

Effects of Passive Dehydration on Neuromuscular Function

By
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Abstract

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Purpose: The purpose of this study was to compare the effects of passive dehydration on neuromuscular function, specifically analyzing maximal voluntary contraction (MVC) peak torque, mean firing rates (MFR) vs. recruitment threshold (RT), motor unit action potential amplitudes ($MUAP_{AMP}$) vs RT, recruitment ranges, muscle cross sectional area (mCSA), subcutaneous fat and echo intensity of the vastus lateralis (VL). **Methods:** Nine recreationally trained males (age= 26.89 ± 3.72 yr, weight= 95.52 ± 17.07 kg, height= 180.60 ± 3.76 cm) completed a familiarization and 3 consecutive experimental visits. Participants were euhydrated on visit 1, hypohydrated by 5% of their body mass for visit 2 and rehydrated back to baseline body mass for visit 3. Hypohydration was achieved through fluid restriction and fasting and passive sweat loss in a hot, humid environment room. Each experimental visit consisted of participants completing two isometric MVCs measured by an isokinetic dynamometer, with strong verbal encouragement. The highest peak torque was used for a 70% MVC isometric muscle action. Surface electromyography signals were collected from the VL during the 70% MVC isometric muscle action and each MU was analyzed for $MUAP_{AMP}$ and MFR during the steady force and force at recruitment. Ultrasonography was used in B mode to measure mCSA, subcutaneous fat, and echo intensity of the VL. Urine and blood samples were collected for hydration measurements. Heart rate was

measured at the beginning, during and at the end of each visit. **RESULTS:** Participants experienced significant dehydration from visit 1 to visit 2, verified by Usg (visit 1 = 1.014 ± 0.007 vs. visit 2 = 1.034 ± 0.004 , $p < 0.001$) and body mass loss (visit 1 = 95.5 ± 17.1 kg vs. visit 2 = 90.9 ± 16.4 kg, $p < 0.001$). Participants reached baseline body mass by visit 3 (95.6 ± 17.2 kg), showing no significant differences between body mass between hydration visit 1 or visit 3 ($p = 1.00$). Usg indicated participants were euhydrated (1.019 ± 0.006), and plasma volume significantly increased from visit 2 to 3 (50.3 ± 3.4 to 56.0 ± 4.7 , $p = 0.021$). There was no significant difference in Usg between visit 1 and 3 ($p = 0.675$). HR was significantly higher visit 2 (92 ± 11 bpm) compared to visit 1 (66 ± 6 bpm, $p < 0.001$) and visit 3 (71 ± 12 bpm, $p = 0.024$). HR was also significantly higher post- strength testing visit 2 than visit 1 (94 ± 15 bpm and 76 ± 10 bpm, $p = 0.023$). There was no significant difference in HR visit 1 to visit 3 pre- ($p = 0.597$) or post- ($p = 1.00$) strength testing, or post- strength testing visit 2 to visit 3 ($p = 0.083$). MVC peak torque significantly decreased from visit 1 (263.6 ± 59.6 Nm) to visit 2 (241.5 ± 59.1 Nm), $p = 0.047$. Visit 2 mCSA was significantly smaller (31.4 ± 7.4 cm²) compared to visit 1 (34.0 ± 7.2 cm², $p = 0.003$) and visit 3 (35.2 ± 7.6 cm², $p = 0.003$). There were no significant differences in MFR vs RT or MUAP_{AMP} vs RT relationships, recruitment ranges, subcutaneous fat, or echo intensity. HR) immediately after becoming hypohydrated = 128.9 ± 20 bpm. **Conclusion:** Extreme hypohydration resulted in significantly decreased MVC peak torque and mCSA and increased cardiovascular strain; however, there were no significant changes in MU behavior, subcutaneous fat or echo intensity. It is important for individuals to attenuate the effects hypodration has on the body such as decreased peak torque, decreased muscle size and increased cardiovascular strain especially during exercise or performance. Through maintaining euhydration and rehydrating after

acute bouts of dehydration it will help prevent other serious complications such as exertional heat illness.

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Chapter I

INTRODUCTION

The human body operates most efficiently at a “normal” state of body water content, known as euhydration, with little fluctuations.¹ Once dehydration, or the process of losing body water content, occurs this can have a negative effect on many physiological systems including the cardiovascular, thermoregulatory, immune, and musculoskeletal systems and brain. Decreases in performance start to be recognized at 2% hypohydration and can progress with the increasing level of hypohydration.² Decreases in performance are more commonly seen in cardiovascular exercise, due to the direct strain on the cardiovascular system,³⁻⁵ though other anaerobic and strength measures decline when accompanied with hypohydration.^{6,7}

Previous neuromuscular research has examined the effects of various levels of dehydration (~2-4%) on peak torque and EMG amplitudes. These studies have demonstrated both significant and insignificant changes in peak torque and EMG amplitudes.⁸⁻¹³ However, direct comparison across studies is hard, as there is no consistency in methodologies, and specifically examining motor unit (MU) behavior is often not included. Motor units are characterized by three main principles: the size principle,¹⁴ onion skin scheme,¹⁵ and the common drive.¹⁶ Specific MU characteristics include recruitment threshold (RT), meaning firing rates (MFR), and MU action potential amplitudes (MUAP_{AMP}),¹⁷ which have not been analyzed in dehydration and strength studies. RT is the point of activation for a given MU; lower threshold/small MUs activate first then higher threshold/larger MUs active later. RT is expressed relative to maximum voluntary contraction (MVC). MFR is the rate at which a MU fires at a given RT. Smaller MUs fire at a greater rate than larger MUs. MUAP_{AMP} is the average of peak-to-peak (P-P) amplitude of the MU waveform. Smaller MUs have smaller amplitudes and larger MUs have greater amplitudes.¹⁴⁻¹⁷

When examining an isometric muscle action at a specified steady force (%MVC) it is important that the recruitment ranges of MUs are sufficient. For an isometric muscle action at 70%MVC MUs should be recruited from at least 10% of that muscle contraction through 40% of that muscle action. Understanding the potential changes in MFR vs RT and MUAP_{AMP} vs RT relationships can give a better understanding of the adjustments happening at the MU level during dehydration. For instance, hypohydration may change MU firing times, increase excitation to the motor neuron pool to recruit larger threshold MUs, and/or change MU amplitudes of the low or higher threshold MUs. If MUs increased firing rates, recruited larger MUs and/or increased MUAP_{AMP} and there were no changes in performance this would demonstrate the ability of MUs to adjust and be able to maintain strength and performance. If MU behavior declined and MUAP_{AMP} were lower and MUs had lower firing rates this could help understand why performance decreases while hypohydrated.

The purpose of this study was to examine the effects of an acute bout of 5% hypohydration in resistance trained males on (1) neuromuscular function, specifically MUAP_{AMP} vs RT and MFR vs RT relationships, recruitment ranges, and MVC peak torque of the vastus lateralis; and (2) muscle cross sectional area, subcutaneous fat, and echo intensity of the vastus lateralis. Secondly, we sought to examine the effects of rehydrating after hypohydration on neuromuscular function. We hypothesized that in a hypohydrated state MVC peak torque, MUAP_{AMP}, mCSA and subcutaneous fat would significantly decrease while MFR would significantly increase and no significant changes in echo intensity. We also hypothesized that there would be no differences in dependent variables between the euhydrated (baseline) and rehydration state, suggesting neuromuscular function would recover from the effects of 5% hypohydration with appropriate rehydration.

Chapter II

REVIEW OF LITERATURE

Dehydration is the cause of many physiological disturbances and decrements during performance. Dehydration (even as little as 2% body mass loss) is routinely shown to elicit cardiovascular and thermoregulatory system decrements (e.g. increased heart rate and core temperature) and increased feelings of central fatigue (e.g. higher rating of perceived exertion). The role dehydration plays in neuromuscular function remains unclear. Researchers have shown dehydration to cause either decreases or having no effect on isometric and isokinetic strength performance. To further complicate interpretations of if dehydration alters neuromuscular strength, research methodology used to explore this topic is too widely varied for direct comparison (e.g., passive vs exercise induced dehydration vs percentage of body mass loss). Pinpointing the methodology behind the precise mechanism causing the decrements in neuromuscular strength and performance is complex, as many physiological systems work together simultaneously. Additional research examining passive dehydration on neuromuscular function, specifically motor unit (MU) behavior, is necessary. Discerning the incremental differences in mean firing rates, recruitment thresholds and coactivation would give further understanding to how skeletal muscle reacts under dehydration alone, eliminating some of the confounding variables. Understanding this relationship will make it easier to distinguish what impacts muscle force generation during a hypohydrated state.

OPTIMAL HYDRATION

The human body is composed of many complex physiological and neurological systems, which work together to maintain homeostasis and keep the body functioning at a constant rate. Skeletal muscle, central nervous system (CNS), the cardiovascular, thermoregulatory, immune systems

work most efficiently when the body is euhydrated. Euhydration is a “normal” state of body water content with little fluctuation between the intracellular and extracellular fluid levels.¹ Euhydration can be measured through various methods. The most common methods for measuring euhydration consist of urine specific gravity (Usg), plasma osmolality (Posm) and body weight each defined as ≤ 1.020 , < 290 mOsm/L and $< 2\%$ body mass loss, respectively.¹⁸ Appropriate fluid balance allows the body to perform with optimal strength, speed, and force without additional strain on physiological systems.

Hyperhydration consists of excessive water content in the body, when intracellular and extracellular fluid volumes are expanded.¹ Hyperhydration does not alter neuromuscular function, strength or force output, when compared to a euhydrated state. Though hyperhydration does not appear dangerous, it can lead to exercise-associated hyponatremia (EAH). EAH is described as a serum, plasma or blood sodium ($[Na^+]$) concentration below normal referencing range ($[Na^+] < 135$ mmol/L), which can occur during or up to 24 hours after exercise.¹⁹ Fluid overconsumption is the most common cause of EAH. When the fluid and solute ingestion do not match the amount lost, an imbalance between the intracellular fluid (ICF) and extra cellular fluid (ECF) compartments occurs. While EAH may be symptomatic or asymptomatic and varies between individuals, symptoms typically arises around a Na^+ level of ≤ 130 mmol/L. EAH clinical presentation can be minor (e.g., dizziness, nausea) or severe (altered mental status). When EAH is not recognized or treated appropriately, EAH can lead to serious complications such as exercise associated hyponatremic encephalopathy or cerebral edema, and in some cases, mortality.¹⁹

DEHYDRATION

Hypohydration occurs through dehydration, the active process of decreasing total body water content without adequate fluid replacement. Hypohydration is the loss or deficit of body water due

to dehydration beyond normal limits.² Hypohydration can be defined by a $U_{sg} \geq 1.020$, $Posm > 290$ mOsm/L, and/or $> 2\%$ body mass loss. Generally, water is lost through respiration, urine, feces and vomiting.¹ However, in exercising individuals, additional water may be lost through evaporative water loss (e.g., sweating) during and/or after exercise. Improper fluid replacement strategies during and after exercise contribute to the severity of the negative effects experienced during dehydration and increase the chance of beginning the following exercise session in a hypohydrated state. Beginning exercise in a hypohydrated state will result in increased thermoregulatory, cardiovascular, gastrointestinal and skeletal muscle strain, potentially decreasing athletic performance ability. Furthermore, dehydration is a known risk factor the development of exertional heat illness, including exertional heat stroke.¹

Circulatory System

The circulatory system consists of the heart, arteries, veins and blood. The role of the circulatory system is to deliver oxygen and nutrients to working tissues and organs, remove carbon dioxide and waste products, transport gas, hormones, waste, energy, etc., maintain homeostasis (e.g. body temperature, pH), and prevent illness.³ Blood is the main component of the circulatory system and is made up of plasma and formed elements such as erythrocytes, leukocytes and platelets. Formed elements in blood all play a role in the maintenance and regulation of the body. Oxygen is bound to hemoglobin and carried to the tissues by erythrocytes. Leukocytes protect the body from disease. Blood platelets are cell fragments that help blood coagulate. Blood plasma consists of numerous solutes that include but are not limited to proteins, electrolytes, organic nutrients, and respiratory gases. At rest, the majority of blood remains in the brain, heart, skeletal muscle, gastrointestinal, spleen, and kidneys.³

Cardiac output is defined as total volume of blood pumped by the ventricle per minute; it is the product of heart rate multiplied by stroke volume. Stroke volume is the volume of blood pumped per contraction from the ventricles; end- diastolic volume (EDV) subtracted by end- systolic volume (ESV). EDV is the volume of blood in the ventricle before contraction and ESV is the volume of blood in the ventricle after contraction. Stroke volume is influenced by venous return (preload), contractility, and afterload. Preload is the degree to which the chambers of the heart stretch while blood returns to the heart. Contractility is the strength of the contraction. Afterload is the resistance the heart has to overcome to open up the semilunar valves and pump blood into circulation. External stimuli such as exercise, hyperthermia and/ or hydration state can impact the efficiency of cardiac output.³

The body has three main responses to exercise within the circulatory system: vasodilation of the blood vessels of the skin and muscle, vasoconstriction of non-active tissues, and maintenance of blood pressure.²⁰ When exercise occurs the local environment of the working tissues override the parasympathetic and sympathetic nervous systems through metabolic control mechanisms such as chemical changes in the tissue environment to allow vasodilation of the blood vessels. Vasodilation allows greater blood flow to the working muscle. Simultaneously, there is vasoconstriction of non-active tissues (e.g. gastrointestinal tract and CNS) to meet the demands of the working tissues and to maintain blood pressure. At rest, regulation of blood pressure is primarily regulated by the kidneys. During short term regulation, such as exercise, blood pressure is regulated by baroreceptor control. Decreases in baroreceptor stimulation sends messages to higher centers, vasoconstriction occurs of the skin and muscle to preserve blood pressure and as a result cardiovascular function is maintained.²⁰

Stroke Volume

The human body is comprised of roughly 60% water, intracellular compartments make up 40% and extracellular compartments (15% interstitial fluid and 5% plasma) make up 20%.²¹ The body works most efficiently when all compartments are within normal levels. Individuals with varied training states have different stroke volumes. The stroke volume of a highly trained individual is just shy of twice that of an untrained individual at rest, and twice that at max. Individuals who are trained have other physiological properties that also increase, both in relation and because of the increase in stroke volume. Stroke volume increases due to an increase in plasma volume, which results from an increase in blood volume.³ The larger the blood volume the greater the venous return resulting in increased cardiac output. Stroke volume can also increase because the resting heart rate of a trained individual decreases as the level of training increases. As a result of increased stroke volume and training status, relative VO₂max increases as well. However, when dehydration occurs stroke volume starts to decline. The volume of total water content in the body decreases. The decrease in water content is lost within the intracellular and extracellular compartments, which will create physiological strain on the heart. Lowered body water content decreases blood volume, which in turn decreases plasma volume and results in more viscous blood. Consequently, total peripheral resistance increases (TPR) due to the increased viscosity of the blood. The decrease in blood volume counterbalances the increase in blood viscosity; therefore, TPR decreases slightly. As a result of lowered blood volume, EDV decreases as less blood is returning to the heart between beats. Lowered EDV is the primary reason for decreased stroke volume during dehydration.³ Decreased EDV causes lowered stroke volume, which overall decreases cardiac output. While cardiac output is progressively falling, heart rate increases continuously to compensate for declined stroke volume up to ~30%.^{4,5}

Heart Rate

Studies have shown that for every 1% loss of body mass from water loss, heart rate increase 3-5 beats/minute.²² Though this increase in heart rate may seem small incrementally, when an individual reaches 5% body mass lost, heart rate is 15-25 beats/minute higher than a euhydrated state.²² The increase in heart rate can have implications on performance. Exercising while hypohydrated will cause an elevation in heart rate, which will cause fatigue during exercise quicker compared to exercising in a euhydrated state. Exercising at 80% maximal predicted heart rate (MPHR) for a prolonged duration will have the same exertional effect as exercising at 85-90% MPHR because of the elevated heart rate due to decreased stroke volume. Though heart rate increases to compensate for decreased stroke volume, during maximal exercise heart rate cannot exceed maximal heart rate for that individual; therefore, decreased VO₂ and performance occur because demands cannot be sustained.²⁰

Renal Function

Water excretion in the urine is regulated by the kidneys through antidiuretic hormone, commonly referred to as ADH. The decline in blood volume caused by dehydration is sensed by osmoreceptors in the hypothalamus as they detect an increase in plasma osmolality. ADH is secreted from the posterior pituitary gland to stimulate water retention in the kidneys. ADH stimulation decreases sweat rates as well as urine production through the decrease in free water clearance.²³ Urinary sodium excretion decreases partially due to an increase in aldosterone. Juxtaglomerular cells (cells that detect changes in pressure) in the kidneys produce renin, which downstream produce angiotensin II which stimulates the release of aldosterone from the adrenal

cortex. The release of aldosterone is stimulated when body fluid osmolality increases and there is a reduction in total body water content. The release of aldosterone leads to sodium and water retention and the secretion of potassium to help maintain blood pressure.²⁴

During exercise, blood flow to the kidneys is decreased as the blood is distributed to working tissues. The reduction of renal blood flow during exercise has an associated effect of decreasing the glomerular filtration rate which is involved in the process of creating urine and filtering excess fluid and waste products out of the blood to be eliminated from the body. Through decreasing the glomerular filtration rate and the increased production of aldosterone the body can better retain sodium and water.²⁵

Thermoregulation

Humans create and regulate body temperature through heat production, absorption and loss to maintain resting temperatures $\sim 37^{\circ}\text{C}$.²⁶ Regulation of heat production, absorption and loss are maintained primarily through core temperature (T_c) (abdominal, thoracic, and cranial cavities) and shell temperature (T_s) (skin and muscles).²⁷ T_c is regulated by the CNS, while T_s is affected by the external environment (e.g. dry heat, humid).²⁷ Hypothermia and hyperthermia have varying effects on the body when they reach moderate to extreme temperatures and can be detrimental to the body. Hypothermia ($T_c < 35^{\circ}\text{C}$) impairs the CNS, cardiovascular and respiratory systems. Hyperthermia affects the CNS, can cause systemic inflammation, tissue necrosis and organ failure if T_c elevates considerably ($T_c > 42^{\circ}\text{C}$).²⁸

During exercise metabolic heat production increases 10 to 20 fold, depending on the duration and intensity of exercise,^{29,30} though only 30% of that increase is used as mechanical energy. The remaining increase in metabolic heat production needs to be transferred to the skin to be released to the environment.³¹ Offloading of body temperature to the environment happens through many

process of which are bi-directional, such as conduction, convection and radiation. The process of evaporation accounts for the majority of heat dissipated from the body during exercise. However, evaporation of heat from the skin to the environment is driven by a temperature gradient. Therefore, humidity can inhibit evaporation loss by ~18% compared to a dry hot environment.³² Exercising in a hot and humid environment can lead to increased heat storage and increased Tc. An individual exercising in this environment is at risk for increasing Tc with the lack of heat dissipation from the skin. If the individual is dehydrated while exercising in a hot and humid environment it accelerates the risks and physiological strain placed upon the body.

Comparable to the strain placed on the cardiovascular system, dehydration also compromises the thermoregulatory system. Decreased plasma volume from body water loss hinders sweat rates, which makes it challenging for the body to maintain normal temperature and allows Tc to rise. Decreased sweat rates in a humid environment will intensify the difficulty to offload the heat the body produces. The combination of heat stress and dehydration contributes to a greater increase in Tc.⁹ With every 1% loss in body mass, Tc 0.22C.²² The effects become more prominent with higher ambient temperatures³³ and higher humidity. As exercise duration progresses, body Tc continues to rise; depending upon the duration and intensity, this increase in the body temperature could put an individual at risk for exertional heat illness.³³

Skeletal Muscle

During exercise blood flow is redistributed to different parts of the body for thermoregulatory purposes (e.g. vasodilation of blood vessels in the cutaneous tissue to release heat). The redistribution of blood does not affect blood flow to skeletal muscle during exercise in a euhydrated state.³⁴ However, when the body begins to dehydrate water content from the skin and muscle decreases as extracellular spaces experience fluid loss³⁰ and severe dehydration causes

intracellular fluid stores to reduce as well. Exercising in the heat to elicit a 5% body mass loss was accompanied by an 8% decrease Neuffer et al.³⁵ and a 5% decrease Hackney et al.³⁶ in muscle water content. Equally, Costill et al.³⁷ saw a 7% decrease in muscle water when body weight decreased by 5.8%. Similarly to decreased muscle water content, muscle blood flow also decreases when the degree of dehydration impairs the cardiovascular function. Gonzalez-Alonso, Calbet and Nielsen³⁸ demonstrated that when cardiac output was reduced ~3-4 L/min, blood flow to periphery was significantly reduced, ~2 L/min in the legs, forearm blood flow ~40% lower, and decreased skin blood flow. As dehydration levels continue to increase, blood flow reaching working muscle decreases. This decrease is supplemented by decreased oxygen delivery and oxygen extraction, typically at the end of prolonged moderate exercise in the heat.³⁸

Skeletal muscle is also a source of reactive oxygen species (ROS) during exercise.^{39,40} Exercise has proven to be beneficial in the prevention of oxidative stress. However, if the body is in a compromised state (e.g. dehydrated, hyperthermic, or a compromised immune system) or the exercise intensity or duration is too extreme for an individual, ROS may become more harmful than good.⁴¹ The result of increased reactive oxygen species may not only be damaging to the muscle but may trigger a downstream effect of which would result in an upregulation of pro-inflammatory cytokine production, such as interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF- α).^{40,42}

Performance Limitations

The physiological strain that is placed upon the body induced by dehydration has significant impacts on performance. Impairments of multiple bodily mechanisms include decreased cardiovascular output,⁵ increased metabolic heat production, reduced muscle blood flow that hinders oxygen delivery, impaired thermoregulatory function, fluctuations in sodium and

potassium which may affect neuromuscular function,³⁰ and altered cognition and mood.⁴³ The extent of these impairments to bodily systems is dependent upon the degree of dehydration that occurs, the mode of exercise, and the environment. It can be hard to distinguish the isolated implications of heat and dehydration since they coincide during prolonged exercise.³⁰ Studies have looked at the effect dehydration has on different types of endurance and resistance training performance. Results from various studies have shown both a significant and non-significant difference in performance during endurance performance while hypohydrated. Exercising in a hypohydrated state can limit Vo₂max and mean atrial pressure, this limitation results from a decreased cardiac output and the inability to offload blood to skeletal muscle which hinders oxygen extraction and delivery.⁴⁴

A variety of strength measures have also been researched to see if dehydration impacts strength performance. Judelson et al.⁶ evaluated the difference between 2.5% and 5% body mass loss on strength exercise performance. No significant differences were found between groups for vertical jump height, peak lower-body power, or peak lower-body force. However, when looking at function of total percentage work completed for a back squat protocol, hypohydration significantly decreased muscular endurance performance. Barely et al.⁴⁵ found significant decreases at 5% body mass loss in performance for repeated sled pushes, medicine ball chest pass and handgrip strength, but no significant difference in vertical jump height. Researchers suggest that the findings supporting decreased strength based exercises are less common than endurance exercise because cardiac output is not influencing performance during strength based exercise as it does during endurance exercise. Strength based exercises rely mostly on anaerobic work. Therefore, decreased cardiac output is not influencing anaerobic pathways.¹¹

Individuals or athletes who have multiple sporting events or competitions in 24 hours can be hindered from rehydration from the first event/performance. Although rehydration is vital for returning the body back to a euhydrated state, studies have reported decreases in performance after *ad libitum* rehydration of active and passive dehydration anywhere from 5 to 24 hours post exercise.⁴⁵ Decrements in performance are shown for sport-specific skills^{46,47} repeat- effect capacity,⁴⁵ mood²⁶ and cognitive function.⁴³ These findings pertain to athletes in weight specific sports, who rapidly lose and regain weight in short periods of time,⁴⁵ and also applies to athletes who have multiple competitions in one day. Athletes who engage in sports such as wrestling, boxing and various other combat sports have reported a loss of ~3-5% body mass before competition.⁷ This quick loss in body weight is mostly attributed to rapid water loss leading to a dehydrated state and can effect performance for greater than 24 hours, even with replenishment of water content back to baseline body mass.⁷ For this reason, it is not suggested that athletes rapidly dehydrated themselves to make weight for competitions because their performance can be altered even after rehydration occurs.

CYTOKINES

Cytokines play an integral part of the maintenance and protection of the human body⁴⁸ through innate and adaptive immunity.⁴⁹ Cytokines consist of polypeptides and soluble proteins such as: interferons, interleukins, growth and colony-stimulating factors, chemokines, members of the tumor necrosis factor group and transforming growth factors.^{48,49} Cytokines act as intercellular messengers and mediators in various health and diseases including glucose and lipid metabolism, insulin sensitivity, fatigue, temperature regulation, skeletal muscle hypertrophy and atrophy, etc. Though cytokines play a role in many functions of the human body, their main function is to regulate immune function and homeostatic control of various tissues and organs.^{48,50} Homeostatic

control of tissues and organs is maintained through cell proliferation, differentiation, migration, and survival and apoptosis. When homeostasis is disrupted (e.g. by external stimuli such as heat stress) cytokines help mediate the harmful effects through a variety of mechanisms.

Exercise and Cytokines

Research that originally centered on exercise and cytokines focused on the role cytokines play in mediating inflammatory responses due to exercise induced muscle damage. Since then, evidence has shown that cytokines play a larger role during and after exercise.⁴⁸ Cytokine responses during exercise are dependent upon many variables including the intensity, mode and duration of exercise.⁴⁸ During exercise, cytokines act in a hormone-like behavior, mediating metabolism in working skeletal muscle, liver and adipose tissue, angiogenesis and neurobiology. The local response of skeletal muscle during exercise involves inflammation. Characteristics of inflammation include heat, swelling, redness and pain. As the inflammatory response happens, vasodilation results in higher blood volume in the area of the damage.⁴⁹ The magnitude of the increase in cytokine plasma concentrations is variable and related to exercise intensity⁵¹ and the extent of exercise induced muscle damage.⁵²

Following exercise, the body enters the acute phase of the inflammation response. Increased concentrations of cytokines are released into circulation.⁴⁹ Cytokines released during or after exercise are commonly referred to as pro-inflammatory cytokines, meaning they cause inflammation to occur. The most common pro-inflammatory markers are TNF- α , C-reactive proteins (CRP), and IL-1.⁴⁹ IL-6 dramatically increases after exercise; however, IL-6 is considered a pleiotropic cytokine, meaning that it can act as a pro or anti-inflammatory cytokine⁵³ Biological inhibitors and anti-inflammatory cytokines, TNF- α , IL-1 β , IL-6, and IL-2 receptors, IL-4, and IL-

10 are released into the blood at the same time as the pro-inflammatory cytokines. Findings suggest that anti-inflammatory cytokines are released at the same time as pro-inflammatory cytokines to restrict the magnitude and duration of the inflammatory response to exercise.⁴⁹

Cytokines and thermoregulation

When the body enters hyperthermic conditions it activates responses to lessen the adverse effects hyperthermia can have on the body if not managed properly. Thermoreceptors detect the gradual increase in body heat and respond accordingly. Heat shock factor-1 (HSF-1) and activator protein-1 (AP-1) are required for expression of IL-6 in skeletal muscle.⁴² When temperature is elevated, HSF-1 and AP-1 are activated, which elicits the release of IL-6.⁴⁰ IL-6 may play a vital role in hyperthermic conditions to manage heat stress and prevent destruction of skeletal muscle.^{40,54}

NEUROMUSCULAR FUNCTION

Motor Units

The CNS is the origin of control over human body movement. Motor control performed within a single muscle is regulated by the CNS through the number of active motor units (MU) and their firing rates.^{55,56} A MU consists of an alpha spinal motor neuron and the set of muscle fibers it innervates.⁵⁷ Motor control can be directly reflected through the number of motor unit action potentials (MUAPs) per second or by the sum of firing rates of all active motor units MUAP rate (MR).⁵⁶ The electrical activity from muscles, specifically MUs, can be measured non-invasively through surface electromyography (EMG). EMG is measured through electrodes placed on the surface the skin above a muscle.⁵⁵

Adrian and Bronk⁵⁸ and Seyffarth⁵⁹ demonstrated that as the level of contraction increases, the number of MUs recruited increase and the firing rates of those MUs increase. Work completed by

Henneman¹⁴ demonstrated that in response to increasing excitation, motor neurons are recruited in order of size, from smaller MUs to larger MUs, commonly referred to as the size principle. Once determining the way MUs are recruited, MUs firing rate behavior follow two general properties. The first property, shown through the work of DeLuca and Hostage¹⁷ is that across any given force level there is an inverse relationship between the firing rate and recruitment threshold of MUs. Within a muscle, MUs recruited at a lower threshold maintain higher average firing rates during a contraction at a target force level. Inversely, MUs recruited at a higher threshold maintained lower average firing rates at a targeted force level during a contraction. This property is known as the “onion-skin” scheme¹⁵ However, there are few muscles (e.g. first dorsal interosseous) with slight variations in firing rates and recruitment thresholds compared to the general recruitment and firing rate pattern.¹⁷

The second property pertaining to MUs firing rates is a property called “common drive”. This property demonstrates that changes in MU firing rates of all MUs is associated to changes in the excitation to the motoneuron pool.¹⁶ Therefore, increased excitability to the motoneuron pool will recruit larger MUs and increase the firing rates of the active MUs.¹⁶ Through this mechanism MUs are able to withstand a given force at the onset of fatigue.

Motor Unit Action Potential

The changes generated by a MU are represented by a MU action potential (MUAP). The waveform of a MUAP can be dissected into duration, amplitude, phase, turn and baseline.⁵⁷ Each of these are categorized into three different physiological parameters: size, shape and stability. Size parameters relate to the number and diameter of muscle fibers (MF) and MUs. The size of MUs and MF influence the amplitude and duration. Amplitude of a MUAP goes from the lowest to the highest peak. Amplitude can be influenced by where the electrode is placed on the muscle.

Duration includes the start of activation of MU fibers until the end of the repolarization phase. Shape parameters include the phases, turns and baseline measurements of waveforms. A phase falls between two baseline crossings. A turn is a point of directional change, a normal waveform usually has 2 to 4 turns. Baseline is the normal resting membrane potential, $\sim -70\text{mV}$.⁵⁷ Stability parameters, also referred to as jiggle parameters, are used to quantify the degree of variability in MUAP shape at consecutive discharges.⁵⁷ Anatomically there are variables that can effect different characteristics of a MUAP waveform. For example, muscle atrophy elicits decreased amplitude and decreased duration. Muscle hypertrophy increases amplitude and abnormal neuromuscular transmission causes an increase in jiggle or noise.⁵⁷

Coactivation

Coactivation occurs when an agonist and antagonist muscle simultaneously contract to improve joint stability and movement accuracy.⁶⁰ Research^{16,61} has solidified the two general properties of motor units the “onion-skin” scheme and the “common drive.” These general properties of MUs still hold true as the muscle fatigues. During fatiguing contractions in the vastus lateralis (VL) Contessa et al.⁶¹ examined the role of coactivation of the first dorsal interosseous (FDI), a muscle that typically exhibits different MU firing rate properties than the VL. Results showed motor unit firing rates of the FDI decreased as fatigue increased and there was increased coactivation of the forearm muscles. This evidence shows that as fatigue sets in while exercising, the excitation of the motor neuron pool usually increases to be able to sustain the force needed for contraction. However, when muscles such as the FDI have different MU firing properties, or fatigue is too great, the muscle has to compensate and find other ways to generate force.⁴⁸

Dehydrations effects on neuromuscular function

Recent studies examining the effects of dehydration on neuromuscular function (e.g. isometric strength, isokinetic strength, muscular strength endurance) are yielding inconsistent results. Research teams have used EMG and torque measurements to analyze and quantify neuromuscular strength^{8,9,12,13,55,62,45} and muscular strength endurance.^{9,12,55,62,44}

Isometric strength

Isometric strength is tested at a fixed, or static, angle or muscle length during a contraction. The fixed, or static angle depends on the research team and can vary from study to study. Research teams have evaluated the knee extensors at different angles ranging from 30 to 90 degrees, 0 degrees being in full extension. Biceps brachii are commonly measured at 90 degrees, 180 degrees being full extension. Examining strength performance at a body mass passive loss of 2%, Evetovich et al.⁸ found no significant difference in isometric strength in the biceps brachii. Similarly, with comparable methods of passive dehydration Bigard et al.⁶³ reported no significant difference in isometric maximal voluntary contraction output of the knee extensors, specifically examining the vastus lateralis (VL). Conversely, with an equal passive percent body mass loss of ~2%, Minshull and James¹⁰ yielded a significant decrease in knee extensors volitional static peak force of 7.5%. Rodrigues et al.¹³ and Ftaiti et al.⁹ used methods of exercise (cycle ergometer and running) to elicit 2% in their participants. Rodrigues et al.¹³ compared exercised (knee extensors) to non-exercised (biceps brachii) muscles and concluded a 16% significant decrease in isometric torque of the exercised muscles compared to no significant decreases in the non-exercised muscles. Ftaiti et al.⁹ yielded significant decreases in the knee extensors of 12% of mean peak torque.

Isokinetic strength

Isokinetic strength is performed throughout a dynamic range of motion and allows the muscle to gain strength throughout the movement. Upon testing of isokinetic strength, a predetermined velocity is determined by researchers, ranging from 30°s^{-1} to 240°s^{-1} . At a contraction velocity of 90s^{-1} with 2% passive dehydration Evetovich et al.⁸ found no difference in isokinetic torque of the biceps brachii. However, with exercised induced dehydration Ftaiti et al.⁹ found a 17% significant decrease in knee extensor torque at 60°s^{-1} but no significant differences of torque at 240°s^{-1} . The velocity at which the contractions are performed could influence whether or not decreases in torque are evident due to dehydration. Various velocities (e.g. 240°s^{-1}) may be too rapid to see difference in fluctuating degrees of dehydration.

Muscular strength endurance

Muscular strength endurance is the ability to perform a specific muscle action for a prolonged period of time. This type of strength test is used to examine repeat muscle actions and the fatigability capabilities of the muscle during a specific time frame or amount of repetitions. Muscular endurance can be more aerobically or anaerobically based, dependent upon the test of muscular endurance and the goal of the research team. A study conducted by Barley et al.⁴⁵ observed no significant difference in maximal isometric or isokinetic strength at any time point between a control group and a dehydration group at a loss of $3.2 \pm 1.1\%$ body mass. Though they did observe a decrease in muscular strength endurance. Individuals in the dehydration group performed 28% less unilateral knee extensions at 85% maximum voluntary isometric contraction (MVIC) 3 hours after the dehydration protocol.⁴⁵ Similarly, Ftaiti et al.⁹ measured significant decreases in mean work produced in the both extension and flexion of the knee extensors in comparing the dehydrated to the control visit. The decreases in muscular endurance may be a

product of central fatigue or motivation due perception of fatigue significantly increased throughout the study as individuals were dehydrated.⁶⁴

Results across studies may vary due to the inconsistencies in methods and testing modalities. Inconsistencies include different percent body mass lost, methods to achieve hypohydration, modes of exercise, exercise in different heated and humid environments, or passive dehydration, and a variety of angles and velocities during isometric and isokinetic strength testing. Therefore, the results from each study cannot be compared or generalized since there is not conformity across studies.

Hayes and Morse¹² saw the inconsistency in methods across studies using varying levels of dehydration to measure decreases in neuromuscular performance. This study was one of the first to examine if a dose response relationship exists between the level of hypohydration on maximal strength and activation capacity. Overall dehydration of ~4% body mass loss was elicited through 5 bouts of 20 minutes jogging in a heated environment. After each exercise bout, measures were taken (e.g. body mass and isometric and isokinetic strength tests measured with EMG). The research team yielded results that showed isometric MVC torque was significantly different after bout 1 (body mass decreased $1.0 \pm 0.5\%$) and maximal isokinetic strength at 30°s^{-1} significantly decreased after bout 3 (body mass decreased $2.6 \pm 0.8\%$). There was a decrease in EMG amplitude, yet this change was not significant and dehydration progressed. Hayes and Morse¹² concluded that there is not a dose response relationship between dehydration and neuromuscular performance because of the insignificant decrease in EMG amplitudes despite the significant decreases in maximal isometric torque and maximal isokinetic torque at 30°s^{-1} . However, EMG amplitudes were not decomposed to examine the firing rates of smaller MUs compared to firing rates of larger MUs. As fatigue occurred as higher levels of dehydration progressed firing rates of smaller MUs

to examine the effect of dehydration on MU behavior. Studies have been completed to test isometric and isokinetic strength from passive and exercise induced dehydration, but these studies have not been uniform in their methodologies (e.g. passive dehydration vs. exercise induced dehydration, 2% drop in body mass vs. 5% drop in body mass). These inconsistencies in methodology are the cause of varied results on the impact dehydration has on neuromuscular isometric and isokinetic strength. To have a complete understanding where the physiological change is occurring in the muscle during a hypohydrated state, a more inclusive study needs to examine the changes of motor unit behavior caused by passive dehydration. More specifically, research needs to examine the changes that occur in $MUAP_{AMP}$, firing rates and recruitment thresholds of motor units along with coactivation of the antagonist muscles as the body becomes hypohydrated.

Chapter III

METHODS

Research Design

The study utilized a cross-over design to determine the effects of 5% dehydration and rehydration to baseline on the following dependent variables: isometric strength (MVC), mean firing rates (MFR), motor unit action potential amplitudes (MUAP_{AMP}), recruitment threshold (RT), cross sectional area (mCSA), subcutaneous fat, and echo intensity in the vastus lateralis. The independent variable was hydration status, with participants at 5% body mass loss and then rehydrated to baseline body mass. All participants completed 3 experimental visits: 1) baseline, 2) dehydration, and 3) rehydration over 3 consecutive days.

Participants

Eleven participants consented to participate in this study; 2 participants dropped because of scheduling conflicts. Participants were recruited from the University of Kansas and surrounding communities. Individuals were recruited by word of mouth and posted flyers. Participants were not financially compensated for completion of the study. All participants were screened using a health history questionnaire to determine any exclusion criteria. To be included, participants had to be moderately resistance trained (strength training 3 times a week for at least 1 year) and be free of any cardiovascular, pulmonary, neurological, or other chronic diseases and have no current (within 6 months) musculoskeletal injuries to the ankle, knee, or hip joints. Participants were instructed to abstain from consuming alcohol, caffeine, supplements or NSAIDs, not to exercise 48 hours prior to the baseline visit, and to refrain from these activities throughout the study. The study was approved by the University of Kansas Institutional Review Board.

Procedures

Familiarization Session

The familiarization occurred at least 4 days prior to the baseline visit to ensure there were no lingering effects from being familiarized to the isometric strength testing. Participants were encouraged to report to the familiarization visit in a euhydrated state by consuming ~1 liter of fluid the night before and 1 liter of fluid the morning of the visit.⁶ Body mass was measured and a urine sample was taken to characterize hydration status via urine specific gravity. Participants practiced each of the maximal and submaximal isometric contractions that would be performed during the experimental trials. Participants were also exposed to the environmental room and weighing area.

Baseline (Euhydrated) Visit 1

To promote euhydration for the baseline visit, participants were instructed to drink 1 liter of fluid the night before and morning of the baseline visit. Immediate hydration status was measured using urine specific gravity. If urine indicated the person was euhydrated, body mass was measured and a blood sample was collected. Participants then laid on a flat table in a supine position and ultrasound images of vastus lateralis were taken. Once images were collected, participants then completed isometric strength testing on the Biodex System 3 isokinetic dynamometer. Following strength testing, body mass was recorded and participants were given their target 5% body mass loss for the dehydration visit the next day.

Dehydration (Hypohydrated) Visit 2

Starting immediately after the baseline visit, participants were provided written and verbal instructions to limit fluids and fluid-rich food over the next 24 hours.⁶ They were encouraged to completely eliminate all intake of fluids and food 12 hours prior to arriving to the laboratory. Once

participants arrived to the laboratory, body mass was recorded to see their current hydration status. To assist in achieving the remainder of the passive water loss, participants sat in an environmental room (67-71°C, 8-12% relative humidity) to promote sweating. Body mass was recorded every 30 minutes until the participant reached their target 5% body mass loss. Heart rate was also monitored every 30 minutes to ensure participants remained within safe limits. If heart rate rose above 80% of participant's maximal predicted heart rate and was accompanied by other symptoms such as extreme light headedness or inability to sweat, they were removed from the environmental room and returned to the thermoneutral environment to complete the rest of testing for visit 2.

Once at 5% hypohydration, participants entered a thermoneutral laboratory (~22°C, ~25 relative humidity). Urine and blood samples were collected, and participants then laid supine on a flat table and images of their vastus lateralis taken. Following collection of ultrasound images, isometric strength testing was completed. Prior to leaving and due to the severe hypohydration, participants were required to complete an exit survey to assess any symptoms that could have arisen throughout the visit and to ensure they were capable and safe to leave the laboratory on their own. If participants answered "yes" to any of the survey questions they were required to remain in the lab for 15 more minutes and retake the survey until all symptoms resided and they answered "no" for all survey questions. After participants completed the survey, participants were provided with specific verbal and written instructions to consume fluids in the amount of 150% body mass loss over the next 24 hours in order to return to a euhydrated, baseline body mass.

Rehydration Visit 3

Upon arrival to the laboratory, body mass and a urine sample were measured to ensure the participants achieved their target rehydration body mass and euhydrated urine specific gravity. Participants then provided a blood sample before laying on the ultrasound table to have images

taken of their vastus lateralis. Once images were collected, participants completed the isometric testing. Following testing, body mass was recorded. In the event the individual reported to the laboratory and had not reached baseline body mass, they were provided water in the amount of 3.5 ml/kg (based on euhydrated weight) to consume every 15 minutes. Body mass was measured every 15 minutes and fluid consumption ended once the baseline weight was reached.

Instruments and Measures

Hydration Assessment

Urine, blood, and body mass (weight) were used to determine the participant's hydration status. Urine specific gravity was used as an immediate estimate of hydration status. Participants were instructed void into a urine container at upon arrival to the laboratory for visit 1 and 3 and at 5% hypohydration for visit 2. Urine was analyzed using a clinical refractometer (model REF 312, Atago Company Ltd., Tokyo, Japan). Euhydration was defined as a urine specific gravity \leq 1.025.^{66,18} Blood was collected 3 times: 1) baseline (euhydration), 2) pre-strength testing at 5% hypohydration, and 3) pre-strength testing at rehydrated body mass. Blood was collected using a single needle stick into the cubital vein. At each time point, blood was collected into 1 10 ml and 1 6 ml EDTA vacutainer tubes. EDTA tubes were inverted several times to mix. The 10 ml tubes were centrifuged (Centrifuge 5804R, 15 amp, Hamburg, Germany) at 3000 rpm for 15 min, plasma was pipetted into micro tubes, and stored at -20°C until future inflammatory cytokine analysis. The 6 ml tubes were used to calculate plasma shifts via hematocrit readings. Two micro-hematocrit capillary tubes (Fisher Scientific, Pittsburgh, PA) were filled and spun for 5 minutes in an IEC MB centrifuge (Damon/IEC Division, Needham Hts, MA). After centrifuging, ratios were measured using digital hematocrit reader (CritSpin Reader S120-22, Chatsworth, CA) and averaged together. Hemoglobin was also measured from the 6 ml tubes using a microcuvette (HemoCue AB, Lot

1703045, Angelholm, Sweden) measured in a HemoCue Hb201⁺ analyzer (HemoCue AB, Angelholm, Sweden). Plasma volumes were calculated according to methods described in Dill and Costill.⁶⁶

Body mass was measured using a digital scale (Tanita SC 331S, Tokyo, Japan). A euhydrated body mass from baseline visit was used to calculate the target 5% body mass loss for the dehydration visit. After arrival to the laboratory for the dehydration visit, body mass was assessed and the participant was required to sit in a hot, humid environment until the target body mass was reached. During this time, body mass was measured every 30 minutes. Participants were provided a private area where the scale was set up. The individual was instructed to remove all clothing and towel off all sweat in order to provide an accurate nude weight. After weighing, participants put clothing back on and returned to the environmental room to continue the dehydration protocol or entered the laboratory to continue with testing if the desired body mass loss was reached.

Cardiovascular Function

Heart rate was measured by palpating the radial pulse for 15 seconds and multiplying the number by four. Heart rate was measured upon arrival to the lab, every 30 minutes during neuromuscular strength testing in the thermoneutral environment, and before exiting the lab. Heart rate was also measured every 30 minutes while participants were in the environmental area to ensure they remained within safe limits.

Neuromuscular Function

Muscle Cross Sectional Area (mCSA). Ultrasound was used to measure cross-sectional area, echo intensity, and subcutaneous fat using an imaging device with a multi-frequency linear- array probe

(12 L-RS; 5–13 MHz; 38.4-mm field of view)⁶⁷ in conjunction with GE logiq e Logic View software to generate real-time images. The panoramic function was utilized to attain mCSA images of the vastus lateralis. Participants were instructed to lay on a flat table in supine position for 10 minutes to allow fluid shifts to occur. Fifty percent of the distance from the anterior superior iliac spine to the superior lateral border of the patella was marked for measurement. Ultrasound settings included gain (68 dB), frequency (10 MHz), and depth (8.0 cm) to optimize image quality and were constant across all subjects. Ultrasound images were assessed using ImageJ software (National Institutes of Health, Bethesda, MD). For cross-sectional area measurements the muscles were outlined and measured using the polygon function. Images were taken 3 times: baseline (visit 1), 5% hypohydrated (visit 2), and post-rehydration (visit 3).

Biodex System 3 Isokinetic Dynamometer. The Biodex System 3 isokinetic dynamometer (Biodex Medical Systems, Shirley, NY) was used for testing isometric strength of the leg extensors. The restraining straps were placed over the pelvis, trunk, and the contralateral thigh. The lateral condyle of the femur was aligned with the input axis of the Biodex system in accordance with the User's Guide (Biodex Pro Manual, Applications/Operations, 1998). All isometric knee extensor assessments were performed on the right leg at a flexion of 90°. Isometric strength for the right leg extensor muscles were measured using the torque signal from the Biodex System 3 isokinetic dynamometer.

Isometric Strength Testing. During the baseline (euhydrated) visit, participants performed two extension isometric maximal voluntary contractions (MVCs), with strong verbal encouragement for motivation followed by a submaximal isometric trapezoid muscle action at 70% extension MVC. The highest torque output from the extension MVCs was used to determine the level of the submaximal isometric trapezoid muscle actions for each subject. Trapezoid templates increased

torque at 10% MVC per second until the desired steady torque level was achieved, and remained on a plateau for 12 seconds, then decreased back to baseline at a 10% MVC per second descent. Three minutes of rest were given between each muscle action, and participants were asked to maintain torque output as close as possible to the target torque digitally presented in real time on a computer monitor. If unable to maintain targeted torque, participants were given a second attempt at the template tracing.

Electromyography (EMG) Recording. During the trapezoid muscle actions, surface EMG signals were recorded from the vastus lateralis using a 5-pin surface array sensor (Delsys, Boston, MA). The pins have a diameter of 0.5 mm and are positioned at the corners of a 5 x 5 mm square, with the fifth pin in the center. Prior to sensor placement, the surface of the skin was thoroughly prepared by shaving, removing superficial dead skin with adhesive tape (3M, St Paul, MN), and sterilizing with alcohol. One surface array sensor was placed over the muscle belly of the vastus lateralis at 50% of the distance between anterior superior iliac spine and the lateral condyle of the femur. A 2 x 2 reference electrode (TENSpros, St. Louis, Missouri) was placed over the left patella. The signals from the sensor electrodes were differentially amplified and filtered with a bandwidth of 20 Hz to 9.5 kHz. The signals were sampled at 20 kHz and stored on a computer for off-line analysis.

EMG Decomposition. Processing of EMG signals followed De Luca et al.⁶⁸ and Nawab et al.⁶⁹ Action potentials were extracted into individual firing events of single MUs from the 4 separate EMG signals via the Precision Decomposition (PD) III algorithm. This algorithm is designed for decomposing EMG signals into MUAP trains. Only MUs with > 90% accuracies were used for further analysis. For each MU, RT and MFR during steady force were recorded. MUAP_{AMP} were calculated as described by Pope et al.⁷⁰ The average of the peak-to-peak (P-P) amplitude (mV)

from each of the 4 action potential waveforms was calculated using a custom-written software program (LabVIEW 2015, National Instruments, Austin, TX, USA). Linear regressions were calculated for the MFRs (pps) and MUAP_{AMP} (mV) vs. RT (expressed relative to MVC [%MVC]) relationships for each subject. The y-intercepts and slopes from each subject were used for statistical analysis.

Spike Trigger Average. A secondary validation of the accuracy of the MU firing times and action potential waveforms generated by the Precision Decomposition III algorithm was performed via spike-trigger average protocol. The Precision Decomposition III algorithm derives firing times used as triggers for averaging the 4 raw EMG signals recorded from the surface sensor array.^{71,72} In addition to 90% accuracy in the reconstruct-and-test procedure, the following criteria was required for a MUs inclusion in the subsequent analyses: 1) a high correlation ($r > 0.70$) between the Precision Decomposition III algorithm and spike trigger average derived action potential waveforms and 2) a low coefficient of variation (< 0.30) of the spike trigger average derived action potential P-P amplitude across the contraction time.⁷¹

Signal Processing. Channel 1 was selected from the 5 bipolar EMG channels from the 5-pin surface array sensors to be used for amplitude analyses. The force (N) and the EMG (mV) signals from channel 1 were recorded with a NI cDAQ (National Instruments, Austin, TX USA) during each isometric muscle action. Data was stored on a personal computer (Dell Optiplex 9010; Dell, Inc., Round Rock, TX) for subsequent analysis to ensure protection and prevent data loss. EMG amplitude was expressed as root mean square amplitude values calculated by custom written software (LabVIEW v 15.0; National Instruments, Austin, TX). The sampling frequency for force and EMG signals was 2,000 Hz for the MVCs and 20,000 Hz for submaximal contractions. The EMG signals were bandpassed filtered (zero phase fourth-order Butterworth filter) at 10–500 Hz,

while the force signal is low-pass filtered with a 10-Hz cutoff (zero-phase fourth order Butterworth filter). All EMG calculations were performed on the filtered signals. Peak EMG root mean square is the amplitude that was recorded during the highest 0.25 sec average of force (N) during the 2 MVCs. EMG root mean square for the 70% MVCs is determined by averaging the same 1 second epoch, which is manually selected at the onset of the target steady force to measure MFR. The 70% EMG root mean square values is normalized as a percentage of the peak for further analysis.

Statistical Analysis

Regression analysis for the 70% MVC isometric muscle action was applied against RT to yield slopes and y-intercepts for MUAP_{AMP} vs RT and MFR vs RT. One (variable) x 3 (day) repeated measure ANOVAs were utilized to analyze the neuromuscular data (relative MVC peak torque, MUAP_{AMP} vs RT slope and y-intercept, MFR vs RT slope and y-intercept, and minimum and maximum recruitment ranges). One (variable) x 3 (visit) repeated measure ANOVAs determined differences between mCSA, subcutaneous fat, echo intensity, heart rate, plasma volume, urine specific gravity, and body weight. For significant main effects, post-hoc analyses were conducted using pairwise comparisons and Bonferroni corrections were applied to all analyses excluding MVC peak torque and plasma volume. Greenhouse-Geisser corrections were applied when analyzing urine specific gravity and pre-strength testing heart rate. A priori power analysis indicated a sample size of 15 participants were needed. Data is represented as mean \pm standard deviation. The alpha level was set to $p \leq 0.05$ to determine statistical significance. SPSS statistical software (Version 26; IBM) was used for all statistical comparisons.

Chapter IV

RESULTS

Demographics

Nine recreationally trained males (age = 26.9 ± 3.7 yr, weight = 95.5 ± 17.0 kg, height = 180.6 ± 3.8 cm) completed all aspects of this study. One participant showed up to visit 2 already at their 5% body mass goal, all other participants had to go into the environmental area to attain their desired body mass goal. Participants were in the environmental area for an average duration of 151.8 ± 32.5 minutes. Three participants did not fully reach their target body weight. One was taken out early for personal time restrictions and two were taken out for increased heart rate over 80% maximum predicted heart rate paired with heat illness symptoms. Overall, participants' percent dehydration = 4.9 ± 0.5 . Blood was unable to be drawn from one participant on visit 2 or 3 due to collapsing veins.

Neuromuscular Function

Relative MVC peak torque was affected by hypohydration ($F(2,16) = 3.739$, $p = 0.047$). Relative MVC peak torque was significantly greater on visit 1 (263.6 ± 59.6 Nm) compared to visit 2 (241.5 ± 59.1 Nm, $p = 0.023$). Seven of 9 participants' MVC peak torque decreased when hypohydrated compared to being euhydrated, and 1 subject's peak torque increased slightly. There were no significant differences in MVC peak torque between visit 1 (263.6 ± 59.6 Nm) and visit 3 (254.9 ± 59.8 Nm, $p = 0.385$) or between visit 2 (241.5 ± 59.1 Nm) and visit 3 (254.9 ± 59.8 Nm, $p = 0.90$) (Figure 1). Similarly, there were no significant differences for MUAP_{AMP} vs RT slope ($p = 0.982$) or y-intercept ($p = 0.898$), MFR vs RT slope ($p = 0.674$) or y-intercept ($p = 0.617$) or minimum ($p = 0.159$) or maximum ($p = 0.545$) recruitment ranges.

Muscle cross sectional area (mCSA) significantly changed due to hypohydration ($F(2,16) = 20.455$, $p < 0.001$), significantly smaller on visit 2 (31.4 ± 7.4 cm²) compared to visit 1 ($34.0 \pm$

7.2 cm², $p = 0.003$) and visit 3 (35.2 ± 7.6 cm², $p = 0.003$) (Figure 4). There were no significant differences between mCSA between visit 1 and visit 3 ($p = 0.153$). No significant changes occurred in subcutaneous fat ($p = 0.08$) or echo intensity ($p = 0.42$) across visits.

Hydration

Urine specific gravity, body mass, and plasma volume results are presented in Table 1. Significant changes occurred over days for Usg ($F(1,2,9.759) = 26.488$, $p < 0.001$) and body mass loss ($F(2,16) = 122.0$, $p < 0.001$), indicating participants were hypohydrated at visit 2 and rehydrated to baseline by visit 3 (Table 1). Plasma volume also significantly changed ($F(2,14) = 5.658$, $p = 0.016$), showing participants were rehydrated by visit 3 (Table 1).

Heart rate

Heart rate (HR) immediately after becoming hypohydrated = 128.9 ± 20 bpm. HR was significantly affected by hypohydration pre-strength testing ($F(1.192,9.532) = 19.581$, $p < 0.001$) and post-strength testing ($F(2,16) = 5.899$, $p = 0.012$). Pre-strength testing HR was significantly higher visit 2 (92 ± 11 bpm) compared to visit 1 (66 ± 6 bpm, $p < 0.001$) and visit 3 (71 ± 12 bpm, $p = 0.024$). HR was also significantly higher post-strength testing visit 2 than visit 1 (94 ± 15 bpm and 76 ± 10 bpm, $p = 0.023$). There was no significant difference in HR visit 1 to visit 3 pre- ($p = 0.597$) or post- ($p = 1.00$) strength testing, or post-strength testing visit 2 to visit 3 ($p = 0.083$).

Chapter V

DISCUSSION

The purpose of this study was to examine the effects of an acute bout of 5% hypohydration on neuromuscular function, specifically $MUAP_{AMP}$ and MFR vs RT relationships, MVC peak torque, mCSA, subcutaneous fat, and echo intensity of the vastus lateralis. We also sought to examine the effects of rehydrating after hypohydration on neuromuscular function. The results of the current study indicate that extreme hypohydration significantly decrease MVC peak torque compared to when participants were euhydrated, but the changes in peak torque are not directly attributed to changes in individual MU behavior.

This was the first study, to our knowledge, to examine the effects of 5% hypohydration on neuromuscular behavior. Though no significant differences occurred in MFR vs RT relationships of slope or y-intercepts of a 70% MVC isometric muscle action, it is important to note that in 6 out of 9 participants MFR slopes and y-intercepts increased when hypohydrated, and in 2 participants these values slightly decreased. Changes in y-intercept values may indicate that there is a change in lowest theoretical MU being recruited, suggesting the recruitment threshold decreased and activation of those MUs occurred sooner. Increases in MFR vs RT slope values show that the firing rate of MUs are slightly faster than when participants were euhydrated. Combined, higher threshold MU activation and increased firing times may allow the muscle to be able to reach and sustain a 70% MVC contraction while hypohydrated. Increased MU firing rates and decreased MU recruitment thresholds are in line with previous research examining MU in response to fatigue.⁷³ We can speculate the central nervous system is regulating the MU behavior by adjusting the point of excitation of the motor neuron pool to be able to recruit MUs at a lower threshold to sustain the isometric muscle action at 70% MVC.^{16,61,73} The MFR slope and y-intercept values remained elevated during visit 3 even though participants were rehydrated back

to baseline body weight. Further, MVC peak torque was greater after rehydration compared to hypohydrated but peak torque did not return to baseline values. This suggests that dehydration, though passive, has lingering effects on muscle function and central nervous system control at least 24 hours post hypohydration.⁴⁵

Hypohydration decreased mCSA without significant changes in subcutaneous fat or echo intensity.⁷⁴ Smaller mCSA is normally associated with smaller MUAP_{AMP} and traditionally more type I muscle fibers.⁷⁵ However, we did not observe a decrease in MUAP_{AMP} from visit 1 to visit 2. Though mCSA was smaller, the MUs were firing with the same amplitudes indicating that the MU size did not decrease, but the mCSA did due to the decreased water content within the muscle.⁷⁴ There was decrease in subcutaneous fat from visit 1 to visit 2 though the differences were not significant. A greater amount of water was being drawn out from the muscle compared to subcutaneous fat, which could be expected due adipocytes composed of triglycerides compared to proteins and connective tissue of skeletal muscle. Echo intensity reflects muscle quality. These values remained relatively constant with little fluctuation across visits, demonstrating the quality of the muscle remained the same though the size of the muscle changed.

It is worthy to note that though MUAP_{AMP} or MFR did not significantly change across visits the steady force of the 70%MVC isometric muscle contraction decreased when hypohydrated compared to an euhydrated state. Though there was a decrease in steady force there was not a statistical significant change in MU behavior to justify this change in steady force indicating that MU behavior is changing, however it is not showing significant in the data.

Previous studies have explored the relationship of moderate dehydration on neuromuscular strength. Minshull and James¹⁰ found ~2% passive dehydration elicited a significant decrease of 7.5% in static peak force of the knee extensors. Other research teams, Rodrigues¹³ and Ftaiti,⁹ have

used a variety of modes of exercise (cycling ergometer and running) to induce 2% dehydration and measure peak torque of the knee extensors, finding significant decreases of 16% and 12%. Though our study focused on passive dehydration, significant decreases were still elicited compared to an euhydrated state.

Though the leading mechanism behind decreased MVC peak torque is unknown and could be due to smaller mCSA, previous studies suggest hypohydration associated decreases in performance and strength may be due to glycogen depletion,⁶² reduced blood flow,^{38,44} electrolyte imbalances,³⁷ increased metabolic heat production,³⁹ altered mood, and/or decreased central activation while hyperthermic.⁶⁴ Some of these factors could have affected participants in our study. For instance, fasting and fluid restriction for 12 hours or more prior to visit 2 could have affected glycogen and electrolytes, the significant water loss could have reduced blood flow and oxygen delivery, and decreased central activation could have occurred from being exposed to the hot environment.

Limitations and Future Research

A limitation of the current study is the underpowered sample size. Due to time restraints, the limited testing schedule, and the rigor of the study, it was difficult to schedule subjects. The time it took to dehydrate to 5% body mass loss was variable across participants exposing some to a greater passive thermal strain from sitting in a hot and humid environment for a greater duration of time compared to a subjects who were not in there as long or did not have to enter that environment at all. Not all participants reached their 5% body mass loss goal. Two participants were taken out of the sauna due their inability to continue sweating paired with high heart rate and extreme light headedness, and one subject was removed due to personal scheduling restrictions. One subject completed visit 3 four hours later than everyone else due to a technical issue. Another

limitation to this study is that heart rate was taken manually rather than using a heart rate strap or monitor. Future research can be done to examine the behavior of MUs across visits at an absolute MVC intensity from their baseline visit instead of a relative intensity like the current study and look at the coactivation of other agonist muscles (e.g. rectus femoris) when examining the MUs of the VL when hypohydrated. To understand why MVC peak torque decreased muscle biopsies could be taken from participants to measure muscle glycogen across days to examine if fasting and passive dehydration impact the rate of depletion of glycogen stores. To further examine possible energy depletion, it would be interesting to collect the dietary intake of participants across visits to see if there were differences in caloric consumption across participants and between days. Future research could also be done to examine blood flow, though invasive, it would give insight to the cardiovascular strain placed upon that body in a hypohydrated state and the impact it could have on working muscles.

CONCLUSION

Extreme hypohydration of 5% body mass loss resulted in significantly decreased MVC peak torque and mCSA and increased cardiovascular strain; however, there were no significant changes in MU behavior, subcutaneous fat, or echo intensity. It is important for individuals to attenuate the effects hypohydration has on the body, such as decreased peak torque, decreased muscle size, and increased cardiovascular strain, especially during exercise and performance. Through maintaining euhydration and rehydrating after acute bouts of dehydration it will help in optimizing performance and prevent other serious complications such as exertional heat illness.

Table 1. Hydration status changes across experimental visits.

Visit	Urine specific gravity	Body mass (kg)	Hemoglobin (%)	Hematocrit (g/dl)	Plasma Volume	Plasma Volume Shift (% change)
Visit 1	1.014 ± 0.008	95.5 ± 17.1	16.5 ± 1.0	47.3 ± 2.3	52.8 ± 2.1	
Visit 2	1.034 ± 0.004 ¹	90.9 ± 16.4 ²	17.0 ± 0.9	48.1 ± 2.2	50.3 ± 3.4	-4.4 ± 8.9 ⁴
Visit 3	1.019 ± 0.006	95.6 ± 17.2	16.0 ± 1.5	46.0 ± 2.6	56.0 ± 4.7 ³	11.7 ± 11.1 ⁵

¹ Significantly higher than visit 1 (p < 0.001) and visit 3 (p = 0.001).

² Significantly lower than visit 1 (p < 0.001) and visit 3 (p < 0.001).

³ Significantly higher than visit 2 (p = 0.021).

⁴ Calculated change from visit 1 to visit 2.

⁵ Calculated change from visit 2 to visit 3.

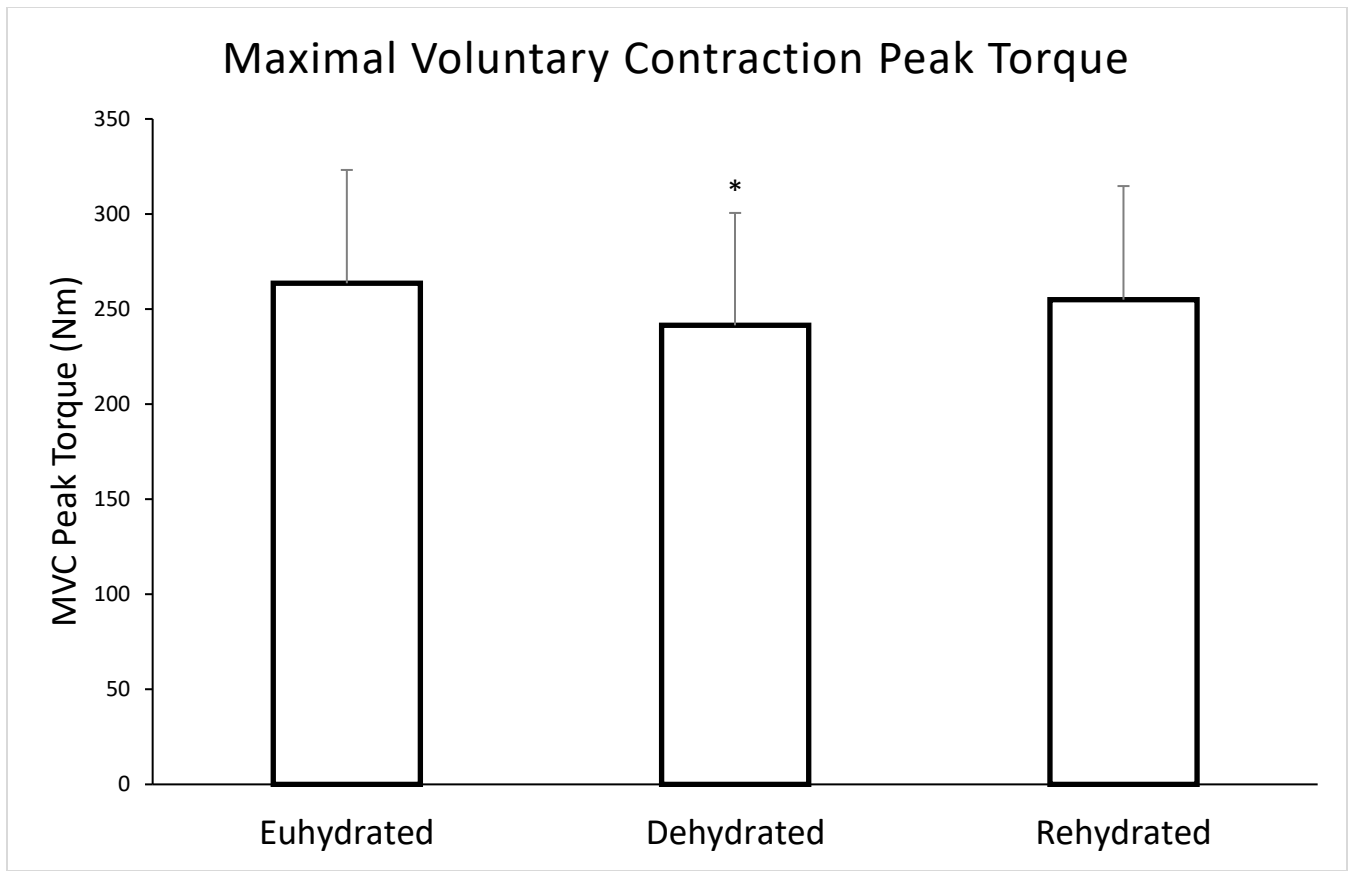


Figure 1. Means and standard deviations (SD) for maximal voluntary contraction (MVC, Newton meters) peak torque across visits. *Significantly weaker than visit 1 ($p = 0.023$).

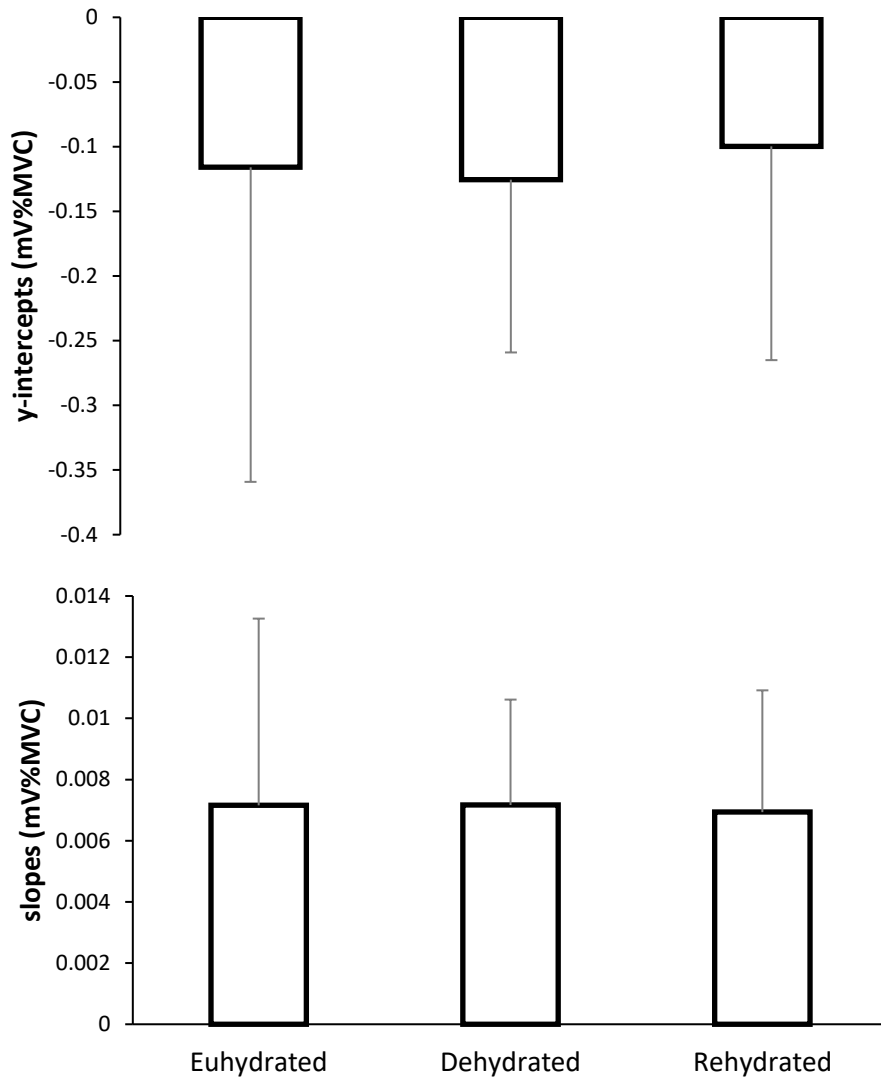


Figure 2. Means and standard deviations (SD) for the slopes and y-intercepts from the motor unit action potential amplitudes ($MUAP_{AMP}$, mV) vs recruitment threshold [(RT) expressed as a function of percent maximal voluntary contraction (%MVC)] relationships. Means and SDs for each visit were developed from each subject's linear regression. There were no significant differences between visits for $MUAP_{AMP}$ vs RT slopes or y-intercepts.

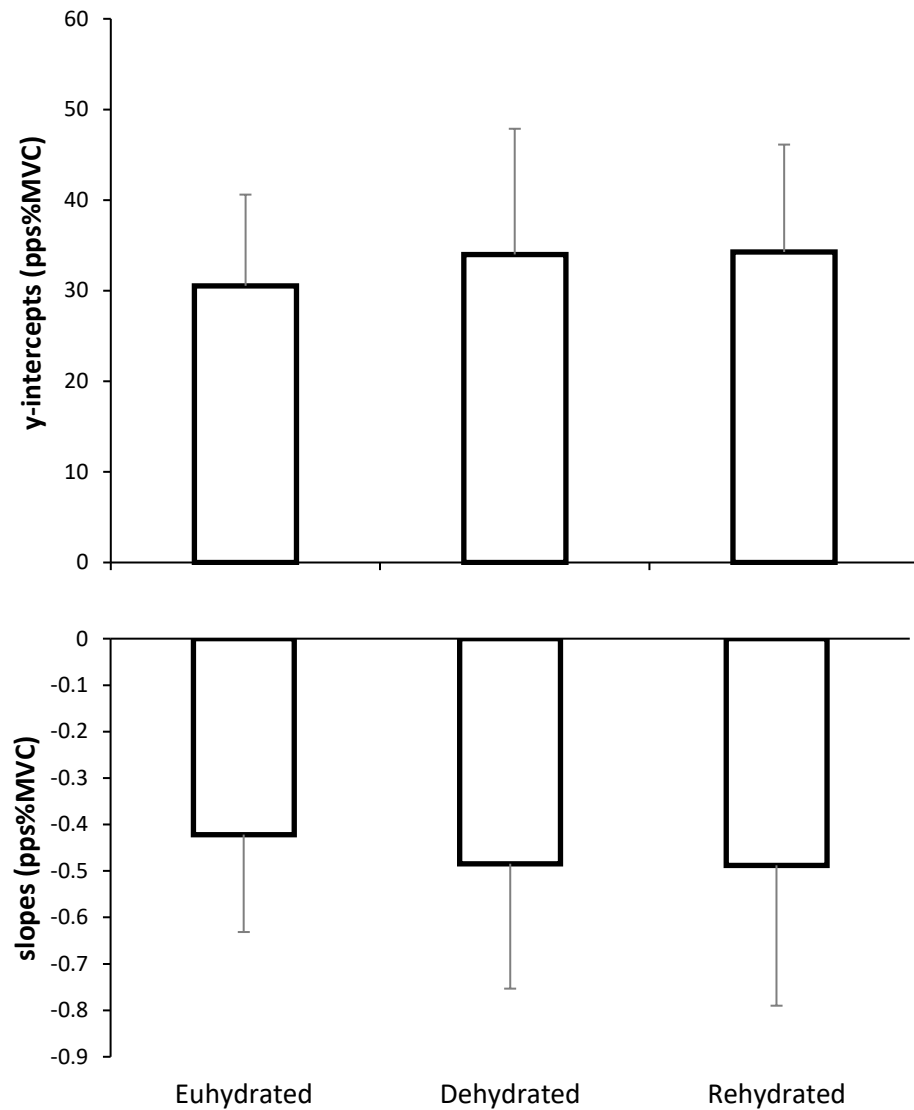


Figure 3. Mean and standard deviations (SD) for the slopes and y-intercepts from the mean firing rate (MFR, pulses per second, pps) vs recruitment threshold [(RT) expressed as a function of percent maximal voluntary contraction (%MVC)] relationships. Means and SDs for each visit were developed from each subject's linear regression. There were no significant differences between visits for MFR vs RT slopes or y-intercepts.

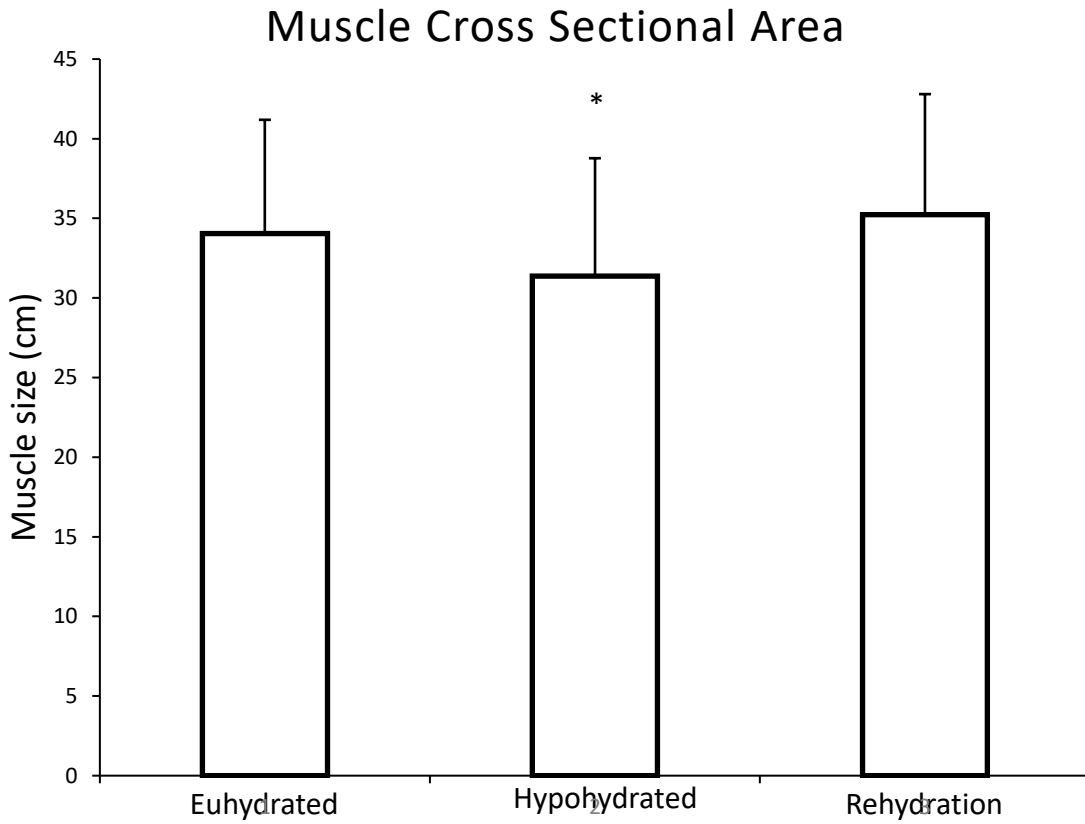


Figure 4. Means and standard deviations of muscle cross sectional area (mCSA) across visits.
*Significantly smaller than visit 1 ($p = 0.003$) and visit 2 ($p = 0.003$).

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APPENDIX
INFORMED CONSENT TO PARTICIPATE IN RESEARCH STUDY

Effects of Passive Dehydration on Neuromuscular Function

KEY INFORMATION

- This project is studying passive dehydration on neuromuscular function.
- Your participation in this research project is completely voluntary.
- Your participation will take 10 hours over a course of 4 days.
- You will be asked to do the following procedures: After a familiarization session, you will report on 3 days in a row. The first day is a hydrated day. Then you will complete a 24 hour dehydration protocol that includes limiting fluids and reporting on the second day to the laboratory where you will sit in a hot, humid environment. The target goal is to be dehydrated by 5% of your weight. For the third day you will be asked to drink fluids in order to rehydrated and reach your baseline body weight. On each of the 3 days, you will have your thigh muscle strength tested, an ultrasound image taken of your thigh muscles, weigh on a scale, and have blood and urine taken. More detailed information on the procedures can be found below.
- The following risks may occur due to participating in this study:
 - Risks that may occur due to the blood draw:
 1. It is likely you will experience mild pain or discomfort when having blood drawn.
 2. It is less likely you will have bruising in your arm where the needle is inserted.
 3. In rare cases you may develop a hematoma (localized swelling filled with blood) at the site in your arm where the needle was inserted.
 - Risks that may occur due to the dehydration protocol:
 1. Light headedness and dizziness may occur as dehydration progresses.
 2. You may feel fatigued.
 3. You may feel nauseated.
 4. Heart rate will most likely increase as dehydration progresses.
 - Risks that may occur due to neuromuscular testing:
 1. Redness or dryness may occur on your thigh where surface electrodes are placed.
 2. Mental and physical stress may occur as you perform strength testing of the thigh muscles.
- There are no benefits to you or financial compensation to you for participating in this study.
- Your alternative to participating in this research study is not to participate.

INTRODUCTION

The Department of Health, Sport, and Exercise Sciences at the University of Kansas supports the practice of protection for human subjects participating in research. The following information is provided for you to decide whether you wish to participate in the present study. You may refuse to sign this form and not participate in this study. You should be aware that even if you agree to participate, you are free to withdraw at any time. If you do withdraw from this study, it will not

affect your relationship with this department, the services it may provide to you, or the University of Kansas.

STUDY PURPOSE

The purpose of this study is to determine the effects of passive dehydration on neuromuscular function.

PROCEDURES

You are being invited to participate in this study because you are between the ages of 18 and 35 years old, are moderately resistance trained (you strength train 3 times a week and have trained for at least 1 year), and are in good general health. If you agree to participate, you will be participating with approximately 30 other people.

Study Participation

After signing this informed consent and upon acceptance into the study, you will complete a familiarization session. The familiarization can be done today or we can schedule to complete it another day. You will then be scheduled to complete 3 additional visits (baseline, dehydration, and rehydration visits) taking place 3 days in a row. Baseline and rehydration visits will take approximately 2 hours each. The dehydration visit will take approximately 4 hours. The total time for the study, from familiarization to the end of rehydration is approximately 10 hours.

Females: If you are a female we will need to schedule your 3-day visits to occur during your follicular phase. The follicular phase is typically the first week after the last day of active menses (bleeding).

Experimental Procedures (Figure 1)

The 4 visits include: 1) familiarization, 2) baseline/hydrated, 3) dehydrated, and 4) rehydrated.

Familiarization. The familiarization session is designed to expose you to the neuromuscular strength testing so you are familiar with the procedures and we can set up the machine specific to anatomical landmarks on your leg. You should be hydrated for this session so we encourage you to drink about 1 liter (4 cups) of fluid the night before this visit and 1 liter (4 cups) the morning before you come. We will take a urine sample to determine whether you are hydrated or not, and then we will weigh you on a scale. We will also take you to the environmental room and the weighing area so you are familiar with both locations and procedures during the dehydration visit. This visit will take approximately 1 hour.

Baseline/Hydrated. To ensure you come to the baseline session hydrated, we encourage you to drink about 1 liter (4 cups) of fluid the night before this visit and 1 liter (4 cups) the morning before you come. Once you arrive, you will provide a urine sample where we will determine whether you are hydrated or dehydrated. You will then weigh and we will take the first blood sample. You will then complete the muscle strength testing. At the end of this session we will give you the target 5% weight loss goal for the dehydration visit. To help you achieve the weight loss, we encourage you to limit fluids and fluid-rich foods (for example: fruits, vegetables, soups, ice cream, yogurt)

over the next 24 hours. At 12 hours before your scheduled time to come to the laboratory, we encourage you to not drink any fluids. This visit will take approximately 2 hours.

Dehydrated. You will report to the laboratory in the morning and we will weigh you to see your current weight to determine how much more weight you need to lose to achieve the 5% goal. You will provide a urine sample. You will then sit in the environmental room (about 97°F and 45% humidity) to promote sweating and the additional weight loss. Every 30 minutes we will take you out of the room and to the weighing area. To make sure we have an accurate weight we ask that you weigh nude. You will be provided a private area where you can remove your clothes and you will have a towel that you should use to wipe off all sweat. Once you weigh, you can put your clothes back on and we will inform you what your weight is. You will continue to sit in the environmental area until one of the following criteria is met: 1) you reach the target 5% weight loss, 2) you experience signs and symptoms such as vomiting or change in consciousness, or 3) you ask to stop the session and stop participating in the study.

Once the target weight is reached, you will be taken to a cool laboratory where you will complete the muscle strength testing then have a urine and blood sample taken. Before leaving, we will give you verbal and written instructions on how much fluid to consume (150% of your weight loss) over the next 24 hours in order to rehydrate to your baseline weight before the next visit. The time for this visit varies and depends on the level of dehydration you report in the morning with. For example, if you come at 2% weight loss the time required to sit in the hot environment and reach the 5% target could take 3 hours. The time to complete the strength testing and all other measures will then take approximately 1.5 hours.

Rehydrated. Once you come to the laboratory we will weigh you to see if you reached your baseline weight. You will then provide a urine sample, complete the strength testing, and have one final urine and blood sample taken. This visit will take approximately 2 hours.

In the event that you do not reach your baseline weight, we will provide you 3.5 ml/kg of water to drink every 15 minutes. For an example of how much water this is, a 149 pound person would need to drink 1 cup of water every 15 minutes. We will give you 1 hour to reach your baseline weight and if you have not reached it by that time the session will end.

Instruments and Protocols

Blood Measures. To obtain blood we will insert a needle into a vein in your arm. We will collect blood 3 times using one single needle stick. For each blood draw we will take 1 10 ml tube (approximately 2.5 tsp) and 1 6 ml tube (approximately 1.2 tsp). The total blood taken for the 3 days is approximately 10 tsp or 3 Tbsp, about 1/9th of what is taken when donating blood. All tubes are labeled with your participant number. We will use blood to measure hydration status and markers of inflammation.

Urine Measures. Urine will be used to determine hydration status. You will use a private restroom to collect the urine sample. Using a urine cup, you will provide 1 sample upon arriving to the facilities and one sample after muscle testing. The cup will be labeled with your participant number. We will measure urine for specific gravity.

Body Weight. A portable body mass scale will be used to determine your weight to characterize hydration. Once you arrive to the facility, you will be asked to void all urine. You will be asked to arrive in minimal clothing (e.g., shorts and t-shirt). On the day of the dehydration protocol, your body weight will be measured every 30 minutes until you reach 5% weight loss. During this portion, you will be given a private area to weigh nude and given a towel to dry off all sweat from your skin.

Cardiovascular. To monitor cardiovascular function, your heart rate (HR) will be continuously monitored during the dehydration protocol using a strap worn around your chest.

Ultrasound. Ultrasound will be used to determine subcutaneous fat, echo intensity and cross-sectional area of the leg extensors (front of thigh muscles) and leg flexors (back of thigh muscles). Images will be taken before baseline, before and after dehydration, and before rehydration.

Neuromuscular Testing. You will complete muscle strength testing on a Biodex Isokinetic Dynamometer. Three surface electrodes will be placed on your leg extensors (front of thigh muscles) and 2 surface electrodes will be placed on the leg flexors (back of thigh muscles). You will perform 3 isometric maximal voluntary contractions followed by submaximal isometric trapezoid muscle actions at 10%, 40% and 70% of your maximum. During the trapezoid testing, templates will increase torque at 10% maximum until you reach the desired steady torque level, you will then hold this for 12 seconds and decrease by 10% maximum back to baseline. You will be given two minutes of rest between each muscle action. Then you will complete 3 maximal voluntary muscle actions. We will familiarize you to this testing during the familiarization visit to ensure you are comfortable with the actions. You will then complete the strength testing each of the 3 days (baseline, dehydrated, and rehydrated).

<u>Familiarization Visit</u>	<u>Baseline/Hydrated Visit</u>	<u>Dehydrated Visit</u>	<u>Rehydrated Visit</u>
1. <u>Weight</u> 2. <u>Urine</u> 3. <u>Muscle testing</u> 4. <u>Visit environmental and weighing areas</u>	1. <u>Weight</u> 2. <u>Urine</u> 3. <u>Blood</u> 4. <u>Ultrasound</u> 5. <u>Muscle testing</u>	1. <u>Weight</u> 2. <u>Continue dehydration in environmental area</u> a. <u>Weigh every 15 min</u> b. <u>Heart rate continuously monitored</u> <u>At 5% weight loss</u> 1. <u>Ultrasound</u> 2. <u>Muscle testing</u> 3. <u>Blood</u> 4. <u>Urine</u>	1. <u>Weight</u> 2. <u>Urine</u> 3. <u>Ultrasound</u> 4. <u>Muscle testing</u> 5. <u>Blood</u>

Figure 1. Data collection procedures during for 4 visits.

RISKS

You may experience one or more of the risks indicated from being in the study. In addition to these, there may be other unknown risks, or risks we did not anticipate, associated with participation in this study.

Risks that may occur due to the blood draw:

1. It is likely to experience mild pain or discomfort when having blood drawn, although we do not anticipate additional blood draws than what is require for normal treatment of care.
2. Bruising at the site in your arm where the needle is inserted is less likely to occur.
3. In rare cases you may develop a hematoma (localized swelling filled with blood) at the site in your arm where the needle was inserted.

Risks that may occur due to the dehydration protocol:

1. Light headedness and dizziness may occur as dehydration levels progress.
2. You may feel fatigued.
3. You may feel nauseated as dehydration progresses.
4. Heart rate will most likely increase as you become more dehydrated.

Risks that may occur due to neuromuscular testing:

1. Redness or dryness may occur at site of surface electrodes.
2. Mental and physical stress may occur as you perform strength testing of the thigh muscles.

BENEFITS

There are no benefits to participating in this study.

ALTERNATIVES TO PARTICPATION

Your alternative to participating in this research study is not to participate

PAYMENT TO PARTICIPANTS

You will not receive any form of compensation for participation in this study.

PARTICIPANT CONFIDENTIALITY

Your name or any other identifiable information will not be associated in any publication or presentation. All information collected about you or with the research findings from this study will be coded with a study number. Only the primary investigators will have access to documents with your identifiable information and documents will be kept in a locked cabinet in a locked office. No identifiable information about you will be shared unless (a) it is required by law or university policy, or (b) you give written permission.

PRIVATE INFORMATION (DATA) AND/OR BIOSPECIMENS

Your identifiable information will be removed from the data and/or biospecimens collected during this project, and the de-identified data and/or biospecimens may be used for future research without additional consent from you.

REFUSAL TO SIGN CONSENT AND AUTHORIZATION

You are not required to sign this Consent and Authorization form and you may refuse to do so without affecting your right to any services you are receiving or may receive from the University of Kansas or to participate in any programs or events of the University of Kansas. However, if you refuse to sign, you cannot participate in this study.

CANCELLING THIS CONSENT AND AUTHORIZATION

You may withdraw your consent to participate in this study at any time. You also have the right to cancel your permission to use and disclose further information collected about you, in writing, at any time, by sending your written request to: Dawn Emerson (contact information below). If you cancel permission to use your information, the researchers will stop collecting additional information about you. However, the research team may use and disclose information that was gathered before they received your cancellation, as described above.

INSTITUTIONAL DISCLAIMER STATEMENT

In the event of injury, the Kansas Tort Claims Act provides for compensation if it can be demonstrated that the injury was caused by the negligent or wrongful act or omission of a state employee acting within the scope of his/her employment.

QUESTIONS ABOUT PARTICIPATION

Questions about procedures should be directed to the researchers listed at the end of this consent form.

PARTICIPANT CERTIFICATION

I have read this Consent and Authorization form. I had the opportunity to ask and I received answers to any questions I had regarding the study. I understand that if I have any additional questions about my rights as a research participant, I may call (785) 864-7429 or (785) 864-7385, or write the Human Research Protection Program on the Lawrence Campus (HRPP), University of Kansas, 2385 Irving Hill Road, Lawrence, Kansas 66045-7568, or email irb@ku.edu.

I agree to take part in this study as a research participant. By my signature I affirm that I am at least 18 years old and that I have received a copy of this Consent and Authorization form.

Participant’s Printed Name

Date

Participant’s Signature

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Health History Questionnaire
Dehydration and Neuromuscular Function Study

INSTRUCTIONS: Complete the following questions the best of your knowledge/ability. Let the investigator know if you need further explanation.

	Yes	No
1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?	<input type="checkbox"/>	<input type="checkbox"/>
2. Do you lose your balance because of dizziness or do you ever lose consciousness?	<input type="checkbox"/>	<input type="checkbox"/>
3. Is your doctor currently prescribing drugs (for example, water pills) for blood pressure or a heart condition?	<input type="checkbox"/>	<input type="checkbox"/>
4. Have you had a medical illness or injury since your last check up or sports physical?	<input type="checkbox"/>	<input type="checkbox"/>
5. Do you have an ongoing chronic illness?	<input type="checkbox"/>	<input type="checkbox"/>
6. Have you ever passed out during or after exercise?	<input type="checkbox"/>	<input type="checkbox"/>
7. Have you ever been dizzy or fainted during or after exercise?	<input type="checkbox"/>	<input type="checkbox"/>
8. Have you ever had pain, discomfort, tightness, or pressure in your chest during or after exercise?	<input type="checkbox"/>	<input type="checkbox"/>
9. Have you ever had racing of your heart or skipped heartbeats?	<input type="checkbox"/>	<input type="checkbox"/>
10. Have you had high blood pressure or high cholesterol?	<input type="checkbox"/>	<input type="checkbox"/>
11. Have you ever been told you have a heart murmur?	<input type="checkbox"/>	<input type="checkbox"/>
12. Has a physician ever denied or restricted your participation for sports or for any heart problems?	<input type="checkbox"/>	<input type="checkbox"/>
13. Have you ever had an unexplained seizure?	<input type="checkbox"/>	<input type="checkbox"/>
14. Have you ever had numbness or tingling in your arms, hands, legs, or feet?	<input type="checkbox"/>	<input type="checkbox"/>
15. Have you ever become ill from exercising in the heat?	<input type="checkbox"/>	<input type="checkbox"/>
16. Do you cough, wheeze, or have trouble breathing during or after activity?	<input type="checkbox"/>	<input type="checkbox"/>
17. Do you have diabetes?	<input type="checkbox"/>	<input type="checkbox"/>
18. Do you have asthma or other lung disease?	<input type="checkbox"/>	<input type="checkbox"/>
19. Do you smoke?	<input type="checkbox"/>	<input type="checkbox"/>
20. Do you have or think you may have any bleeding disorders?	<input type="checkbox"/>	<input type="checkbox"/>
21. Do you get frequent muscle cramps when exercising?	<input type="checkbox"/>	<input type="checkbox"/>

- | | | |
|---|--------------------------|--------------------------|
| 22. Do you or someone in your family have sickle cell trait or disease? | <input type="checkbox"/> | <input type="checkbox"/> |
| 23. Have you been diagnosed with a gastrointestinal disorder such as inflammatory bowel disease, gag reflex disorder, hypomotility, diverticulitis, or other? | <input type="checkbox"/> | <input type="checkbox"/> |
| 24. Have you been diagnosed with a chronic inflammatory disease such as rheumatoid arthritis, lupus, psoriasis, or other? | <input type="checkbox"/> | <input type="checkbox"/> |
| 25. Have you been diagnosed with kidney disease? | <input type="checkbox"/> | <input type="checkbox"/> |
| | Yes | No |
| 26. Do you have any other chronic illnesses such as cardiovascular or respiratory disease, diabetes or other metabolic disorders, neurological disorders, or other? | <input type="checkbox"/> | <input type="checkbox"/> |
| 27. Do you have an implanted electromedical device? | <input type="checkbox"/> | <input type="checkbox"/> |
| 28. Do you currently have an injury in muscles, tendons, bones, or joints? For example, muscle strain, ankle sprain, stress fracture, shin splints, herniated disc, plantar fasciitis, etc. | <input type="checkbox"/> | <input type="checkbox"/> |

Explain any “Yes” answer here:

Heat acclimatization occurs when your body has adapted to exercise in the heat. A gradual increase in exercise intensity and heat stress over 10-14 days helps to facilitate this.

29. Based on this, do you think you are heat acclimatized? Yes No

Exertional heat exhaustion is the inability to continue exercising in the heat with signs and symptoms including, but not limited to, fatigue, low blood pressure, headache, dizziness, vomiting, and nausea. The person typically has a high core body temperature that is less than 40.5°C/104.5°F.

30. Have you ever experienced heat exhaustion when exercising? Yes No

Exertional heat stroke is the most serious heat illness that occurs during physical activity. The body reaches temperatures greater than 40.5°C/104.5°F. The person also experiences central nervous system dysfunction,

with signs and symptoms including, but not limited to, loss of consciousness, aggressiveness, confusion, and seizure.

31. Have you ever experienced heat stroke when exercising? Yes No

Exertional hyponatremia occurs when sodium levels in blood drop dangerously low. The condition often occurs because of drinking too much fluids during exercise. Signs and symptoms include, but are not limited to, nausea, dizziness, tingling or swelling in arms or legs, headache, disorientation, loss of consciousness, pulmonary or cerebral edema.

32. Have you ever experienced hyponatremia during or immediately after exercising? Yes No

33. Are you currently sick or experiencing any symptoms of an illness? Yes No

If yes, What illness(es) do you have? _____

Medications and Nutrition

34. Are you allergic to any medications? Yes No

If yes, Name of medication

35. Are you currently taking any **prescription** medications, pills, or inhalers on a regular basis? Yes No
This includes birth control.

If yes, Name of medication

36. Are you currently taking any **non-prescription** (over the counter) medications or pills on a regular basis? Yes No

If yes, Name of medication

37. Are you taking any **supplements, vitamins, or other substances** on a regular basis? For example: multi-vitamin, iron, diet pills, protein powder, etc. Yes No

If yes, Name of supplement

Demographics

38. Date of birth
(MM/DD/YY)

Sex refers to your biological characteristics (ie, anatomy, hormones, etc.)

39. What is your sex? Female MtF Female
(check one) Male FtM Male
 Intersex

FEMALES ONLY

40. Do you have a regular menstrual cycle? Yes No

41. What is the first day of your last period? (MM/DD/YYYY) _____

42. Are you currently on birth control or other hormone supplementation? Yes No

43. Are you currently pregnant? Yes No

I hereby state that, to the best of my knowledge, my answers to the above questions are complete and correct.

Signature

Printed Name

Date

Effects of Passive Dehydration on Neuromuscular Function
Exit Survey – Dehydration Visit

Participant # _____

Date _____

Directions: Complete each question and answer regarding how you *currently* feel. Let the investigator know if you need further explanation.

	Yes	No
44. Are you nauseous or feel like you need to vomit?	<input type="checkbox"/>	<input type="checkbox"/>
45. Are you blacking out or feel like you are about to faint?	<input type="checkbox"/>	<input type="checkbox"/>
46. Are you light-headed or dizzy?	<input type="checkbox"/>	<input type="checkbox"/>
47. Are you confused about where you are or what you are doing?	<input type="checkbox"/>	<input type="checkbox"/>
48. Are you having trouble breathing or feel out of breath?	<input type="checkbox"/>	<input type="checkbox"/>
49. Do you feel anxious or under abnormal mental stress from completing study?	<input type="checkbox"/>	<input type="checkbox"/>
50. Do you have any muscle cramps?	<input type="checkbox"/>	<input type="checkbox"/>
51. Do you have blurred or double vision?	<input type="checkbox"/>	<input type="checkbox"/>
52. Do you feel uncomfortable leaving the laboratory alone?	<input type="checkbox"/>	<input type="checkbox"/>
