tr>
</tbody>
</table>

2. Are you allergic to any medications? If yes, please list medications and reaction.

3. Please list any allergies, including food allergies that you may have?

4. Are you a current or former smoker? If former, how long has it been since you quit?

5. Are you currently enrolled in another clinical research study?

6. Do you currently drink > 8oz/day of either green or black tea?

7. In the past two years have you been diagnosed with cancer? If so, what type?

8. Are you currently on a diet regimen including but not limited to, Atkins, South Beach, Intermittent Fasting, etc?
9. Have you donated blood or plasma recently? If so when?

10. Do you currently have any chronic illness that requires continuous medical care? If so what is the illness?

11. Have you ever been hospitalized? If yes, please explain.

<table>
<thead>
<tr>
<th>Year of hospitalization</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
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</tbody>
</table>

12. Illnesses and Other Health Issues

List any chronic (long-term) illnesses that have caused you to seek medical care.

Have you ever had (or do you have now) any of the following. Please circle questions that you do not know the answer to.

- Sickle cell anemia: yes no
- Cystic fibrosis: yes no
- Water retention problems: yes no
- Heart pacemaker: yes no
- Epilepsy: yes no
- Convulsions: yes no
- Dizziness/fainting/unconsciousness: yes no
- Asthma: yes no
- Shortness of breath: yes no
- Chronic respiratory disorder: yes no
- Chronic headaches: yes no
- Chronic cough: yes no
- Chronic sinus problem: yes no
- High blood pressure: yes no
- Heart murmur: yes no
<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart attack</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>High cholesterol</td>
<td>yes</td>
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<td>Diabetes mellitus or insipidita</td>
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<tr>
<td>Rheumatic fever</td>
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<tr>
<td>Emphysema</td>
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<td>Hepatitis</td>
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<td>Bladder problems</td>
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<tr>
<td>Tuberculosis (positive skin test)</td>
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<tr>
<td>Yellow jaundice</td>
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<td>Auto immune deficency</td>
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<tr>
<td>Anemia</td>
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<td>Endosseuma</td>
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<td>Stomach/intestinal problems</td>
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<td>Hepatic encephalopathy</td>
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<td>Mania</td>
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<td>Hypermania</td>
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<td>Monosodium glutamate hypersensitivity</td>
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<td>Seizure disorders</td>
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Any others (specify):__________________________

---

Do you smoke cigarettes or use any other tobacco products?  yes no
Do you have a history of drug or alcohol dependency?  yes no
Do you ever have any pain in your chest?      yes no
Are you ever bothered by racing of your heart? yes no
Do you ever notice abnormal or skipped heartbeats? yes no
Do you ever have arm or jaw discomfort, nausea, or vomiting associated with cardiac symptoms? yes no
Do you ever have difficulty breathing? yes no
Do you ever experience shortness of breath? yes no
Do you ever become dizzy during exercise? yes no
Are you pregnant? yes no
Human Performance Laboratory
University of Central Florida

Is there a chance that you may be pregnant? yes no
Have you ever had any tingling or numbness in your arms or legs? yes no
Has a member of your family or close relative died of heart problems or sudden death before the age of 50? yes no
Has a health care practitioner ever denied or restricted your participation in sports for any problem yes no
If yes, please explain: ________________________________

What is your current level of physical activity (hours per week, intensity, mode of exercise)?

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

Are you presently taking any nutritional supplements or ergogenic aids? (if yes, please detail)

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________
APPENDIX E: PHYSICAL ACTIVITY READINESS QUESTIONNAIRE
PAR-Q & YOU

A Questionnaire for People Aged 15 to 69

Physical Activity Readiness Questionnaire - PAR-Q (Revised 2007)

Regular physical activity is fun and healthy, and encourages more people to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 65 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

You may be able to do any activity you want — as long as you start slowly and build up gradually. Do you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow their advice.

Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to the PAR-Q questions, you can be reasonably sure that you can start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.

Take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to be active. It is also highly recommended that you have your blood pressure measured. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

IMPORTANT: Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing the PAR-Q, consult your doctor prior to activity.

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Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.
APPENDIX F: VISUAL ANALOG SCALE
APPENDIX G: DIETARY RECALL INSTRUCTIONS
Dietary Recall Instructions:

1. Use the forms provided to record everything you eat or drink on the days instructed.

2. Indicate the name of the FOOD ITEM, the AMOUNT eaten, how it was PREPARED (fried, boiled, etc.), and the TIME the food was eaten. If the item was a brand name product, please include the name. Try to be accurate about the amounts eaten. Measuring with measuring cups and spoons is best, but if you must make estimates, use the following guidelines:
   - A fist is about 1 cup
   - Tip of your thumb is about 1 teaspoon
   - A thumb represents about 1 ounce of cheese
   - A golf ball represents about 1 tablespoon
   - The palm is about 3 ounces of meat (roughly the size of a deck of cards)

3. Try to maintain your normal diet, and be honest about what you eat. The information you provide is confidential.

4. Follow the specific instructions below when reporting foods:
   - MILK – indicate % fat, source (e.g., cow, almond, coconut), and flavoring (if any).
   - FRUITS & VEGETABLES – an average serving size of cooked or canned fruits and vegetables is ½ cup. Fresh, whole fruits and vegetables should be listed as small, medium, or large. Be sure to indicate sugar or syrup is added to fruit and list if any margarine, butter, cheese sauce, or cream sauce is added to vegetables. When recording salad, list items comprising the salad separately and be sure to include salad dressing used.
   - EGGS – indicate whole or whites only, method of preparation (e.g., scrambled, fried, poached), and number eaten.
   - MEAT/POULTRY/FISH – indicate approximate size or weight, in ounces, of the serving. Be sure to include any gravy, sauce, or breading added and preparation method.
   - CHEESE – indicate kind, number of ounces or slices, and whether it is made from whole milk, part skim, or is low calorie.
   - CEREAL – specify kind, brand, whether cooked or dry, and measure in terms of cups or ounces. *Consuming 5 oz of cereal is not the same as consuming 1 cup of cereal. Be sure to include any milk consumed with cereal (see MILK).
   - BREADS – specify kind (e.g., whole wheat, enriched wheat, white) and number of slices.
   - BEVERAGES – include everything drink, excluding water. Be sure to record cream and sugar used in tea and coffee, whether juices are sweetened or unsweetened, and whether soft drinks are diet or regular.
   - FATS - record any butter, margarine, etc. or other fats used in cooking or on food.
   - PREPARED DISHES/CASSEROLES – list the main ingredients, approximate amount of each ingredient to the best of your ability, and brand (if applicable).

5. Express approximate measures in cups (c), tablespoons (T), teaspoons (t), grams (g), ounces (oz), pieces, etc.

6. If you are unsure of how to report any food item you consume, please take pictures of the packaging and Nutrition Facts panel, when possible.
### Day 1

<table>
<thead>
<tr>
<th>Meal</th>
<th>Time</th>
<th>FOOD/BEVERAGE DESCRIPTION</th>
<th>AMOUNT</th>
<th>Total kcal (from label)</th>
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<tbody>
<tr>
<td>Breakfast</td>
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</tr>
<tr>
<td>Lunch</td>
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</tr>
<tr>
<td>Dinner</td>
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</tr>
<tr>
<td>Snacks</td>
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<tr>
<td>Meal</td>
<td>Time</td>
<td>FOOD/BEVERAGE DESCRIPTION</td>
<td>AMOUNT</td>
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<tr>
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Day 2                  Date: ________
## Day 3

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<td>Snacks</td>
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REFERENCES


