ABSTRACT

The Effects of Hypohydration on Muscular Performance and Markers of Catabolism in Resistance-Trained Females

Joshua J. Gann, Ph.D.

Dissertation Chairperson: Darryn S. Willoughby, Ph.D.

The purpose of this study was to determine the effects of previous night dehydration on muscular strength, endurance, and lower-body power, perceptual measures, and markers of catabolism in resistance-trained females. Ten healthy, resistance-trained females completed two bouts of resistance exercise, either dehydrated (~3% body weight) (DT) or heat exposed with fluid replacement (HT). Each exercise bout consisted of one rep maximum (1RM) followed by five sets to failure of 75% of 1RM for bench press and leg press, and vertical jump assessment. Muscle and blood samples were obtained prior to and 1hr following exercise. Blood samples were obtained to examine cortisol. From each muscle sample, glucocorticoid receptor-DNA (GR-DNA) binding and mRNA expression were determined. Bench press 1RM ($p = 0.04$) was significantly lower for DT compared to HT. No significant difference was found for leg press 1RM. There was no difference in total reps completed for bench press or leg press. No significant differences were found for total volume lifted for bench press or leg press or vertical jump. There were no significant interactions

between session and time for any markers of mRNA expression. There was no significant interaction or main effects for session and time for serum cortisol. There was a significant main effect for session for GR- DNA binding (p = 043). GR-DNA binding was significantly elevated post exercise for DT (p = .016). Current results suggest that hypohydration may have a negative impacton bench press 1RM performance. Though the only performance measure to reach a statistically significant difference was bench press 1RM, there was a reduction in leg press 1RM and total volume lifted for both bench press and leg press. While this was an acute bout of only two exercises, it would be reasonable to suggest that this reduction in volume would continue for other exercises across a full workout. Additionally, GR-DNA binding was increased with hypohydration. Theoretically, if an individual were to be chronically hypohydrated, this reduction in volume and increase in GR-DNA binding could diminish the anabolic response to resistance exercise and potentially lead to muscle atrophy.

The Effects of Hypohydration on Muscular Performance and Markers of Catabolism in Females

by

Joshua J. Gann, M.S., B.S.

A Dissertation

Approved by the Department of Health, Human Performance, and Recreation

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Paul Gordon, Ph.D., Chairperson

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Approved by the Dissertation Committee

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Darryn Willoughby, Ph.D., Chairperson

_____

Yunsuk Koh, Ph.D.

_____

Brian Leutholtz, Ph.D.

_____

Andrew Gallucci, Ph.D.

_____

Mar Magnusen, Ph.D.

Accepted by the Graduate School

August 2017

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J. Larry Lyon, Ph.D., Dean

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1RM - 1 repetition maximum

ACTH – adrenocorticotropic hormone

ANOVA – analysis of variance

BMI – body mass index

cDNA – complementary deoxyribonucleic acid

DEXA – dual energy x-ray absorptiometry

DNA – deoxyribonucleic acid

Foxo1 – forkhead box O

FSH – follicle-stimulating hormone

GnRH – gonadotropin-releasing hormone

GR – glucocorticoid receptor

HR – heart rate

IGF-1 – insulin-like growth factor-1

LH – luteinizing hormone

MPS – muscle protein synthesis

mRNA – messenger ribonucleic acid

mTORC1 – mammalian target of rapamycin complex 1

MAFbx – muscle atrophy box

MuRF1 – muscle ring finger-1

PCR – polymerase chain reaction

PI3K – phosphatidylinositol-3 kinase

PRS – perceived recovery status

RPE – ratings of perceived exertion

REDD1 – regulated in DNA development-1

RM – repetition maximum

RNA – ribonucleic acid

S6K – S6 kinase

sRPE – session RPE

UPS - ubiquitin protease system

USG – urine specific gravity

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CHAPTER ONE

Introduction

The effects of hypohydration on aerobic performance have been well established (American College of Sports Medicine et al., 2007). However, less is known about the effects on anaerobic performance (e.g. high intensity, short duration performance such as sprinting and weight lifting). Many exercise modes have been utilized to assess the impact on anaerobic performance including repeated sprints (Gann et al., 2016), resistance exercise (Judelson et al., 2008; Kraft et al., 2010), and isokinetic exercise (Hayes & Morse, 2010). In a review, Kraft et al. (2012) found that the effects of hypohydration on anaerobic exercise may not be seen until 3% body mass loss. Additionally, a meta-analysis found that when hypohydration was achieved ≥ 8 hr prior to exercise testing with an overnight sleep, residual effects of heat exposure and exercise were nullified (Savoie, Kenefick, Ely, Cheuvront, & Goulet, 2015). Hypohydration is a common problem among athletes and recreational exercisers. Many of these individuals fail to rehydrate adequately between exercise sessions (Thigpen et al., 2014). In addition to performance decrements, hypohydration can lead to diminished cognitive performance, nausea, increased risk of heat illness, and in extreme cases, coma or death (Casa et al., 2015; Gann et al., 2015). Moreover, moderate dehydration (3%) has been shown to negatively affect ratings of perceived exertion (RPE), session RPE (sRPE), and perceived recovery prior to exercise (PRS), meaning participants perceived the exercise

to be more difficult and expected negatively-altered performance due to dehydration (Gann et al., 2016; Kraft et al., 2010, 2011).

Both men and women have been shown to underestimate sweat loss during exercise (O'Neal et al., 2012; Thigpen et al., 2014). This underestimation could lead to insufficient fluid intake post-exercise and subsequent hypohydration. This suggests that females are at the same risk as males in suffering from progressive dehydration. However, to date, virtually all research focusing on hypodration's effects on anaerobic exercise performance has been performed on male participants. A potential reason for the lack of research focusing on the effects of hypohydration on anaerobic exercise in females could be in part due to the menstrual cycle. Ovarian hormones fluctuate predictably over 23–28 days, on average. The menstrual cycle is divided into four phases: menstrual phase, follicular phase, ovulation, and luteal phase (Nader, 2012; Sherman & Korenman, 1975). While fluid balance between phases could be a concern when performing hypohydration research with female participants, current research from our lab (McKinley-Barnard et al., manuscript in review) has shown no difference in total body water between follicular and luteal phases. Additionally, menstrual cycle phase does not seem to effect exercise performance or perceptual response to exercise (Marsh & Jenkins, 2002) or replacement of fluid losses following exercise (Maughan, McArthur, & Shirreffs, 1996). Further, thermoregulatory differences (Kaciuba-Uscilko & Grucza, 2001) between males and females warrants investigation into the effects of hypohydration on females during anaerobic exercise.

Much of the previous literature has resulted in equivocal results in regards to hypohydration and catabolic responses to exercise. Previously, Judelson et al. (2008)

examined hypohydration's impact at 5% of body mass reduction and demonstrated an increase in levels of cortisol post resistance exercise (e.g. catabolic state). However, this level of hypohydration is unlikely and typically limited to weight-cutting sports (e.g. wrestling, boxing, mixed martial arts). Yamamoto et al. (Yamamoto et al., 2008) examined the impact of hypohydration (2.5% and 5% body mass reduction) on muscle damage utilizing blood markers of damage (myoglobin and creatine kinase) following lower-body anaerobic exercise and found no statistical difference between all trials. Although, it should be noted these markers can be poor indicators as indirect evidence to myofibril damage and potential protein degradation. Hayes et al. (2010) examined progressive dehydration and found a dose-dependent relationship for maximal power output, demonstrating a lower force production for isometric leg extension at only a 1% reduction of body mass via dehydration. However, no hormonal data was collected for this study.

The glucocorticoid, cortisol, is elevated in the circulation during stress-related situations including nutritional deficits. Cortisol operates through its intracellular glucocorticoid receptor (GR) and acts as an inhibitor of muscle protein synthesis (MPS) through varied mechanisms. Firstly, cortisol decreases the rate of MPS (limits amino acid transport into the muscle), thereby increasing the rate of muscle protein breakdown. This leaves an individual in a state of catabolism which, over the course of time, can equate to muscle atrophy (Löfberg et al., 2002). Secondly, cortisol inhibits the stimulatory effects of insulin, insulin-like growth factor 1 (IGF-1), the amino acid, L-leucine, and the phosphorylation of two key controlling factors for translation initiation (4E-BP1 and S6K1) and subsequent MPS (Zhenqi Liu, Li, Kimball, Jahn, & Barrett, 2004). Additional

evidence suggests that the cortisol-induced up-regulation on muscle proteolysis also occurs with activation of the ubiquitin protease system (UPS), the lysomal system (autophagy), and the calcium-dependent system (calpains) (Tiao et al., 1996). In regards to the PI3K/Akt pathway (mediator for the anabolic actions of insulin and IGF-1), evidence suggests that cortisol exerts its catabolic actions by causing a decreased expression of insulin receptor substrate 1 (IRS-1) protein, which operates upstream in the PI3K/Akt pathway (Nakao et al., 2009). The result of this inhibition on the PI3K/Akt pathway is an inhibition on mRNA translation and subsequent MPS (potentially leaving an individual in a state of catabolism). Currently, it appears that no published data are available investigating the catabolic hormonal response to an acute bout of anaerobic exercise at moderate hypohydration (3% body mass lost) in females.

Purposes of the Study

The purpose of this study is to compare the impact of hypohydration on skeletal muscle exercise performance and the catabolic hormonal responses to resistance exercise. The specific aims of the proposed study are to examine the impact hypohydration has on: 1) muscle performance (strength, power, and endurance), 2) perceptual responses responses [rating of perceived exertion (RPE), session RPE (sRPE), and perceived recovery status (PRS)], 3) serum cortisol as a marker of catabolism, 4) the extent of GR-DNA binding in skeletal muscle, and 5) mRNA expression of proteolytically-related genes.

Hypotheses

H_1: There will be no significant differences in isotonic muscular strength, muscular endurance, power, or heart rate between hydrated and hypohydrated trials.

H_2: There will be no differences for RPE, sRPE, or PRS between hydrated and hypohydrated trials.

H_3: There will be no differences in serum cortisol levels between hydrated and hypohydrated trials.

H_4: There will be no differences in the protein content of the GR and extent of GR-DNA binding in skeletal muscle between hydrated and hypohydrated trials.

H_5: There will be no differences in mRNA gene expression of proteolytically-related genes between hydrated and hypohydrated trials.

Delimitations

1. Ten apparently healthy females between the ages of 18 to 30 who were recreationally resistance-trained [persons who resistance train for general health and body composition purposes (e.g. 3 to 6 days per week for at least 6 months prior to the onset of the study), yet did not perform, with consistency, the volume of resistance training normally required in order to compete in professional strength or bodybuilding competitions or competitive athletic events] participated in the study.
2. Participants were recruited from Baylor University and within the surrounding Waco, TX area by flyers.
3. Participants were excluded from the study if they are amenorrheic.

4. All participants were considered low risk for cardiovascular disease, with no contraindication to exercise as outlined by the American College of Sports Medicine (ACSM).
5. All participants were tested at the Baylor Laboratory for Exercise Science and Technology (BLEST) and Exercise Nutritional Biochemical Laboratory (EBNL) in accordance with Helsinki Code after signed university approved informed consent documents.
6. A night's sleep was given following dehydration protocol to eliminate any potential residual effects from heat exposure.
7. Volume load for each of the testing sessions was identical, relative to 1RM.
8. All participants replicated their diet the day before and day of dehydration protocol for each trail.

Limitations

1. The results of the study are only applicable to the larger population of recreationally, resistance-trained women between 18 and 30 years of age.
2. Inferences are limited to the time points at which samples were collected.
3. A negative placebo effect may have been seen due to the inability to blind participants to which trial was being completed.
4. Each participant's difference in inherent circadian rhythm due to sleep schedule and daily stresses may have affected criterion variables.
5. The biopsy procedure may have caused trauma (inflammation) to the site of extraction; to minimize any possible stress response, additional samples were taken from at least 0.5 cm proximal or distal to the original biopsy site.

Assumptions

1. All laboratory equipment was functioning properly to produce valid and reliable measurements. Proper calibration and the use of trained research staff minimized any potential for errors.
2. Participants put forth maximal effort during all exercise sessions.
3. All participants followed the hydration protocol as instructed.
4. All participants followed all guidelines provided for completion of the study.
5. All participants maintained their normal dietary habits throughout the study.
6. All participants refrained from exercise for 48 before each of the testing sessions.
7. All participants had adequate sleep (7 to 8 hours) before each of the testing sessions.
8. Participants accurately answered all relevant questions regarding medical history and resistance training experience.

CHAPTER TWO

Literature Review

Dehydration and Anaerobic Performance

Dehydration is a condition caused by an excessive loss of body fluids and is commonly experienced by athletes, individuals regularly participating in exercise, those who work in hot or humid conditions, and those whose work requires equipment that may hinder thermoregulation (e.g. firefighters, military). Athletes in weight class sports, such as boxing, wrestling, and mixed martial arts often intentionally expose themselves to extreme conditions (fluid restriction, saunas, or hot water baths) to decrease body water in order to compete in lower weight classes (Gann et al., 2016; Hall & Lane, 2001; Hickner et al., 1991; M. Smith et al., 2001; M. S. Smith, Dyson, Hale, Harrison, & McManus, 2000). While athletes in other sports do not intentionally dehydrate themselves, they often unintentionally train or compete in a dehydrated state. Professional, college, and youth athletes have all been shown to be chronically dehydrated before practices and games (Da Silva et al., 2012; Osterberg, Horswill, & Baker, 2009; Silva et al., 2011; Thigpen et al., 2014; Volpe, Poule, & Bland, 2009).

The detrimental effects of dehydration on aerobic performance (e.g. impaired performance, increased cardiovascular strain, etc.) are seen at a loss of 2% body mass (Cheuvront, Kenefick, Montain, & Sawka, 2010). However, information on the effects of dehydration on anaerobic performance is less clear. Results to this point have been equivocal. This is possibly due to a variety of potentially confounding factors. Research

has shown dehydration ≥ 3% loss of body mass is the critical level to diminish anaerobic performance (Kraft et al., 2012; Yoshida, Takanishi, Nakai, Yorimoto, & Morimoto, 2002). However, there have been instances in which anaerobic performance was diminished with < 3% body mass lost (Gann et al., 2016; Schoffstall, Branch, Leutholtz, & Swain, 2001). Even if this critical level of dehydration is reached, it is still difficult to determine the exact effects of dehydration on anaerobic performance. There are numerous other possible confounders including: variations in modalities used to achieve dehydration (exercise-induced, diuretic-induced, or passive), variations in modalities used to measure anaerobic performance, heat stress influence, participant fitness level, inter-individual variability, and a possible duration component (Joshua J. Gann et al., 2016; Kraft et al., 2012; Savoie et al., 2015).

Besides the level of dehydration, another possible explanation for these differing results could possibly depend on which of the variety of modes used for measuring anaerobic performance during each study. Tests of anaerobic performance include one-repetition maximum weight lifting (Schoffstall et al., 2001), full-body resistance exercise protocol (Kraft et al., 2010), repeated back squats (Judelson et al., 2007), unilateral leg extensions (Hayes & Morse, 2010), vertical jump (Cheuvront, Kenefick, Ely, et al., 2010; Hayes & Morse, 2010; Judelson et al., 2007; Watson et al., 2005), 15-second Wingate anaerobic tests (Cheuvront, Carter, Haymes, & Sawka, 2006; Kraft et al., 2011), 30-second lower-body Wingate anaerobic tests, 30-second upper body Wingate anaerobic tests (Jones, Cleary, Lopez, Zuri, & Lopez, 2008), single bout sprints (Watson et al., 2005), and repeated effort sprints (Davis et al., 2015; Gann et al., 2016). Since a variety of modalities have been used to measure anaerobic performance, it is possible that

exercise mode could be a determining factor on whether dehydration has a negative impact on performance (Kraft et al., 2012; Savoie et al., 2015). Specifically, body weight-dependent exercises (exercises in which performance is dependent on the participant moving his/her on body weight, e.g. sprints, jumps) may not be negatively impacted by dehydration and performance and may potentially benefit from the loss of body mass associated with dehydration (Savoie et al., 2015).

Along with the exercise modality chosen to measure performance and the level of dehydration achieved, numerous other possible confounders exist that could possibly effect previous results. Using modes of active dehydration (exercise-induced dehydration) or passive dehydration (sauna or hot water bath) on the same day as exercise testing can have a negative influence on performance (Savoie et al., 2015). The exercise used to induce dehydration could lead to fatigue that hinders performance. Similarly, the heat exposure used in both active and passive dehydration could potentially lead to diminished performance in excess of the potential effects of dehydration. In order to attempt to isolate the effects of dehydration, protocols to promote dehydration protocols should be passive and conducted on the day prior to exercise testing to allow the participants an overnight sleep to recover from any possible residual effects of the heat exposure used to induce dehydration (Savoie et al., 2015).

Kraft et al. (Kraft et al., 2012) suggested that a time component (time of anaerobic performance and recovery intervals during repeated bouts) may influence the effects of dehydration on anaerobic performance. Anaerobic bouts lasting < 30 seconds might experience no impairment when dehydrated, while those ≥ 30 seconds will. Similarly, it is suggested that a work-to-recovery ratio exists for repeated bouts of anaerobic exercise,

and the length of the recovery periods between repeated bouts may potentially influence performance in a dehydrated state. If the length of recovery is extended, the deleterious effects of the dehydration may potentially be mitigated (Kraft et al., 2012).

Additionally, inter-individual variability may play a part in how dehydration affects an individual. Gann et al. (2016) found no differences in overall sprint performance, but individual results revealed a majority of participants experienced a practically significant change (± 0.1s) in sprint performance. It has been suggested that training status may possibly influence the impact dehydration has on anaerobic performance. This is a potential explanation for some of the inter-individual variability. Untrained individuals seem to be more susceptible to the adverse effects on performance from dehydration than trained. Moreover, the negative effects of dehydration appear to be more pronounced in untrained and anaerobically-trained participants during repeated anaerobic bouts than those who were aerobically-trained (Kraft et al., 2012; Savoie et al., 2015).

Research suggests that there is a critical level of body mass loss (~ 3%) for anaerobic performance decrements to appear. Likewise, a multitude of other factors are possible confounders in research pertaining to dehydration and anaerobic performance. The potential confounders should be taken into account when investigating the effects of dehydration on anaerobic performance.

Effect of Dehydration on Muscular Strength

The majority of research investigating the effects of dehydration on muscular strength has focused on isometric and isokinetic strength, with equivocal results. The majority of studies found no significant differences in maximal isometric strength or

isokinetic strength (Kraft et al., 2012). However, decreases in isokinetic strength have been seen at slower velocities of 30 º/s, 60 º/s (Hayes & Morse, 2010).

Though little research exists, dehydration has been shown to negatively impact isotonic muscular strength. Following passive dehydration in a sauna, participants' bench press one repetition maximum (1RM) was significantly reduced (Schoffstall et al., 2001). Though the level of dehydration (1.5%) did not reach the apparent threshold to see decrements in anaerobic performance (≥ 3% loss of body mass), bench press 1RM was decreased by 5.6%. These negative effects, however, were negated when participants were allowed two hours to rest and rehydrate. It is plausible that if participants had reached the proposed critical level of dehydration, performance would not have returned to baseline levels following the rest and rehydration, though this is only speculative.

Conversely, it is conceivable that the decrements in bench press 1RM were due to the residual effects of heat exposure from the sauna-induced dehydration protocol. Participants were not exposed to heat for euhydrated trials and for the dehydrated trials, and the 1RM trials were performed on the same day (once body temp returned to baseline) as the dehydration protocol. Though only speculative, it is plausible that with sufficient recovery time following the dehydration protocol, that no differences in performance would have been observed. Further research, accounting for possible confounders, is needed to determine the effects of dehydration on isotonic strength.

Effects of Dehydration on Muscular Endurance

Though research is limited, the effects of dehydration on muscular endurance are more established compared to muscular strength. Two studies have compared the effects of dehydration on multi-set resistance exercise paradigms (Judelson et al., 2007; Kraft et

al., 2010). When completing six sets of back squats under three conditions (euhydrated, dehydrated 2.5% via exercise in a heat chamber, and dehydrated 5% via exercise in a heat chamber), participants experienced diminished performance during both dehydrated trials compared to euhydrated. The 5% dehydration trials yielded greater decreases in performance compared to euhydrated than did the 2.5% dehydration trials (Judelson et al., 2007). Similarly, Kraft et al. (2010) found that passive dehydration (3%) via hot water submersion significantly impaired participants' performance during a full-body resistance exercise protocol when compared to a euhydrated after heat exposure trial. Participants completed 14.9% less total repetitions when dehydrated (Kraft et al., 2010). This decreased muscular endurance performance is potentially, at least in part, due to the increased reliance on aerobic pathways over the course of the exercise bouts (Kraft et al., 2012).

Effects of Dehydration on Power

Vertical Jump

Vertical jump height is commonly used as an indicator of anaerobic power and has been included in the methodology of various studies, with all reaching the same conclusion; no significant change between dehydrated and control trials (Hayes & Morse, 2010; Judelson et al., 2007; Watson et al., 2005). Cheuvront et al. (Cheuvront, Kenefick, Ely, et al., 2010), however, took a different approach. It was hypothesized that dehydration does affect vertical jump height despite previous results showing otherwise. Since, when dehydrated, body mass is lower, if power was not affected then, theoretically, vertical jump height would improve. Previous results have shown no

significant change; therefore, this suggests that there is a deterrent effect on anaerobic power when dehydrated. Subjects performed trials in a euhydrated and a dehydrated state. In the dehydrated trial the subjects performed vertical jumps, with and without a weighted vest to simulate the mass lost from the dehydration protocol. As expected, the jumps performed without the weighted vest showed no significant difference from the euhydrated trial. The jumps performed with the vest, however, showed a 4% reduction in vertical jump height. This suggests that the hypothesis is confirmed, and power output is reduced. This reduction in power, however, is offset by the loss of body mass (Cheuvront, Kenefick, Ely, et al., 2010).

Wingate Anaerobic Tests

Wingate anaerobic tests are another commonly accepted modality for testing anaerobic power. Cheuvront et al. (Cheuvront et al., 2006) examined the effects of dehydration on performance of 15-second Wingate anaerobic tests. Subjects performed a dehydrated (2.7%) and euhydrated trial. During each trial, subjects performed four 15-second Wingate anaerobic tests. No significant difference was found between peak power produced or mean power produced in the two trials (Cheuvront et al., 2006).

In a similar study, Kraft et al. (2011) tested the effects of dehydration on repeated Wingates. Participants completed three trials: control, heat exposed with rehydration, and dehydrated. Similarly to Cheuvront et al. (2006) 15-second Wingate tests were performed; however, six 15-second Wingate tests were performed rather than four, and subjects were dehydrated to 3% rather than 2.7%. Kraft et al. (2011) found that mean power and peak power were significantly reduced for the dehydrated trials compared to the control. Though the difference between control and the other two trials became

noticeable with the third and fourth Wingate tests, the largest differences were observed in the fifth and sixth Wingate tests. These larger differences during the fifth and sixth Wingate tests suggests that the negative effects of dehydration are worsened with subsequent anaerobic exercise bouts (Kraft et al., 2011).

These differing results seem to possibly indicate two things. First, it seems that the higher level of dehydration, 3% as opposed to 2.7%, may perhaps have been a factor in the decreased performance. Likewise, another study found that dehydration > 3% (3.1%) significantly decreased mean and peak power produced in 30-second lower body Wingate anaerobic tests, and 30-second upper-body Wingate anaerobic tests (Jones et al., 2008). These findings are in accordance with what is believed to be the critical level of dehydration (~3% body mass loss) that was not reached by participants in Cheuvront et al. (2006). Secondly, another possible explanation for the differing outcomes is the increase in the amount of Wingate tests performed. This suggests that dehydration possibly elicits the effects of fatigue in repeated trials more rapidly than would be seen when euhydrated (Kraft et al., 2012).

Sprint Performance

Sprint performance, similarly to vertical jump performance, is dependent on individuals propelling their own body weight. Theoretically, sprint performance could potentially be improved when dehydrated. With decreased body mass, the individual should be able to complete the sprint in a shorter amount of time.

Watson et al. (Watson et al., 2005) examined the influence of diuretic-induced dehydration on sprint performance at distances of 50, 200, and 400 meters. Simulated races were performed at each distance during a dehydrated and a control trial. Results

showed that 2.2-2.5% dehydration did not alter sprint performance. The lack of diminished performance could have possibly been the outcome of subjects not reaching a level of dehydration or number of trials needed to induce a negative response (Watson et al., 2005).

Davis et al. (2015) tested the effects of dehydration (3%) on repeated 30 meter sprint performance (3 bouts x 8 sprints) following previous night dehydration via exercise in the heat. No significant differences were found for the first bout of sprints, but sprint times were significantly slower for bouts two and three during the dehydrated trial. Similarly, Gann et al. (2016) used passive dehydration (3%) via hot water bath the night prior to each trial of two bouts of ten 40 yd. sprints: dehydrated (3%) and heat exposed with fluid replacement. Results showed mean sprint performance was not significantly different between dehydrated (3%) and euhydrated trials, though participants reported increased measures of perceptual feelings of difficulty during the dehydrated trial. However, when individual participant's data was considered, a greater effect was observed; 58% of participants experienced a ≥ 0.1 s decrease in mean sprint time for at least one bout, and 25% of participants experienced a ≥ 0.1 s decrease in mean sprint time for both bouts. These results suggest that it is plausible that due to inter-individual variability, the group means disguised the effects observed in many of the participants.

These differing results could potentially be due to the differences in mode of dehydration. Both studies used previous night dehydration, which would mitigate the possible effects of heat exposure on performance. Davis et al. (2015) used exercise in the heat, whereas Gann et al. (2016) used passive heat exposure. Though participants were given overnight to recover, it is still possible that participants were still fatigued from the

previous night's exercise. Another, possible cause for the differing results is the homogeneity of participants used by Davis et al. (2015) compared to Gann et al. (2016). Davis et al. (2015) used only college baseball players, as opposed to Gann et al. (2016) using a mixture of college athletes from various sports (football, basketball, soccer, and track). While all athletes used where anaerobically fit, training for each sport was varied, with some (e.g. basketball, soccer, and baseball) utilizing more aerobic conditioning than others (e.g. football players and track sprinters). These differences in training are a potential confounding factor in dehydration research (Kraft et al., 2012).

Considerations for Research with Female Participants

Menstrual Cycle

The menstrual cycle is a series of changes that occur in the female reproductive system to prepare for a potential reproduction. The first menstrual cycle, or menarche, occurs with the onset of puberty and continues throughout the female's life until menopause. The typical menstrual cycle lasts approximately 28 days, though can vary from 23-35 days depending on the individual and is divided into four phases: menstrual phase, follicular phase, ovulation, and luteal phase (Nader, 2012; Sherman & Korenman, 1975). This cycle is governed by various hormonal changes. The stimulation and inhibition of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland is controlled by gonadrotropin-releasing hormone (GnRH). FSH and LH, in turn, regulate the release of estrogen and progesterone, which direct the movement of the menstrual cycle through each of the four stages (Dalkin et al., 1999; Sharma et al., 2012).

During a normal 28 day cycle, the menstrual phase begins on the first day of menstruation and continues until day five. This phase is commonly known as the woman's "period". During this phase, the endometrium (uterine lining) is shed and is expelled out of the vagina as menstrual fluid. The expulsion of menstrual fluid, known as menses, lasts for five days, though the majority of discharge occurs in the first three days (Sharma et al., 2012; Sherman & Korenman, 1975).

The follicular phase also begins on day one and lasts through day 13. During this phase, the hypothalamus releases GnRH, stimulating FSH production. Numerous follicles in the ovaries are stimulated by FSH. These follicles produce large amounts of estrogen. Eventually, all but one of these follicles cease development and a dominant follicle emerges. This dominant follicle continues to grow and will produce a mature egg. Rising estrogen levels results in the growth of the endometrium to prepare for the potential pregnancy (Lenton, Landgren, Sexton, & Harper, 1984; Midgley & Jaffe, 1968; Sharma et al., 2012; Sherman & Korenman, 1975).

During the ovulation phase (Day 14), an increase in estrogen stimulates the production of LH. This release of LH assists in maturing and weakens the wall of the follicle and leads to the mature egg being released from the ovary. Once released the egg is moved into the fallopian tube. Here, the egg can live up to 24 hours awaiting fertilization. If fertilization occurs, the egg attaches to the endometrium and pregnancy begins. If the egg is not fertilized, it degenerates and the endometrium begins to break down (Midgley & Jaffe, 1968; Sherman & Korenman, 1975).

The final phase of the menstrual cycle is the luteal phase (Day 15-28). The remainder of the follicle that released the egg is altered to become the corpus luteum. The

newly formed corpus luteum produces progesterone and suppresses the production of FSH and LH. Decreased levels of FSH and LH cause the corpus luteum to atrophy, which prompts a drop in progesterone levels. The decreased levels of progesterone initiate the breakdown of the endometrium and the beginning of menstruation (Lenton, Landgren, & Sexton, 1984; Midgley & Jaffe, 1968; Sharma et al., 2012; Sherman & Korenman, 1975).

Physiological Response to the Menstrual Cycle

Hormonal changes during the various stages of the menstrual cycle impact the reproductive system, as well as various other systems throughout the body. The sex hormones, estrogen and progesterone, have been shown to have an influence on the central nervous system, respiratory system, cardiovascular system, and the renin-aldosterone system (Bayliss & Millhorn, 1992; McEwen & Alves, 1999; Oelkers, 1996; Stumpf, 1990). However, evidence suggests that these changes in hormonal levels across the different phases of the menstrual cycle only result in few significant changes in physiological responses (Marsh & Jenkins, 2002).

Menstrual cycle phase appears to have little, if any, effect on cardiovascular response. While studies have shown differences in resting and exercise heart rate between phases (Hessemer & Brück, 1985, 1985), the majority of research found no significant differences (Bemben, Salm, & Salm, 1995; Galliven et al., 1997; Jurkowski, Jones, Toews, & Sutton, 1981; Lebrun, McKenzie, Prior, & Taunton, 1995; Marsh & Jenkins, 2002). Similarly, the majority of studies have reported no differences in plasma volume (Chapman et al., 1997; De Souza, Maguire, Rubin, & Maresh, 1990; Horvath & Drinkwater, 1982; Stachenfeld, Silva, & Keefe, 2000) or hematocrit (Chung, Goldfarb, Jamurtas, Hegde, & Lee, 1999; Hackney, McCracken-Compton, & Ainsworth, 1994;

Lebrun et al., 1995; McCracken, Ainsworth, & Hackney, 1994; Tenaglia, McLellan, & Klentrou, 1999) between menstrual cycle phases; although a few studies have found decreased plasma volume (Calzone, Silva, Keefe, & Stachenfeld, 2001; Stephenson & Kolka, 1988) and increased hematocrit (Stachenfeld et al., 2000) during the luteal phase. Overall, it seems that cardiovascular physiology is mostly unaffected by menstrual cycle phase (Marsh & Jenkins, 2002),

Like cardiovascular response, the menstrual cycle has little effect on respiratory response. While studies have shown an increase in oxygen consumption (VO_2) at rest during the luteal phase (Hessemer & Brück, 1985; Williams & Krahenbuhl, 1997), and maximal oxygen consumption (VO_{2Max}) expressed in absolute terms (L/Min) during the early follicular phase (Lebrun et al., 1995), the majority of research show no differences in VO_2 or VO_{2Max} (Bemben et al., 1995; De Souza et al., 1990; Galliven et al., 1997; Hackney et al., 1994; Horvath & Drinkwater, 1982; Jurkowski et al., 1981; Schoene, Robertson, Pierson, & Peterson, 1981; Stephenson & Kolka, 1988).

Body weight, blood lactate concentrations, sweat rate, and ratings of perceived exertion (RPE) have also been found to be unaffected by menstrual cycle phase (Marsh & Jenkins, 2002). However, an increased threshold for the onset of sweating (Hessemer & Brück, 1985; Inoue et al., 2005; Marsh & Jenkins, 2002; Stephenson & Kolka, 1985) and increased core temperature during the luteal phase have been observed (Coyne, Kesick, Doherty, Kolka, & Stephenson, 2000; Hessemer & Brück, 1985, 1985; Pivarnik, Marichal, Spillman, & Morrow, 1992; Stephenson & Kolka, 1993). With this increased sweat threshold and increased core temperature, increased care should be taken during

heat exposure, both active (e.g. exercise in the heat) and passive (e.g. sauna or hot bath) to reduce any potential risk of heat illness during the luteal phase.

Research suggests fluid balance and hydration status are not impacted by menstrual cycle phase. Hydration status measured via urine specific gravity (USG) was not different across menstrual cycle phases before or after exercise (Yasuda, Kawai, Hara, Iide, & Matsumura, 2012). Similarly, total body water was not significantly different between mid-follicular and mid-luteal phases (McKinley-Barnard et al., manuscript in review). Following exercise-induced dehydration, fluid replacement was not affected by menstrual cycle phase (Maughan et al., 1996).

Exercise Performance and the Menstrual Cycle

Fluctuations of hormone levels throughout the menstrual cycle have been theorized to possibly change the physiological responses of the female body, but these fluctuations appear to have little effect on the body's physiology (Marsh & Jenkins, 2002). These same hormone changes have been theorized to possibly affect exercise performance in females depending on the phase of the menstrual cycle.

As previously noted, menstrual cycle phase appears to have little influence on resting VO_2 or VO_{2Max} (Bemben et al., 1995; De Souza et al., 1990; Galliven et al., 1997; Hackney et al., 1994; Horvath & Drinkwater, 1982; X. A. K. Janse de Jonge, 2003; Jurkowski et al., 1981; Marsh & Jenkins, 2002; Schoene et al., 1981; Stephenson & Kolka, 1988). Likewise, menstrual cycle phase has little to no effect on factors affecting VO_{2Max} and maximal performance (heart rate, plasma volume, hematocrit, body weight, blood lactate concentrations, sweat rate, and RPE) (Bemben et al., 1995; Chapman et al., 1997; Chung et al., 1999; De Souza et al., 1990; Galliven et al., 1997; Hackney et al.,

1994; Horvath & Drinkwater, 1982; Jurkowski et al., 1981; Lebrun et al., 1995; Marsh & Jenkins, 2002; McCracken et al., 1994; Stachenfeld et al., 2000; Tenaglia et al., 1999).

Similarly, submaximal and steady-state aerobic exercise seems to be minimally affected by menstrual cycle phase; although, some discrepancies exist (Janse de Jonge, 2003). The majority of research show no differences between menstrual cycle phase for steady state VO_2 (De Souza et al., 1990; Dombovy, Bonekat, Williams, & Staats, 1987; Galliven et al., 1997; Hackney et al., 1994; Jurkowski et al., 1981; Nicklas, Hackney, & Sharp, 1989; Pivarnik et al., 1992), time to exhaustion (Jurkowski et al., 1981; Lebrun et al., 1995; McCracken et al., 1994; Nicklas et al., 1989), heart rate (De Souza et al., 1990; Dombovy et al., 1987; Galliven et al., 1997; Jurkowski et al., 1981), or RPE (De Souza et al., 1990; Galliven et al., 1997; Hackney et al., 1994). However, there have been instances of contradictory findings where VO_2 was increased during steady state aerobic exercise (Hessemer & Brück, 1985; Williams & Krahenbuhl, 1997). Despite these conflicting results, it seems menstrual cycle phase has little effect on submaximal or steady state aerobic exercise.

The effect of menstrual cycle phase on muscular contractile characteristics has been equivocal. In a review, the authors suggested that these discrepancies were due to methodological deficiencies (Janse de Jonge, 2003). No changes were seen for electrically stimulated muscular contractile strength (Janse de Jonge, Boot, Thom, Ruell, & Thompson, 2001; White & Weekes, 1998), isokinetic strength (Gür, 1997; Janse de Jonge et al., 2001; Lebrun et al., 1995), and fatigability (Gür, 1997; Janse de Jonge et al., 2001) across menstrual cycle phases. Similarly, no differences were seen in muscular

strength or hypertrophy across menstrual cycle phases following a 12-week resistance training protocol (Sakamaki-Sunaga, Min, Kamemoto, & Okamoto, 2016).

Gender Differences in Thermoregulation

Thermoregulation is the process in which the human body maintains a homeostatic temperature through heat gain and heat loss. When core temperature is elevated, the body's primary mode of cooling is through evaporation of sweat. Due to gender related differences in physiology, body composition, and anthropometric characteristics, thermoregulation may transpire differently in males and females.

Thermoregulatory responses during exercise are dependent on fitness level, heat acclimatization, body mass, body surface area, body composition and surface-to-mass ratio (Andérson, Ward, & Mekjavić, 1995; Havenith, Luttikholt, & Vrijkotte, 1995). Females, in general, have less body mass and body surface area than men. Less mass is advantageous in regards to thermoregulation due to lessened heat production. However, having a smaller surface area also results in reduced heat dissipation (Kenney, 1985). Females also have a greater surface-to-mass ratio compared to males. This is a thermoregulatory advantage due to a greater relative area for heat dissipation (Green, Bishop, Muir, & Lomax, 2000; Shapiro, Pandolf, Avellini, Pimental, & Goldman, 1980). Also, as stated above, core temperature and sweat threshold are increased during the luteal phase of the menstrual cycle (Coyne et al., 2000; Hessemer & Brück, 1985, 1985; Inoue et al., 2005; Marsh & Jenkins, 2002; Pivarnik et al., 1992; Stephenson & Kolka, 1985, 1993).

Compared to men, women produce a lower total volume of sweat (Green et al., 2000; Kenney, 1985) but have a greater total number of sweat glands and a greater

density of sweat glands (Bar-Or, Magnusson, & Buskirk, 1968). Also, women have shown a delayed sweat response compared to men, meaning females have an increased sweat response threshold (Bittel & Henane, 1975; Grucza, 1990). However, both genders respond similarly to increases in core temperature, besides sweat volume differences, when matched for fitness level, body composition, and body size (Green et al., 2000).

Differences in thermoregulation do exist due to differences in body mass, surface area, body composition and fitness levels. Women have a lower total volume of sweat compared to men, but have a greater total of active sweat glands as well as a greater surface-to-mass ratio than men. Despite these differences, thermoregulatory effectiveness is similar between genders.

Effects of Dehydration on Females

To date, the majority of research pertaining to the effects of dehydration has focused mainly on male participants. However, female athletes and exercisers have been shown to suffer from dehydration (Thigpen et al., 2014; Volpe et al., 2009) and to underestimate sweat loss during exercise (O'Neal et al., 2012; Thigpen et al., 2014), which could lead to further dehydration.

Research has shown that dehydration in females can increased perceived difficulty of tasks, worsen mood, and hinder concentration of females with as little as 1-2% loss of body mass (Armstrong et al., 2012). Likewise, loss of 2% body mass has been shown to diminish skill performance and decision making time tests in elite female athletes (MacLeod & Sunderland, 2012). Sawka et al. (1983) found that female participants appear to respond to similarly to male participants physiologically when dehydrated. A 2% loss of body mass resulted in an increase in heart rate, core

temperature, and RPE after 120 minutes of steady-state cycling (Logan-Sprenger, Heigenhauser, Killian, & Spriet, 2012).

Exercise-induced dehydration was found to significantly increase resting, exercise, and recovery heart rate following a modified Harvard step test and decrease strength measures by 7-10%; although the differences were not statistically significant (Greenleaf, Prange, & Averkin, 1967). These results are potentially misleading due to methodological issues. A between-subjects design was used. This would not account for any individual physiological differences between subjects in each group.

A 4% reduction in body mass via exercise in the heat did not significantly affect muscular strength. It did, however, reduce muscular endurance by 15% compared to euhydration (Montain et al., 1998). This reduction in muscular endurance could potentially be attributed to possible confounders in the study design. Participants were dehydrated via exercise in the heat. It is plausible that diminished muscular endurance could potentially be a result of fatigue from the exercise protocol or residual effects related to the heat exposure. .

Grip strength and row strength were not affected by a loss of 1.4% body mass following passive heat exposure. However, vertical jump performance was improved by 5.9% compared to euhydration (Gutiérrez, Mesa, Ruiz, Chirosa, & Castillo, 2003). The lack of difference found for strength measures could possibly be explained by the participants not reaching the proposed critical level of dehydration (≥3%). Interestingly, vertical jump was improved by the lowered body mass, unlike previous studies involving men.

To date, little research exists investigating the effects of dehydration on female participants. It appears that females respond to dehydration similarly to males in regards to variables affecting aerobic performance, mood, concentration, and skill performance. Studies investigating the effects of dehydration on females during anaerobic performance are minimal. The few studies that have been conducted all have methodological issues that could potentially confound the outcomes.

Catabolic Response to Exercise

The hypothalamus-pituitary-adrenal (HPA) axis is a key governing system in the body that connects the central nervous system and the endocrine system. The HPA axis is responsive to stress and helps the body maintain homeostasis and normal physiological function. In response to stress, the hypothalamus releases corticotropin-releasing hormone (CRH). This CRH, in turn, stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary. ACTH prompts the adrenal cortex to secrete steroid hormones called glucocorticoids, the end product of the HPA axis (Kudielka & Kirschbaum, 2005; Nicolaides, Kyratzi, Lamprokostopoulou, Chrousos, & Charmandari, 2015). In situations to physical stress, HPA axis response does not differ between genders (Kudielka & Kirschbaum, 2005).

The primary glucocorticoid is cortisol, the majority of which is bound in the blood to binding proteins, with only ~10% circulating as free cortisol. This circulating free cortisol is elevated during times of psychological and physical stress (Kraemer & Ratamess, 2005). Through an intercellular glucocorticoid receptor (GR), cortisol works as an inhibitor of muscle protein synthesis (MPS) through multiple mechanisms. The GR

is expressed throughout the body and regulates the expression of glucocorticoid responsive genes.

Cortisol has been shown to limit amino acid transport into skeletal muscle by blunting amino-acid induced $p70^{S6k}$ phosphorylation and inhibiting mRNA translation initiation, and by impeding the binding of mRNA to the 43S preinitiation complex. This limiting of amino acid transport leads to a diminished rate of MPS, an increase in the net protein degradation in the muscle, and an increase in the rate of muscle protein breakdown, thereby leaving the individual in a catabolic state. If this continues over a period of time, muscle atrophy could potentially occur (Zhenqi Liu et al., 2004; Löfberg et al., 2002).

In addition to limiting amino acid transport, cortisol impedes the stimulatory effects of various moderators of MPS. Increased cortisol blunts the amino acid-induced phosphorylation of 4E-BP1 and S6K1, downstream effectors of mammalian target of rapamycin (mTOR). Likewise, increased cortisol results in insulin resistance and the inhibition of leucine uptake, as well as blunting the effects of insulin-like growth factor 1 (IGF-1) (Liu et al., 2001; Liu et al., 2004; Louard, Bhushan, Gelfand, Barrett, & Sherwin, 1994; Simmons, Miles, Gerich, & Haymond, 1984).

There is evidence suggesting the up-regulation of muscle proteolysis via cortisol occurs through the activation of the ubiquitin protease system (UPS), autophagy, and calcium-dependent proteolytic system (calpains) (Tiao et al., 1996). Cortisol binds to nuclear receptors known as glucocorticoid receptors (GR). The primary function of the GR complex is regulation of gene expression through transactivation or transrepression, inducing or repressing gene transcription by directly binding to DNA response elements,

or physically associating with other transcription factors (e.g. NF-κB, AP-1) (Schakman, Gilson, & Thissen, 2008). The GR complex upregulates gene transcription associated with the UPS that increase rates of protein ubiquitination or directly increase proteolytic activities of the proteasome. Calpains are thought to dissociate actin and myosin to be degraded by the UPS. Additionally, in the lysosomal system, the GR complex is also believed to up-regulate expression of enzymes related to protein degradation (cathepsin L) (Schakman et al., 2008; Tiao et al., 1996).

The phosphatidyl inositol-3 kinase (PI3K)/Akt pathway is involved with the downstream activation of mTOR and is a mediator for the anabolic effects of insulin and IGF-1. Insulin receptor substrate 1 (IRS-1) plays a critical role as a signal intermediator between insulin and IGF-1 and the PI3K/Akt pathway. The catabolic effects of cortisol are theorized to be a result of causing a decreased expression of IRS-1, and thus an inhibition of the PI3K/Akt pathway. This results in an inhibition on mRNA translation and ensuing MPS, which can possibly lead to a catabolic state (Nakao et al., 2009).

Catabolic Response to Dehydration

To date, research investigating the catabolic response to dehydration is limited and has yielded equivocal results. Cortisol levels were significantly elevated in elite wrestlers using dehydration for rapid pre-competition weight loss. A correlation was found between dehydration level and cortisol levels (e.g. higher levels of dehydration equated higher cortisol levels) (İrfan, 2015).

Equivocal results have been found in regards to cortisol response to aerobic exercise in a dehydrated state. Moderated dehydration (1.3%-3.9%) has been shown to have no effect on cortisol during low- and moderate-intensity aerobic exercise (Hoffman

et al., 1994; Svendsen, Killer, & Gleeson, 2014). Conversely, Maresh et al. (2006) found that plasma cortisol levels were elevated pre- and post-treadmill running in a dehydrated state, regardless of exercise intensity.

Research suggests that acute bouts of resistance exercise alone result in significant increases in cortisol and the cortisol stimulating hormone, ACTH (Kraemer & Ratamess, 2005). However, it appears that only one investigation on the catabolic response to dehydration and anaerobic performance is currently available. Judelson et al. (2008) investigated the effects of dehydration on resistance exercise induced markers of anabolism, and catabolism. Participants completed six sets of back squats (80% of 1RM) in three different hydration states (euhydrated, 2.5% dehydrated, 5% dehydrated). It was found that dehydration of 4.8% increased markers of catabolism (cortisol, epinephrine, and norepinephrine) and decreased markers of anabolism (testosterone). This suggests that dehydration negates the resistance exercise-induced increases in circulating testosterone and increases the stress hormone response. Though research is limited, dehydration appears to significantly increase catabolic hormones and blunt the anabolic response during resistance exercise (Judelson et al., 2008).

Conclusions

Current literature suggests that a 3% loss of body mass or more is needed to impair the different dimensions of anaerobic performance. In addition to a critical level of dehydration, various other possible confounders exist that could influence research including: variations in modalities used to achieve dehydration (exercise-induced, diuretic-induced, or passive), variations in modalities used to measure anaerobic performance, heat stress influence, participant fitness level, inter-individual variability,

and exercise duration. Menstrual cycle phase appears to have little effect on physiological and performance variables despite fluctuations in sex hormone levels; however, core temperature and sweat activation is elevated during the luteal phase. Though limited research is available, dehydration seems to exhibit similar effects on females as men. Cortisol is released in response to both physical and psychological stress. Elevated cortisol levels results in diminished MPS and muscle protein degradation. Prolonged periods of catabolism can potentially lead to muscle atrophy. Current research on the influence of dehydration on markers of catabolism during exercise is limited and has produced equivocal results. Further research is needed to determine the effects of dehydration on anaerobic performance and markers of catabolism in female participants.

CHAPTER THREE

Methods

Participants

Ten apparently healthy, resistance-trained (2-3 times per week for at least 6 months) females between the ages of 18-30 and a body mass index between 18.5-30 kg/m2 completed the study. Enrollment was open to women of all ethnicities. Only participants considered as low risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM) were allowed to participate. All participants were eumenorrheic and had not taken any form of birth control for at least 6 months prior to participation. Participants began the study upon cessation of menses and completed all trials during the follicular phase of the menstrual cycle. All participants provided written informed consent and were cleared for participation by passing a mandatory medical screening. All eligible subjects signed university-approved informed consent documents and approval was granted by the Institutional Review Board for Human Subjects. Additionally, all experimental procedures involved in the study conformed to the ethical consideration of the Declaration of Helsinki.

Experimental Approach

In a randomized, cross-over design, participants visited the laboratory on 4 separate occasions over a 2-week period in the following manner: Visit 1 = dehydration with or without fluid replacement, Visit 2 = resistance training testing, vertical jump

testing, blood draw, muscle biopsy, urine specific gravity, plasma volume, and perceptual measures, Visit 3 = dehydration with or without fluid replacement, Visit 4 = resistance training testing, vertical jump testing, blood draw, muscle biopsy, urine specific gravity, plasma volume, and perceptual measures. Participants began testing sessions upon cessation of menses. At visit 1, participants were randomly determined using a random number generator (www.random.org) to complete the dehydration procedures with or without fluid replacement first. The entire duration of the study was approximately one year.

Muscle Strength and Endurance Assessment

In order to determine possible effects of the hypohydration on muscular strength, participants performed 1 repetition maximum (1-RM) tests on the free-weight bench press and angled leg press exercises at visit 2 and 4. Participants warmed up by completing 5 to 10 repetitions at approximately 50% of the estimated 1-RM. The participant rested for 1 minute, and then completed 3 to 5 repetitions at approximately 70% of the estimated 1-RM. The weight was then increased conservatively, and the participant attempted to lift the weight for one repetition. If the lift was successful, the participant rested for 2 minutes before attempting the next weight increment. This procedure continued until the participant failed to complete the lift. The 1-RM was recorded as the maximum weight that the participant was able to lift for 1 repetition. In order to assess muscle endurance, using the bench press and angled leg press exercises, participants performed as many repetitions as possible with 75% of their 1-RM for 5 sets. A recovery period of 2 minutes was given between each set.

Vertical Jump

Vertical jump height was assessed using a Vertec (Sports Imports, Columbus OH) jump height measurement system. Participants performed 3 countermovement jumps from a standing position. The highest attempt was recorded. Participants completed this test 3 times with a recovery period of 1 minute was given between attempts.

Dehydration Procedures

Prior to arrival, participants were encouraged to aggressively hydrate during the day and consume a minimum of 500 ml of fluid 1 hour before arriving. Upon arrival, participant's pre-treatment weight was recorded wearing the swimming suit they wore during treatment. Participants were then submerged in a hot water bath (~40º C). Core temperature and water temperature were continuously monitored. If core temperature reached or exceeded 38.9º C, participants were removed from the water and allowed to cool before returning to the water. This safety temperature is the temperature used in previous investigations (Gann et al., 2016; Kraft et al., 2010, 2011). Also, if participants experienced any adverse feelings (nausea, light headedness etc.) they were removed from the water. Participants exited the water and toweled off and weighed every 30 minutes, before returning to the water. For the euhydrated/heat exposed trial, participants consumed water equal to 75% of the fluid they lost during the next 30 min submersion interval. This continued until their total sweat loss minus the fluid replacement equaled a loss of ~3.5% body mass. Upon completion, participants were given an amount of fluid to consume overnight to equal 125% of total fluid lost minus fluid given during treatment. Participants consumed this water before returning, but were allowed/encouraged to drink extra as the purpose of this trial is to have participants

exposed to heat but not be dehydrated. Dehydration procedures for the hypohydrated trial were performed in the same manner as described above minus the fluid replacement. Participants were given one 500 ml bottle of water to consume before returning.

Body Composition Assessment

Body composition assessment was performed in line with our previous studies (Willoughby & Leutholtz, 2013; Willoughby, Spillane, & Schwarz, 2014) at Visit 1. Total body mass (kg) was determined on a standard dual beam balance scale (Detecto Bridgeview, IL). Percent body fat, fat mass, and fat-free mass were determined using DEXA (Hologic Discovery Series W, Waltham, MA). Quality control calibration procedures were performed on a spine phantom (Hologic X-CALIBER Model DPA/QDR-1 anthropometric spine phantom) and a density step calibration phantom prior to each testing session. The DEXA scans were segmented into regions (right & left arm, right & left leg, and trunk). Each of these segments were analyzed for fat mass and fat-free mass. Based on previous studies in our laboratory, the accuracy of the DEXA for body composition assessment is ± 3.7% as assessed by direct comparison with hydrodensitometry and scale weight. Total body water was determined by bioelectric impedance analysis (Tanita Inc., Arlington Heights, IL). Total body water was determined with bioelectrical spectroscopy (Tanita Inc., Arlington Heights, IL) using a low energy, high frequency current (500 micro amps at a frequency of 50 kHz).

Venous Blood Sampling

At Visit 2 and 4, venous blood samples were obtained from the antecubital vein into one 10 ml serum collection tube using a standard vacutainer apparatus. The serum

tubes were allowed to stand at room temperature for 10 min, and then centrifuged at 2,000 rpm. The serum was removed and frozen at -80°C for later analysis. For each exercise session, blood samples were obtained immediately pre-exercise and 1 hour post-exercise.

Percutaneous Muscle Biopsies

At Visit 2 and 4, percutaneous muscle biopsies (20-30 mg) were obtained from the middle portion of the right vastus lateralis muscle at the midpoint between the patella and the greater trochanter of the femur at an approximate depth between 1 and 2 cm using a 14-gauge fine needle aspiration biopsy instrument (Tru-Core I Biopsy Instrument, Medical Device Technologies, Gainesville, FL) based on our previously-published procedures (Schwarz et al., 2013). A small area of the skin (cleaned with alcohol and betadine) was anesthetized with a subcutaneous injection of the topical anesthetic 1% Lidocaine. After the initial biopsy, the next biopsy attempts extracted tissue from approximately the same location as the initial biopsy by using the pre-biopsy markings and depth markings on the needle. After removal, adipose tissue was removed and muscle specimens were immediately stored at -80°C for subsequent analysis. For each exercise session, muscle samples were obtained immediately pre-exercise and 1 hour post-exercise.

Urine Specific Gravity Assessment

Participants were asked to provide a urine sample prior to treatment in order to determine urine specific gravity (USG) (the concentration of particles in the urine) to assess pre-treatment hydration status using an automated urine analyzer (Clinitek Status

+, Siemens, Malvern, PA). Upon completion of USG analysis, the urine sample was safely discarded in an appropriately-marked biohazard waste container.

Perceptual Measures

Following each set during the resistance training protocol, participants were asked to estimate ratings of perceived exertion (RPE) a 10-point omni scale. Participants were shown the scale for reference. Ten minutes following completion of each resistance training session participants were asked to estimate their session RPE (sRPE) for the entire session using the 10-point omni scale. Prior to resistance training sessions, participants also estimated subjective feelings for thirst, and previous night's sleep quality using a 10cm visual analog scale. Perceived recovery status (PRS) and perceived readiness was estimated prior to resistance exercise testing using a scale developed by Laurent et al. (2011).

Assessment of Hematocrit

From the blood samples obtained at the testing sessions, hematocrit was determined by, first, transferring a portion of the whole blood into a capillary tube. This tube was then centrifuged (Hettich Haematokrit 200 Tabletop Centrifuge, Hettich Instruments, Beverly, MA) to separate blood plasma from red blood cells. Hematocrit was measured manually via buffy coat analysis using the method established by Dill & Costill (1974). Total red blood cell volume was subtracted from total blood volume. The remaining volume was recorded as hematocrit.

Assessment of Serum Cortisol

From the blood samples obtained at the testing sessions, cortisol levels were determined using a commercially available enzyme-linked immunoabsorbent assay (ELISA) kit (Cayman Chemical Company, Ann Arbor, MI, #5000360). Standards (8 total) were made via serial dilution starting at a concentration of 4,000pg/ml and ending at 6.6pg/ml. After 50µl of sample per well were added, 50µl of the cortisol AChE tracer was added to all wells except for the blank wells. Next, 50µl of the monoclonal antibody were added to all wells except for the nonspecific binding and blank wells. The plates were then covered with a plate film and incubated overnight at 4°C. The following morning the plates were then emptied and rinsed five times with wash buffer. The plates were then reconstituted with 200µl of Ellman's Reagent and 5µl of tracer was added to the total activity wells. The plates were then covered with a plastic film and placed on an orbital shaker for 90 minutes to develop in the dark. All samples were run in duplicate and the assays were performed at 450 nm wavelength with a microplate reader (iMark, Bio-Rad, Hercules, CA). Hormone concentrations were determined using data reduction software (Microplate Manager, Bio-Rad, Hercules, CA) and final cortisol concentration expressed relative to plasma volume.

Skeletal Muscle Cellular and Nuclear Extraction and Protein Content

Portions of each muscle sample were homogenized using a cell extraction buffer (item # FNN0011, Life Technologies, Grand Island, NY, USA) and a tissue homogenizer. The cell extraction buffer was supplemented with phenylmethanesulphonyl fluoride (PMSF) (item # P7626, Sigma Chemical Company, St. Louis, MO, USA) and a protease inhibitor cocktail (item # P2714, Sigma Chemical

Company, St. Louis, MO, USA) with broad specificity for the inhibition of serine, cysteine, and metallo-proteases (Ferreira, 2014; Taylor, 2012). Remaining muscle samples were homogenized in nuclear extraction buffer (item # 40410, Active Motif, Carlsbad, CA). The nuclear extracts were supplemented with a phosphatase inhibitor buffer (Bufford, 2010; Cooke, 2011). Total protein content for cellular- and nuclear-extracted samples were analyzed in duplicate and determined spectrophotometrically using the Bradford assay at a wavelength of 595 nm and using bovine serum albumin as the standard (Ferreira 2014).

Assessment of Skeletal Muscle Transcription Factor Protein Levels

Protein levels of GR/DNA binding were determined using commercially-available ELISA kits (Active Motif, Carlsbad, CA, # 45496). This assay contains an oligonucleotide containing a GRE consensus sequence, and GR contained in nuclear extract binds specifically to this oligonucleotide. A total of 30 µl of complete binding buffer was added to each well. Next 20 µl of sample was added to the sample wells and 20µl of complete lysis buffer was added to the blank wells. Plates were sealed with adhesive cover and incubated for 1 hour at room temperature with mild agitation (100 rpm on rocking platform). Following incubation, 100 µl of diluted GR antibody was added to all wells, incubated for 1 hour and then washed 3 times with 200 µl of wash buffer. Next, 100 µl of diluted HRP-conjugated antibody were added to all wells, incubated for 1 hour at room temperature, and then washed 4 times with 200 µl of wash buffer. Finally, with 100 µl of developing solution was added to all wells and incubated for 20 minutes before 100 µl of stop solution was added. The plates were then read at 450 nm wavelength and in duplicate according to manufacturer instructions with a microplate

reader (iMark, Bio-Rad, Hercules, CA). Protein concentrations were determined using data reduction software (Microplate Manager, Bio-Rad, Hercules, CA), and the final concentration was expressed relative to muscle protein content.

Assessment of Skeletal Muscle Gene Expression

From the four muscle tissue samples obtained at each of the two testing sessions (eight total), mRNA expression of myostatin, MuRF1, MAFbx, Foxo1, REDD1, and Atrogin-1 were analyzed using reverse transcription real-time polymerase chain reaction (RT-PCR) utilizing our standard laboratory procedures (Buford 2010).

Total RNA Isolation from Skeletal Muscle Samples

Approximately 10 - 15 mg of muscle tissue was used for biochemical analysis. Total cellular RNA was extracted from homogenate of biopsy samples with a monophasic solution of phenol and guanidine isothiocyanate contained within the TRI-reagent (Sigma Chemical Co., St. Louis, MO). Five hundred μL of TRI-Reagent was added to each tube, and then muscle samples were homogenized using a pestle. One hundred μL of chloroform was added to each tube and shaken, then allowed to sit for 15 minutes. This process separated the samples into three distinct phases, a lower (pink) organic phase which contains the protein, a middle (gray) interphase containing the DNA, and an upper (clear) aqueous phase containing the RNA. Using a sterile transfer pipette, the clear aqueous phase was transferred into a new microfuge tube. The remaining interphase and organic phase was stored in an ultra-low freezer at -80^{o}C. Subsequently, 250 μL of 100% isopropanol was added to each tube and allowed to sit at room temperature for 5 to 10 minutes. Samples were then centrifuged at 12,000 x g at 2 to 8^{o}C

for 10 minutes, allowing for the formation of a RNA pellet. The supernatant was discarded, then 500 µL of 75% ethanol was added then vortexed to wash the pellet. The samples were then centrifuged at 7500 x g at 2 to 8ºC for 5 minutes then the supernatant discarded. The washing procedure was repeated twice. The pellet was allowed to air dry for 5 to 10 minutes, then 50 µL of nuclease free water was added to the microtube. Total RNA concentrations from each sample were determined spectrophotometrically with an optical density of 260 nm (OD260) and to verify RNA integrity and absence of RNA degradation, indicated by an OD_{260}/OD_{280} ratio of approximately 2.0. The final concentration adjusted to 200 ng•µl-1 by diluting the crude total RNA extracts into DEPC-treated nuclease-free H2O. The RNA samples were stored at -80°C until later analyses.

Reverse Transcription and Complementary DNA Synthesis

A reverse transcription reaction mixture [e.g., 1 µl of total cellular RNA, 4 µl 5× reverse transcription buffer, a dNTP mixture containing dATP, dCTP, dGTP, and dTTP, MgCl2, RNase inhibitor, an oligo(dT)15 primer, 10 µL of nuclease-free H2O and 1 U•µl-1 MMLV reverse transcriptase enzyme (Bio-Rad, Hercules, CA)] were incubated at 42°C for 40 minutes, heated to 85°C for five minutes, and then quick-chilled on ice yielding the complementary DNA (cDNA) product. The standardized cDNA solutions were frozen at -80°C until real-time RT-PCR was performed.

Oligonucleotide Primers for Polymerase Chain Reaction (PCR)

The mRNA sequences of human skeletal muscle REDD1 (NC_000010.11), MuRF1 (NC_000894.1), Myostatin (NC_030685.1), Atrogin-1 (GCF_000001405.33),

MAFbx (GCF_000001405.33), Foxo1 (GCF_000001405.33) , and GAPDH (GCF_000001405.33) published in the NCBI Entrez Nucleotide database (www.ncbi.nlm.nih.gov) were used to construct PCR primers using Beacon Designer software (Bio-Rad, Hercules, CA, USA), and then commercially synthesized (Integrated DNA Technologies, Coralville, IA). These primers (Table 1) amplify fragments of base pairs for myostatin, MuRF1, MAFbx, Foxo1, REDD1, and Atrogin-1 mRNA.

Table 1
Primer Sequences

GAPDH	Forward	5'- AAA GCC TGC CGG TGA CTA AC -3'
	Reverse	5'- CGC CCA ATA CGA CCA AAT CAG A -3'
Myostatin	Forward	5'- CCA GGA GAA GAT GGG CTG AA -3'
	Reverse	5'- CAA GAC CAA AAT CCC TTC TGG AT -3'
MuRF1	Forward	5'- CCT GAG AGC CAT TGA CTT TGG -3'
	Reverse	5'- CTT CCC TTC TGT GGA CTC TTC CT -3'
MAFbx	Forward	5'- CCC AAG GAA AGA GCA GTA TGG AGA -3'
	Reverse	5'- GGG TGA AAG TGA AAC GGA GCA -3'
Foxo1	Forward	5'- TTG TTA CAT AGT CAG CTT G -3'
	Reverse	5'- TCA CTT TCC TGC CCA ACC AG -3'
REDD1	Forward	5'- TGA GGC ACG GAG TGG GAA -3'
	Reverse	5'- CAG CTC GAA GTC GGG CAA -3'
Atrogin-1	Forward	5'- GCA GCT GAA CAA CAT TCA GAT CAC -3'
	Reverse	5'- CAG CCT CTG CAT GAT GTT CAG T -3'

RT-PCR

Aliquots of cDNA were added to each of the PCR reactions for, myostatin, MuRF1, MAFbx, Foxo1, REDD1, Atrogin-1, and GAPDH. Specifically, each PCR reaction contained the following mixtures: 2 µl of cDNA template along with 10.0µl of 2× SYBR Green Supermix (Bio-Rad, Hercules, CA) [100 mM KCl mixture, 40 mM Tris-

HCl, 0.4 mM of each dNTP, 50 U•ml-1 of iTaq DNA polymerase, 6.0 mM MgCl2, SYBR Green I, 20 nM flourescein], 1.5 µl of sense and anti-sense primers, and 5.0 µl nuclease-free dH2O. Each PCR reaction was amplified with a thermal cycler (Bio Rad, Hercules, CA) and the amplification sequence involved a denaturation step at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds, and extension at 72°C for 60 seconds. RT-PCR was performed over 40 cycles with emitted fluorescence from the SYBR green fluorophore being measured after each cycle. An emission of fluorescence occurs due to the integration of the SYBR green into the double-stranded cDNA produced during the PCR reaction. GAPDH was used as an external reference standard for detecting relative change in the quantity of target mRNA due to its consideration as a constitutively expressed housekeeping gene (Thellin et al., 1999). All C_T values were assessed in the linear portion of amplification and a DNA melting curve analysis was performed after amplification to assure that the single gene products were amplified in absence of primer-dimers. Quantification of all mRNA were expressed relative to GAPDH expression using the delta C_T protocol.

Oligonucleotide primers were designed using known human mRNA sequences available online through the NCBI database. The expression of mRNA was determined from the ratio of the C_T values relative to GAPDH. Delta changes from rest were expressed by subtracting the baseline GAPDH/target C_T ratio from the GAPDH target C_T ratio for each time point. The specificity of the PCR was demonstrated with an absolute negative control reaction containing no cDNA template, and single gene products confirmed using DNA melt curve analysis.

Statistical Analysis

Data for all blood and muscle markers was analyzed with separate 2 (trial) x 2 (time) analysis of variance (ANOVA) to determine any significant differences for the main effects of trial and time and for the trial x time interaction. Significant interactions were further analyzed with paired t-tests. However, to protect against Type I error, the conservative Greenhouse-Geisser correction factor was used to evaluate observed within-group F-ratios. Data for muscular strength, endurance, power, and perceptual measures was analyzed with separate paired t-tests. Data for all heart rate measures was analyzed with separate 2 (trial) x 5 (time) analysis of variance (ANOVA) to determine any significant differences for the main effects of trial and time and for the trial x time interaction. Significant interactions were further analyzed with one-way ANOVA. Statistical Package for Social Sciences (SPSS) for Windows Version 20.0 software (SPSS, Chicago, IL) was used for all analyses. Significant differences among groups were identified by a Tukey HSD post-hoc test. However, to protect against Type I error, the conservative Greenhouse-Geisser correction factor was used to evaluate observed within-group F-ratios. For statistical procedures, a probability level of ≤ 0.05 was adopted throughout the study.

CHAPTER FOUR

Results

Consort Data

In all, 12 participants were screened for the study, with two being unable to participate due to use of birth control. All ten eligible participants that began the study continued until completion.

Anthropometric Data

Physical and anthropometric data for the participants is expressed in Table 2.

Table 2
Anthropometric Data

Variable	Mean ± SD
Age (years)	22.0 ± 2.1
Height (cm)	164.5 ± 5.0
Bodyweight (kg)	61.9 ± 19.0
Body Fat %	26.7 ± 2.9

Note : SD = standard deviation; cm = centimeters; kg = kilograms; % = percentage.

Hydration Measures

Each of the 10 participants hydration status was assessed via dehydration percentage (DEHY %) total body water (TBW), total body water percentage (TBW %), hematocrit (Hct), and 4 participants hydration status was assessed via urine specific gravity (USG). Paired-samples t tests revealed no significant differences for TBW ($p = 0.360$) and TBW% ($p = 0.099$). However, DEHY % ($p < 0.001$), Hct ($p = 0.041$), and

USG ($p < 0.001$) were significantly higher for DT compared to HT. Data for all measures of hydration status are expressed in Table 3.

Table 3
Hydration Data

Variable	DT	HT
DEHY %	3.3 ± 0.6*	0.6 ± 0.9
TBW	30.8 ± 3.3	31.0 ± 2.7
TBW%	51.8 ± 3.1	51.1 ± .8
Hct	42.0 ± 3.3*	40.4 ± 3.3
USG	1.030 ± 0.002*	1.018 ± 0.002

Note: $p \leq 0.05$ indicated by *; DEHY = dehydration; TBW = total body water; TBW % = total body water percentage; Hct = hematocrit; usg = urine specific gravity.

Performance Measures

Paired t-tests revealed bench press 1RM ($p = 0.04$) was significantly lower for DT compared to HT. No significant difference was found for leg press 1RM ($p = 0.22$). There was no difference in total reps completed for bench press ($p = 0.44$) or leg press ($p = 0.21$) for DT compared to HT. No significant differences were found for total volume lifted for bench press ($p = 0.36$) or leg press ($p = 0.13$) for DT compared to HT. There was no significant difference ($p = 0.11$) for vertical jump height. Data for muscular strength, endurance, and power is expressed in Table 4.

Table 4

Performance Data

Variable	DT	HT
Bench 1RM	42.7 ± 14.5 kg*	44.1 ± 13.9 kg
Bench Reps	33.5 ± 5.0	33.0 ± 5.5
Bench Volume	1067.0 ± 355.3 kg	1102.0 ± 390.1 kg
Leg Press 1RM	216.1 ± 55.0 kg	223.4 ± 55.7 kg
Leg Press Reps	42.6 ± 20.3	45.8 ± 19.7
Leg Press Volume	6831.6 ± 3849.1 kg	7564.1 ± 4007.9 kg
Vertical Jump	45.8 ± 5.2 cm	46.9 ± 6.0 cm

Note: $p \leq 0.05$ indicated by *; 1RM = one rep maximum

Perceptual Measures

RPE was not significantly different following bench press ($p = 0.36$) or leg press ($p = 0.50$). SRPE was not significantly different ($p = 0.20$) for DT vs HT. Significant differences for PRS ($p = 0.004$) and PR ($p = 0.001$) indicate participants expected impaired performance during DT. Feelings of thirst were significantly higher ($p < 0.001$) for DT vs HT. Estimations of sleep quality were significantly lower ($p = 0.02$) for DT vs HT. Data for perceptual measures is expressed in Table 5.

Table 5

Perceptual Data

Variable	DT	HT
Bench RPE	7.5 ± 1.2	7.4 ± 0.8
Leg Press RPE	7.5 ± 1.4	7.5 ± 1.7
Session RPE	7.2 ± 1.1	6.9 ± 1.9
PRS	5.1 ± 2.2*	7.2 ± 1.1
Perceived Rediness	4.2 ± 1.0*	2.5 ± 0.5
Thirst	6.7 ± 2.5*	2.2 ± 2.4
Sleep	4.6 ± 3.1*	7.6 ± 1.8

Note: $p \leq 0.05$ indicated by *; RPE = rating of perceived exersion; PRS = perceived recovery status

Heart Rate Measures

Bench Press Heart Rate

The heart rate data for each set of bench press is presented in Figure 1. No significant interaction between session and time for heart rate was observed [$F(4, 36) = 1.088$, $p = .720$, partial $\eta 2 = .036$]. The main effect of session revealed no statistically significant difference in heart rate between trials [$F(1, 9) = .637$, $p = .445$, partial $\eta 2 = .066$]. The main effect of time demonstrated no statistically significant difference in heart rate between time points [$F(4, 36) = .461$, $p = .763$, partial $\eta 2 = .235$]. Mauchly's test of sphericity indicated that the assumption of sphericity had been violated for the interaction between session and time [$\chi 2(9) = 18.994$, $p = .029$; therefore, a Greenhouse-Geisser correction was applied ($\varepsilon = 0.507$).

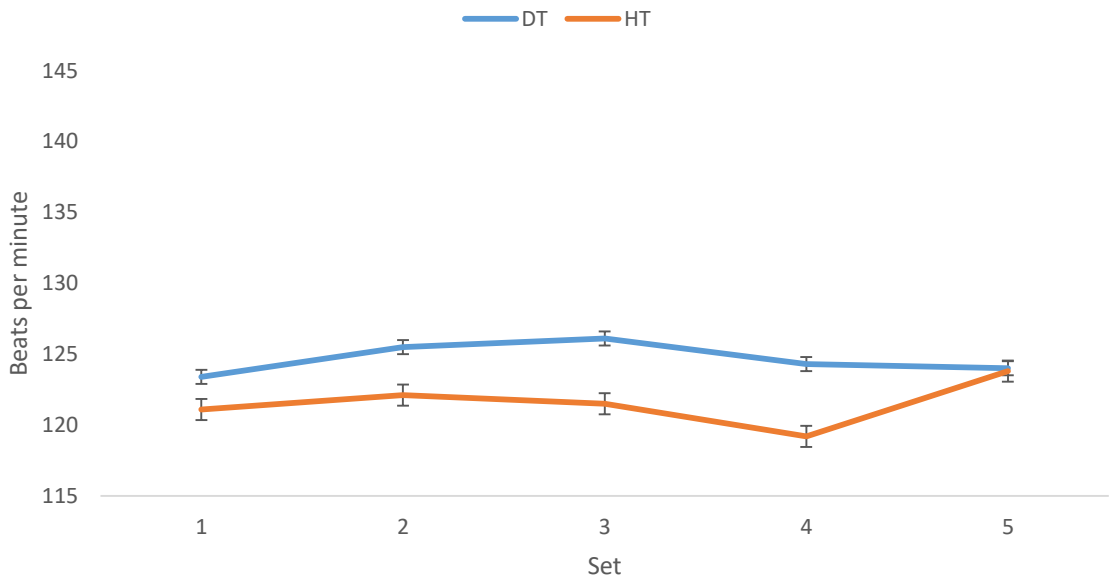

Figure 1. Heart rate in beats per minute following each set of bench press. * = significantly different from baseline.

Leg Press Heart Rate

The heart rate data for each set of leg press is presented in Figure 2. No significant interaction between session and time for heart rate was observed [F(4, 36) = 1.493, p = .314, partial η2 = .499].The main effect of session revealed no statistically significant difference in heart rate between trials [F(1, 9) = .037, p = .852, partial η2 = .004]. The main effect of time demonstrated a statistically significant difference in heart rate between time points [F(4, 36) = 4.05, p = .042, partial η2 = .310]. Mauchly's test of sphericity indicated that the assumption of sphericity had been violated for time [χ2(9) = 23.130, p = .007]; therefore, a Greenhouse-Geisser correction was applied (ε = 0.448). Post-hoc analyses revealed significantly higher heart rate for set 5 vs. set 1 (p = .024), vs. set 2 (p = .037), vs. set 3 (p = .045), and vs. set 4 (p = .026).

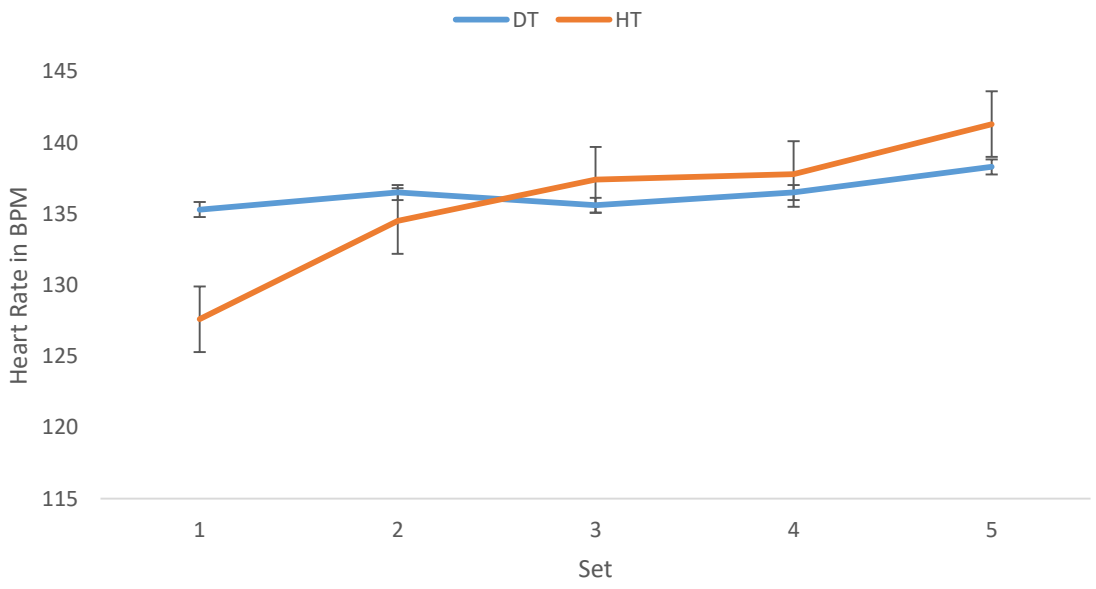

Figure 2. Heart rate in beats per minute following each set of leg press. * = significantly different from baseline.

Serum Cortisol

Serum cortisol pre- and post- each exercise session are presented in Figure 3. There was no statistically significant interaction between session and time [F(1, 9) = .020, p = .890, partial η2 = .002]. The main effect of session revealed no statistically significant difference between trials [F(1, 9) = .067, p = .801, partial η2 = .007]. The main effect of time demonstrated no statistically significant difference between time points [F(1, 9) = 1.666, p =.229, partial η2 = .156].

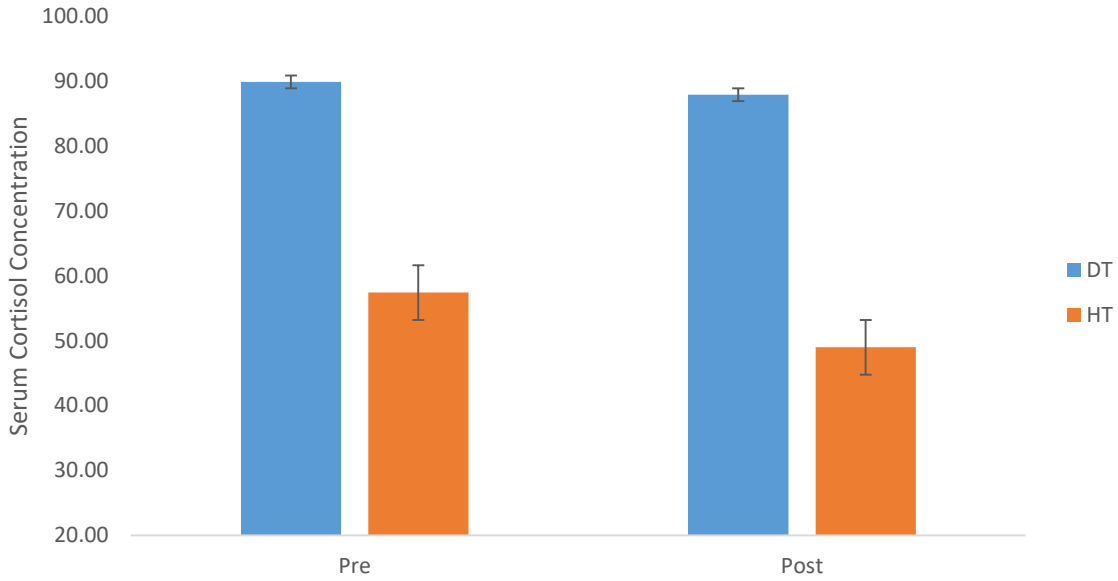

Figure 3. Serum cortisol concentrations for each time point. * = significantly different from baseline.

Glucocorticoid Receptor-DNA Binding

The binding of GR-DNA pre- and post- each exercise session is presented in Figure 4. There was no statistically significant interaction between session and time [F(1, 9) = .018, p = .898, partial η2 = .002]. The main effect of session revealed a statistically significant difference between trials [F(1, 9) = 5.569, p = .043, partial η2 = .382]. Paired-samples t tests revealed a significant difference post-exercise between DT and HT (p =

.016). The main effect of time demonstrated no statistically significant difference between time points [F(1, 9) = 2.533, p =.146, partial η2 = .220].

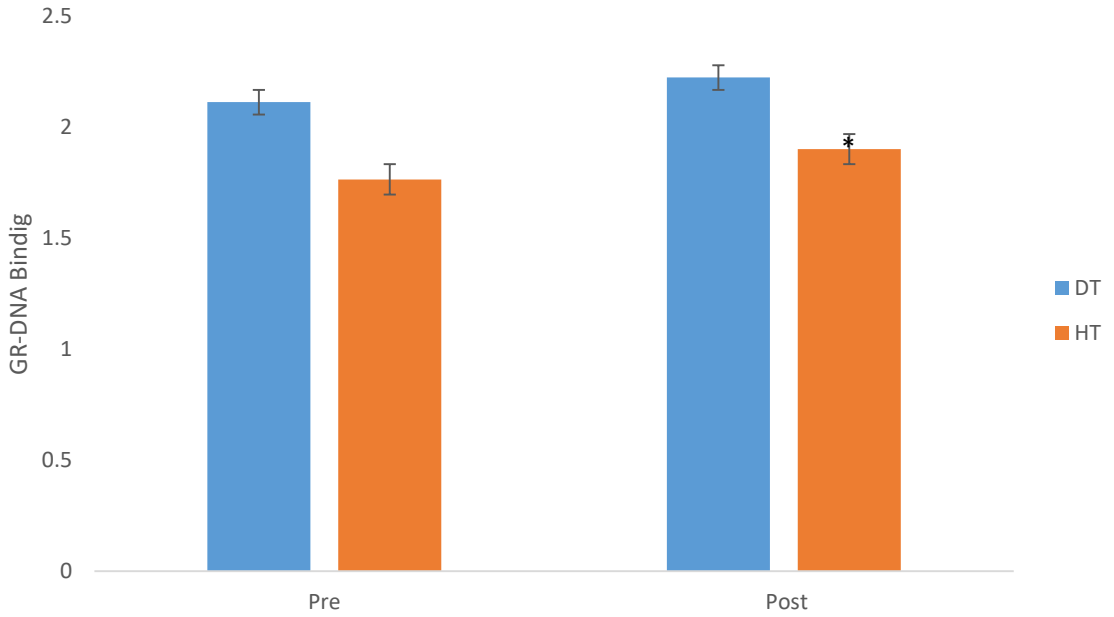

Figure 4. GR-DNA binding for each time point. * = significantly different between trials.

Gene Expression

MuRF1 mRNA Expression

The mRNA expression of MuRF1 pre- and post- each exercise session is presented in Figure 5. There was no statistically significant interaction between session and time for MuRF1 mRNA expression [F(1, 9) = .692, p = .427, partial η2 = .071]. The main effect of session revealed no statistically significant difference in MuRF1 mRNA expression between trials [F(1, 9) = 2.244, p = .168, partial η2 = .200]. The main effect of time demonstrated no statistically significant difference in MuRF1 mRNA expression between time points [F(1, 9) = .646, p =.442, partial η2 = .067].

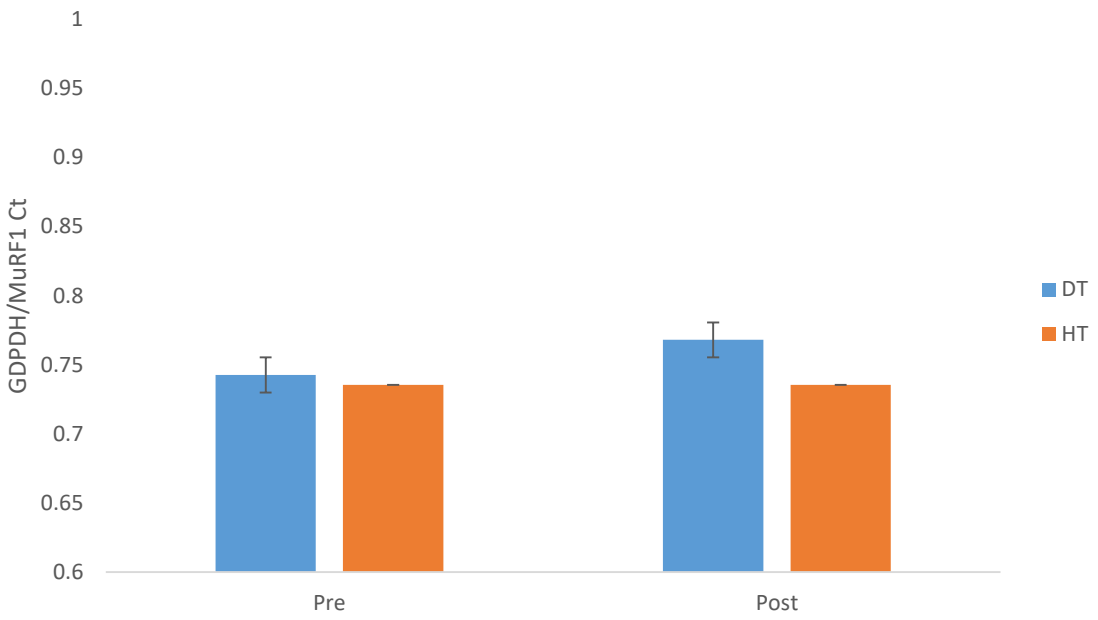

Figure 5. MuRF1 mRNA expression (GDPDH/MuRF1 Ct Ratio) for each time point. Ct = threshold cycle. * = $p \leq 0.05$.

MAFbx mRNA Expression

The mRNA expression of MAFbx pre- and post- each exercise session is presented in Figure 6. There was no statistically significant interaction between session and time for MAFbx mRNA expression [$F(1, 9) = .254$, $p = .627$, partial $\eta 2 = .027$]. The main effect of session revealed no statistically significant difference in MAFbx mRNA expression between trials [$F(1, 9) = .299$, $p = .598$, partial $\eta 2 = .032$]. The main effect of time demonstrated a statistically significant difference in MAFbx mRNA expression between time points [$F(1, 9) = 14.922$, $p = .004$, partial $\eta 2 = .624$]. Paired t-tests revealed significant increases for MAFbx mRNA expression from pre vs. post for DT ($p = .006$).

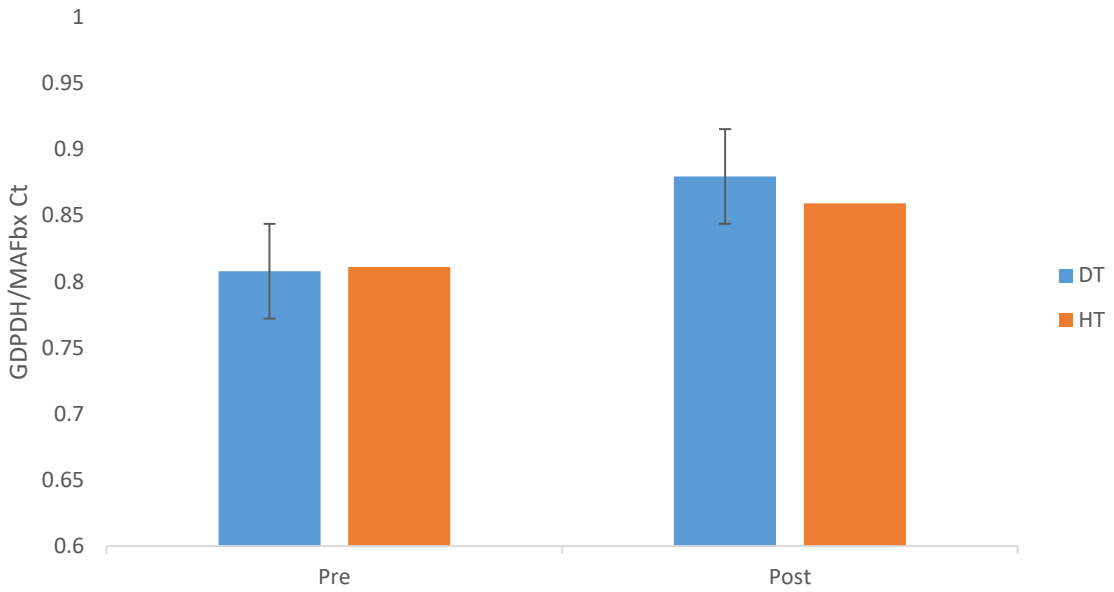

Figure 6. MAFbx mRNA expression (GDPDH/MAFbx Ct Ratio) for each time point. Ct = threshold cycle. * = $p \leq 0.05$.

Myostatin mRNA Expression

The mRNA expression of Myostatin pre- and post- each exercise session is presented in Figure 7. There was no statistically significant interaction between session and time for Myostatin mRNA expression [$F(1, 9) = 1.247$, $p = .293$, partial $\eta 2 = .122$]. The main effect of session demonstrated no statistically significant difference in Myostatin mRNA expression between time points [$F(1, 9) = .143$, $p = .714$, partial $\eta 2 = .016$]. The main effect of time revealed a statistically significant difference in Myostatin mRNA expression between sessions [$F(1, 9) = 16.871$, $p = .003$, partial $\eta 2 = .652$]. Post-hoc analyses revealed significant increase for Myostatin mRNA expression from pre vs. post for DT ($p = .009$), and HT ($p = .031$).

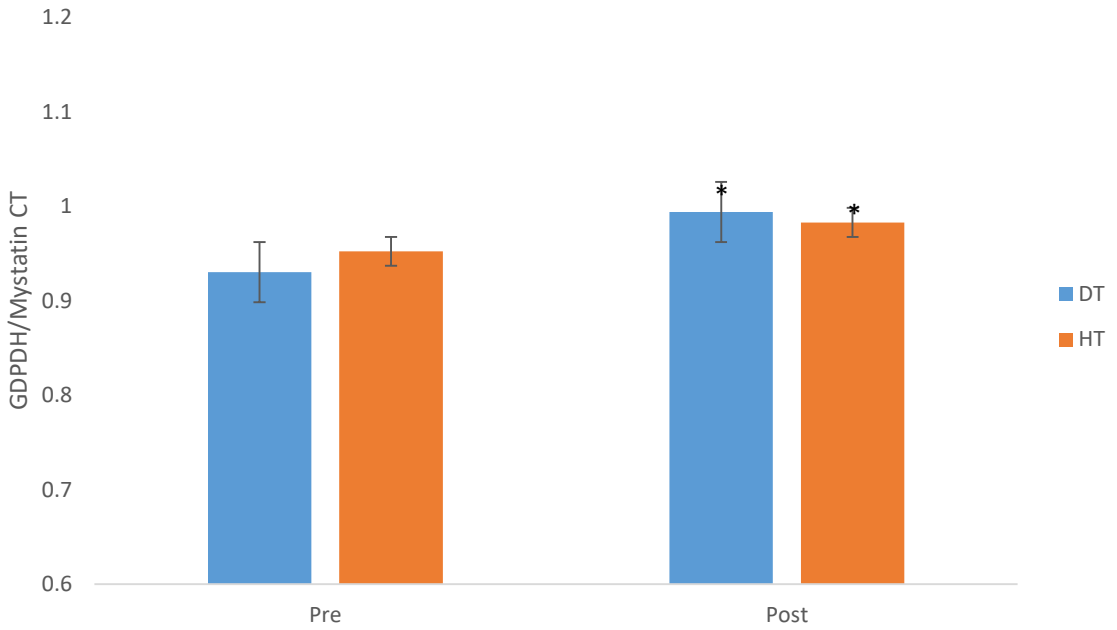

Figure 7. Myostatin mRNA expression (GDPDH/Myostatin Ct Ratio) for each time point. Ct = threshold cycle. * = $p \leq 0.05$.

Atrogin-1 mRNA Expression

The mRNA expression of Atrogin-1 pre- and post- each exercise session is presented in Figure 8. There was no significant interaction between session and time for Atrogin-1 mRNA expression [$F(1, 9) = .478$, $p = .507$, partial $\eta 2 = .050$]. The main effect of session revealed no statistically significant difference in Atrogin-1 mRNA expression between trials [$F(1, 9) = 020$, $p = .890$, partial $\eta 2 = .002$]. The main effect of time demonstrated no statistically significant difference in Atrogin-1 mRNA expression between time points [$F(1, 9) = .182$, $p = .680$, partial $\eta 2 = .020$].

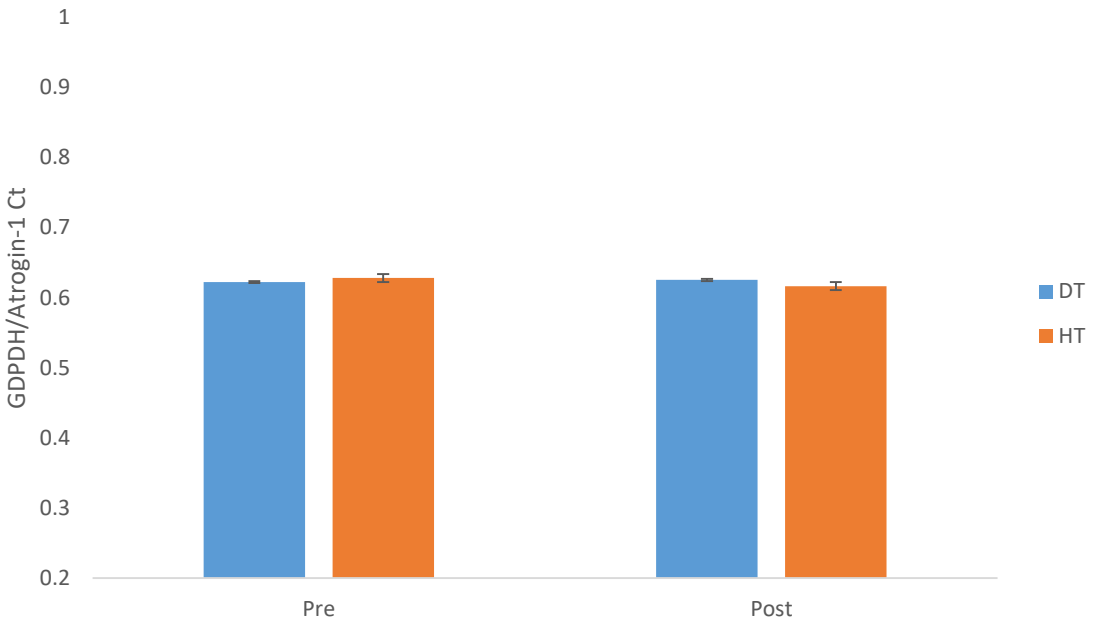

Figure 8. Atrogin mRNA expression (GDPDH/Atrogin Ct Ratio) for each time point. Ct = threshold cycle. * = $p \leq 0.05$.

Redd1 mRNA Expression

The mRNA expression of REDD1 pre- and post- each exercise session is presented in Figure 9. There was no statistically significant interaction between session and time for REDD1 mRNA expression [$F(1, 9) = 3.824$, $p = .082$, partial $\eta 2 = .298$]. The main effect of session revealed no statistically significant difference in REDD1 mRNA expression between trials [$F(1, 9) = .115$, $p = .742$, partial $\eta 2 = .061$]. The main effect of time demonstrated a statistically no significant difference in REDD1mRNA expression between time points [$F(1, 9) = .001$, $p = .973$, partial $\eta 2 = .000$].

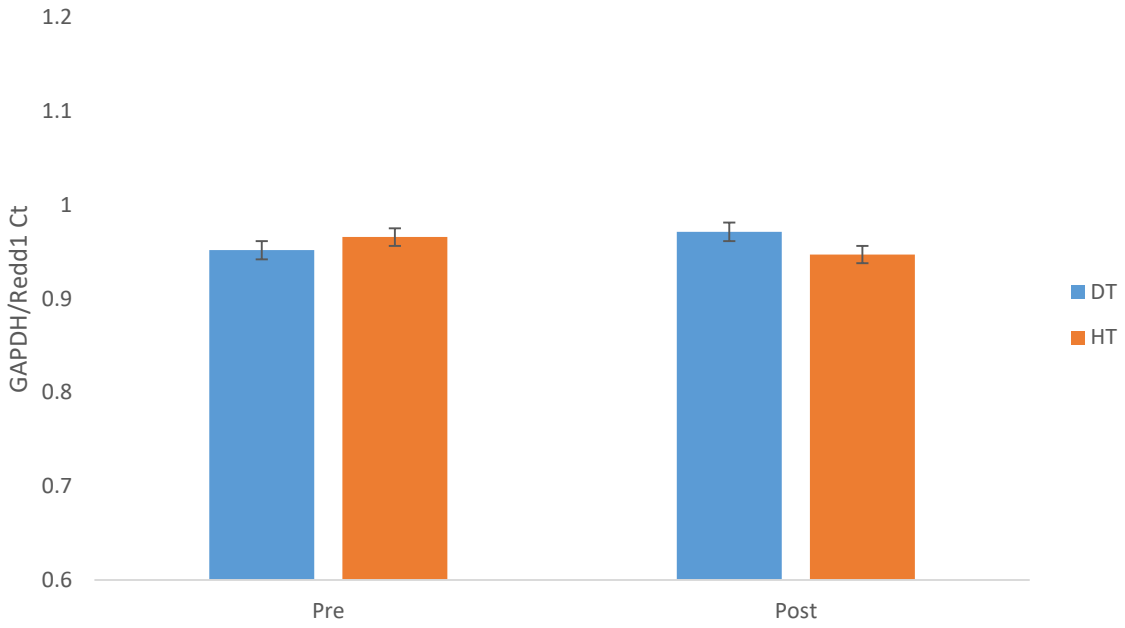

Figure 9. Redd1 mRNA expression (GDPDH/Redd1 Ct Ratio) for each time point. Ct = threshold cycle. * = $p \leq 0.05$.

FoxO1 mRNA Expression

The mRNA expression of FoxO1 pre- and post- each exercise session is presented in Figure 10. There was no statistically significant interaction between session and time for Foxo1 mRNA expression [$F(1, 9) = 1.395$, $p = .268$, partial $\eta 2 = .134$]. The main effect of session revealed no statistically significant difference in Foxo1 mRNA expression between trials [$F(1, 9) = .415$, $p = .55$, partial $\eta 2 = .044$]. The main effect of time demonstrated no statistically significant difference in Foxo1 mRNA expression between time points [$F(1, 9) = 1.113$, $p = .319$, partial $\eta 2 = .110$].

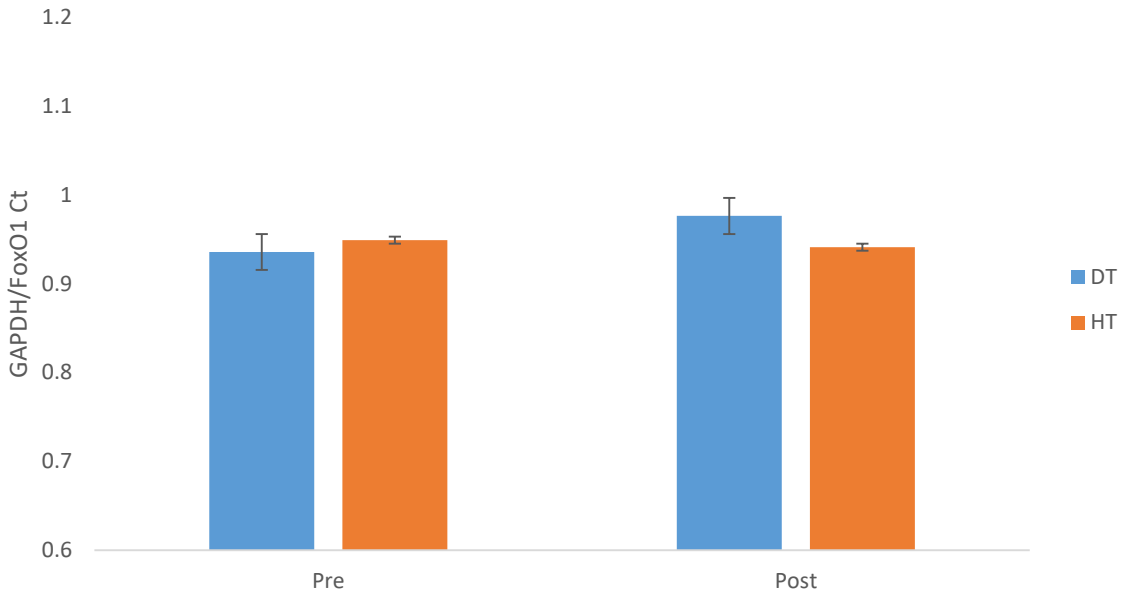

Figure 10. FoxO1 mRNA expression (GDPDH/FoxO1 Ct Ratio) for each time point. Ct = threshold cycle. * = $p \leq 0.05$.

CHAPTER FIVE

Discussion

Introduction

Hypohydration is a common problem for athletes, those participating in exercise programs and those that work in the heat. Hypohydration has been shown to diminish aerobic performance with loss of 2% body mass (American College of Sports Medicine et al., 2007), and anaerobic performance with loss of 3% body mass (Kraft et al., 2012). Hypohydration has been previously shown to negatively affect muscular strength (Schoffstall et al., 2001), muscular endurance (Kraft et al., 2010), and muscular power (Kraft et al., 2011). Likewise, dehydration has been shown to negatively affect perceptual measures of perceived difficulty and recovery (Gann et al., 2016; Kraft et al., 2010, 2011). It is possible that negative effects of some previous studies could potentially be influenced by residual effects of heat exposure or exercise from the dehydration protocol unless an overnight rest was given (Savoie et al., 2015).

Underestimation of sweat loss can potentially lead to inadequate fluid replacement following exercise and has been shown to affect both men and women (O'Neal et al., 2012; Thigpen et al., 2014). This suggests that females are at an equal risk in experiencing progressive dehydration. Despite this, virtually all research pertaining to the effects of dehydration on anaerobic performance has been performed using male participants only. This lack of can potentially be attributed to the possible difficulties that accompany using female participants (menstrual cycle variability, hormone fluctuations across phases, etc.). Despite these potential confounders, further research is warranted

due to the thermoregulatory differences between males and females (Kaciuba-Uscilko & Grucza, 2001).

Cortisol, a glucocorticoid, is elevated during physically and mentally stressful situations and operates through its intracellular glucocorticoid receptor (GR). Through the GR, cortisol inhibits muscle protein synthesis (MPS). This leaves an individual in a catabolic state and over time could lead to muscle atrophy (Löfberg et al., 2002). Previous research concerning hypohydration and catabolic exercise response has thus been equivocal (Judelson et al., 2008; Yamamoto et al., 2008). To date, it appears that no published data are available investigating the catabolic hormonal response to an acute bout of anaerobic exercise at moderate hypohydration (3% body mass lost) in females..

Performance Measures

Previous research has found hypohydration of ~3% loss of body mass to negatively affect anaerobic performance across various modes of testing (Kraft et al. 2012). To date this is the first study to examine the effects of hypohydration on muscular strength, endurance, and power in females. The current study measured muscular strength via 1RM testing for bench press and leg press. Bench press 1RM was significantly reduced when hypohydrated. No statistical differences were seen for leg press 1RM. However, when one outlier was removed from analysis, the difference approached significance ($p = 0.06$). These findings are in agreement with the Schoffstall et al. (2001), who found bench press 1RM to be reduced when hypohydrated. One major difference between the current study and the study performed by Schoffstall et al. (2001) was the dehydration protocol used by each. Schoffstall et al. (2001) used same day passive dehydration, while the current study used previous night passive dehydration. The current

design allowed for an overnight sleep to help eliminate the potential lasting effects of heat exposure as a potential confounder and isolate the effects of hypohydration.

Total repetitions and total volume lifted during five sets or both bench press and leg press were modalities used to test muscular endurance in the current study. No significant differences were found for total repetitions or total volume for either bench press or leg press. Participants completed 5 sets of each exercise using 75% of their measured 1RM. While there was no statistical difference, total repetitions were reduced for leg press, even though the average weight lifted was less due to lower average 1RM. Coinciding with this, total volume was not significantly lower; however, bench press volume was reduced by an average of 35.0 kg and leg press was reduced by an average of 732.5 kg. Previously, hypohydration has been shown to reduce the number of reps completed across a full-body exercise protocol (Kraft et al., 2011). Though only two exercises were completed in the current study, it is plausible that over and entire workout this trend would continue and each additional exercise would experience a reduction of reps and/or volume. If hypohydration were to be a chronic issue and this decrease in mechanical stimuli were to continue, it is plausible to assume that muscular hypertrophy could be hindered and potentially result in muscular atrophy.

Vertical jump is commonly used as a measure of anaerobic power. In the current study, vertical jump was not significantly different for DT compared to HT. Previous studies using vertical jump as a modality to test anaerobic power all reached similar conclusions (Hayes & Morse, 2010; Judelson et al., 2007; Watson et al., 2005).

Heart rate was not significantly different between trials. Previous studies have found an increase in heart rate coinciding with hypohydration (Kraft et al., 2010, 2011;

Gann et al., 2016). While the differences between trials was not significantly different, heart rate was increased during DT. When combined with the reduced performance during DT, it appears that while not statistically significant, hypohydration does increase the physiological strain experienced during exercise. Furthermore, it is plausible that over the course of a full body workout, that this trend would continue.

Perceptual Measures

Previous research has determined that hypohydration has a negative impact on both RPE and sRPE during various modes of anaerobic exercise (Gann et al., 2016; Kraft et al., 2010, 2011). The current study, however, found no differences for RPE for bench press, leg press, or sRPE. During a full body resistance training protocol, RPE was unchanged while total repetitions were significantly lowered when hypohydrated (Kraft et al., 2010). Similarly, the current study observed no change in RPE or sRPE with hypohydration, but performance was decreased. This suggests that hypohydration did negatively impact perceived exertion. That is, hypohydration resulted in decreased performance, yet this lower volume of work was perceived as similarly difficult.

Recovery plays an important role in physical performance. During the current study, PRS and perceived readiness were significantly impaired prior to participating in DT compared to HT, meaning participants felt perceptually less recovered when hypohydrated. Though only one previous study has examined the effects of hypohydration on perceived recovery (Gann et al., 2016), both have found that participants feel less recovered when hypohydrated and subsequently perform worse compared to HT. It appears that the PRS and PR scales are sensitive to hypohydration

and can potentially be used to predict performance; however further research is needed before a definitive conclusion can be reached.

Serum Cortisol

Cortisol is elevated during times of physical, mental and emotional stress, and has been shown to inhibit MPS (Kraemer & Ratamess, 2005). The current study found no differences in serum cortisol; although, serum cortisol was elevated both pre- and post-exercise sessions during DT compared to HT. Even though not statistically significant, increased serum cortisol prior to exercise when hypohydrated suggests that there is an increased physiological or psychological strain associated with hypohydration.

Prior research on hypohydration's effect on cortisol levels, though limited, has been equivocal. Hypohydration has been found to be significantly raised in wrestlers who used dehydration as a means of pre-competition weight loss, with a significant correlation found between higher levels of cortisol and higher levels of hypohydration (Irfan et al., 2015). Cortisol has also been found to be elevated prior to and post aerobic exercise following dehydration (Maresh et al., 2006). Conversely, hypohydration has been shown to have no effect on cortisol during aerobic exercise (Hoffman et al., 1994; Svendsen, Killer, & Gleeson, 2014). Judelson et al. (2008) found that hypohydration increased cortisol and blunted the anabolic response during resistance exercise. Further research is needed to determine the extent that cortisol levels are affected by hypohydration.

Glucocorticoid Receptor-DNA Binding

Cortisol works as an inhibitor of MPS through binding to its glucocorticoid receptor. The GR is expressed throughout the body and regulates the expression of

glucocorticoid responsive genes. The GR prompts activation of various proteolytic genes and induces muscle proteolysis (Taio et al., 1996). To date, few studies have investigated the effect of exercise on GR-DNA binding and to our knowledge, the current study is first to investigate the effects of hypohydration on GR-DNA binding. GR-DNA binding was elevated for DT compared to HT with a significant difference found post exercise. Though not significant, GR-DNA binding was increased following exercise in both trials. This supports the findings of Willoughby et al. (2003). This study induced muscle damage through eccentric exercise. It is possible that the lack of significant difference in the current study could be potentially due to not inducing enough mechanical stress with only two exercises being performed. Though only speculative, it is possible that a greater difference would be observed with a total body workout. Further research is needed to determine the efficacy of this theory. It should be noted that GR-DNA binding was elevated prior to exercise for DT. This suggests that hypohydration induces a physiological stress response even without the presence of physical stress (e.g. exercise). This supports the findings that of Savoie et al. (2015) that an overnight recovery negates the effects of heat exposure as a result of the dehydration protocol.

Gene Expression

MuRF1

The MuRF1 gene is a proposed regulator of the atrophy process and is expressed in skeletal muscle. It has been suggested that the MuRF1 gene is synergistically activated by FoxO1 and GR-DNA binding (Mascher et al., 2008; Waddell et al., 2008). To our knowledge, the current study is the first to investigate the effects of hypohydration on

MuRF1 mRNA gene expression. No significant interaction was found for time and session and no main effects were found for session or time. Previously, it has been shown that MuRF1 mRNA expression was increased after a bout of resistance training. However, expression was decreased following a subsequent bout of resistance training (Mascher et al., 2008). In the current study, all participants were resistance-trained, thus, potentially explaining the lack of MuRF1 mRNA expression. Though GR-DNA binding was increased in the current study, FoxO1 mRNA expression was not. Waddell et al. (2008) suggested that FoxO1 is a critical component to achieve full induction of MuRF1 activation. The lack of expression of FoxO1 mRNA could potentially be responsible for the lack of expression of MuRF1 mRNA.

MAFbx

The ubiquitin protease system (UPS) is a primary pathway involved in protein degradation in skeletal muscle. Increased glucocorticoid release has been associated with muscle atrophy through the activation of the UPS. MAFbx is expressed through this pathway (Foletta, White, Larsen, Léger, & Russell, 2011). In the current study, MAFbx mRNA expression was minimal and no significant interactions or main effects were found. MAFbx expression was not altered following a single bout of resistance exercise. However, MAFbx and MuRF1 expression have been shown to be diminished by repeated bouts of resistance exercise (Mascher et al., 2008). While only an acute bout of resistance exercise was performed in the current study, all participants had been participating in regular resistance exercise. It is possible that this chronic resistance training could have potentially been responsible for the lack of MAFbx mRNA gene expression observed.

Myostatin

Mysostatin expression has been linked to negative regulation of muscle hypertrophy. The expression of mysostain increases with increases in the release of glucocorticoids and has been suggested to operate through GR-mediated mechanisms, thus resulting in muscle proteolysis and atrophy (Roth et al., 2003; Willoughby, 2004). To our knowledge, the current study is the first to investigate the effects of hypohydration on myostatin mRNA expression. The current study found no significant differences between sessions; however, myostatin mRNA expression was increased post-exercise for both DT and HT compared to pre-exercise. Previous research has found mysostatin mRNA expression to be reduced with chronic resistance-training in both males and females (Roth et al., 2003). However, the current results concur with the findings of Willoughby (2004) that resistance training up-regulates myostatin mRNA expression. However, it has been suggested that this up-regulation in myostatin mRNA expression is lessened by increased levels of follistatin-like related gene (FLRG) and down-regulation of the activin IIb receptor (Willoughby, 2004).

Atrogin-1

Atrogin-1 has been associated with muscle proteolysis and atrophy through the UPS (Foletta et al., 2011). The current study is the first to examine the effects of hypohydration on atrogin-1 mRNA expression. No significant interactions or main effects were found for session or time for atrogin-1 mRNA expression in the current study. This lack of expression can possibly be attributed to the current study using resistance-trained individuals. Prior research has shown that chronic resistance training in rat models can blunt the expression of atrogin-1 mRNA. (Zanchi et al., 2009).

Redd1

Research has shown Redd1 expression to be induced following cellular stress and has been shown to be an inhibitor of mTOR phosphorylation and MPS (Brugarolas et al., 2004; Wang, Kubica, Ellisen, Jefferson, & Kimball, 2006). To our knowledge, this is the first study to examine the effects of hypohydration on Redd1 mRNA expression. No significant interacitons or main effects were found for either session or time. Redd1 mRNA expression has been shown to be reduced with chronic resistance training (Drummond et al., 2008). Drummond et al. (2008) found that Redd1 expression is diminished even with low intensity resistance exercise. However, in the current study no differences were seen in any condition. It is possible that the participant's training level was sufficient enough to blunt Redd1 expression and thus, see no differences during the current study. Drummond et al. (2008) found that Redd1 expression is diminished even with low intensity resistance exercise.

FoxO1

FoxO1 has been shown to be an important regulator in gene expression during muscle atrophy (Hribal, Nakae, Kitamura, Shutter, & Accili, 2003; Waddell et al., 2008). FoxO1, with the GR, has also been shown to activate MuRF1, MAFbx and Atrogin-1 expression (Foletta et al., 2011; Waddell et al., 2008) To our knowledge, this is the only study to examine the effects of hypohydration on FoxO1 mRNA expression. No significant interacitons or main effects were found for either session or time. In the current study, expression of MuRF1, MAFbx, and Atrogin-1 expression was minimal. Due to the role FoxO1 plays in activating the expression of these genes, it is plausible that their lack of expression could potentially be due to insufficient FoxO1 mRNA

expression. Without the stimulus of FoxO1, these expression of these genes may not have been activated, even with increased GR-DNA binding.

Conclusions and Future Directions

The current results suggest that previous night dehydration may have a negative impact on both muscular strength and endurance, as well as perceptual feelings of exertion and recovery in resistance trained females while increasing GR-DNA binding. Though the only performance measure to reach a statistically significant difference was bench press 1RM, there was a reduction in leg press 1RM and total volume lifted for both bench press and leg press. While this was an acute bout of only two exercises, it would be reasonable to suggest that this reduction in volume would continue for other exercises across a full workout. Additionally, GR-DNA binding was increased with hypohydration. Hypohydration appears to have little effect on proteolytic gene expression. Though, it is possible that gene expression was suppressed due to all participants being resistance-trained. Further research is needed to determine if hypohydration effects proteolytic gene expression in untrained individuals. Theoretically, if an individual were to be chronically hypohydrated, this reduction in volume and increase in GR-DNA binding could diminish the anabolic response to resistance exercise and potentially lead to muscle atrophy. Further research is needed to confirm this theory. Additionally, further research is needed to determine in the additional mechanical stress from a full body workout would result in proteolytic gene expression.

APPENDICES

APPENDIX A

Informed Consent Form

Baylor University
[HEALTH, HUMAN PERFORMANCE AND RECREATION]

Consent Form for Research

Protocol title: The Effects of Hypohydration on Muscular Performance and Markers of Catabolism in Females

Principal investigator: Darryn Willoughby, Ph.D.**,** Professor, Department of Health, Human Performance, and Recreation, Baylor University

Supported by: National Strength and Conditioning Association, Exercise and Biochemical Nutrition Lab, Baylor University

Introduction

Please read this form carefully. The purpose of this form is to provide you with important information about taking part in a research study. If any of the statements or words in this form are unclear, please let us know. We would be happy to answer any questions. You have the right to discuss this study with another person who is not part of the research team before making your decision whether or not to be in the study.

Taking part in this research study is up to you. If you decide to take part in this research study we will ask you to sign this form. We will give you a copy of the signed form.

The person in charge of this study is Darryn Willoughby, Ph.D. We will refer to him as the "researcher" throughout this form.

Why is this study being done?

Dehydration is a common problem among athletes and exercisers. Many of these individuals fail to rehydrate between exercise sessions. Dehydration has been shown to have a negative effect on aerobic performance. However, less is known about the impact on anaerobic performance (e.g. high intensity, short duration performance such as sprinting and weight lifting). Current research on dehydration and anaerobic performance has been done only on males. However, both men and women have been shown to underestimate sweat loss during exercise and thus, are equally susceptible to dehydration.

Many investigations to date often examine levels of dehydration that are unrealistic (> 5% of body mass lost) in anaerobically active exercisers or are limited to extreme cases in athletes (e.g. weight-cutting sports). However, results show dehydration places individuals in a state of catabolism (muscle break down). Research has also shown that the negative effects of dehydration on anaerobic exercise may not occur until 3% of body mass is lost. Thus, justification exists for further understanding the impact of dehydration at moderate realistic levels (3% of body mass) on acute resistance exercise and hormonal levels. Currently, it appears that no published data are available investigating the catabolic hormonal response to an acute bout of anaerobic exercise at moderate hypohydration (3% body mass lost) in females. Therefore, the purpose of this study is to compare the impact moderate hypohydration has on the catabolic hormonal and molecular responses to resistance exercise.

You have been asked to volunteer for this study because you are a healthy female between the ages of 18-30. You are of normal weight, defined as having a body mass index (BMI) between 18.5-30 kg/m^2. You have had regular periods for the past 6 months. You are physically active and resistance-trained, defined as participating in a structured resistance training program at least 3 times a week for at least 6 months prior to the study. You have not consumed any nutritional supplements (excluding multi-vitamins) for one month or anabolic steroids 3 months prior to the study. You are a non-smoker and considered as low risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM). You are free from orthopedic problems that would inhibit you from participating in upper- and lower-body resistance training exercises.

We are asking you to take part in this study because you:

You will be one of 10 apparently healthy, resistance trained females between the ages 18 to 30 who will participate in this study. You have had regular periods for the past 6 months. You are not currently taking any medications known to influence the cardiac or endocrine systems (e.g., blood pressure, heart rate, thyroid, etc.). You are free from orthopedic problems that would inhibit you from participating in upper or lower-body exercise. During an initial familiarization session, you will be informed of the requirements of the study and sign an informed consent statement. A trained individual will examine you to determine if you are qualified to participate in this study. If you are cleared to participate in the study, you will be familiarized to the testing procedures. This will take approximately 20 minutes to complete. Once you complete the familiarization, you will complete baseline testing.

How long will I take part in this research study?

We expect that you will be in this research study for a total of **14 days/2 weeks.** During this time, we will ask you to make **4 study visits** to Marrs McLean Gym, room 120 [Exercise and Biochemical Nutrition Lab (EBNL) at Baylor University].

What will happen if I take part in this research study?

We expect that your participation in this study will last for 2 weeks. During this time, we will ask you to make four visits to the EBNL and Baylor Laboratories for Exercise Science & Technology (BLEST) at Baylor University. Data collection will occur in Marrs-McLean Gym, rooms 120 and 233.11. During the familiarization meeting, you will be provided with an overview and familiarization of the study so that you can choose to participate if eligible. You will sign an informed consent form, and then you will complete a medical history questionnaire and undergo a general physical exam to determine if you qualify to participate in the study. This will take approximately 20 minutes. Data collection will occur at the remainder of the 4 visits. We anticipate the 2nd and 4th visits will take approximately 90 minutes each. We anticipate that the 1st and 3rd visits will take approximately 3 hours. The total lab time required to complete this study is approximately 9 hours.

Below is a breakdown of each study visit.

VISIT 1

Visit 1 will take roughly 3 hours to complete the dehydration procedures. Following this visit you will be given a volume of water to drink before returning the following morning.

At this visit, we will ask you to do the following procedures:

- Measure your vital signs: blood pressure, temperature, heart, breathing rates
- Measure your weight and ingest a small sensor to monitor your internal temperature.
- Complete dehydration procedure:
    - Dehydration Procedure
        - You will be weighed wearing the swimsuit to be worn during session. You will then sit in a hot water bath and will be removed from the bath and weighed every 30 minutes until you lose 3.5% of your body mass. During the hydrated trial (either Visit 2 or Visit 4), you will receive water to drink following each time you weigh. Following both trials (Visit 2 and Visit 4), you will be given a volume of water to drink before returning the following morning.

VISIT 2

Visit 2 will take roughly 90 minutes to complete the resistance training. You will be required to return the morning following visit 1.

At this visit, we will ask you to do the following procedures:

- Measure your vital signs: blood pressure, temperature, heart, breathing rates
- Measure your height, weight, DEXA scan, and total body water.
- Provide a urine sample. This sample will be used to measure your hydration status only.
- Provide estimations for perceived exertion, sleep, and thirst.
- Perform a resistance exercise session consisting of bench press, and leg press
    - You will warm up by completing 5 to 10 repetitions at approximately 50% of the estimated 1-RM. You will rest for 1 minute, and then complete 3 to 5 repetitions at approximately 70% of the estimated 1-RM. The weight will then be increased conservatively, and you will attempt to lift the weight for one repetition. If the lift is successful, you will rest for 2 minutes before attempting the next weight increment. This procedure will be continued until you fail to complete the lift. The 1-RM will be recorded as the maximum weight that you are able to lift for 1 repetition. In order to assess muscle endurance, using the bench press and angled leg press exercises, you will perform as many repetitions as possible with 75% of your 1-RM for 5 sets.
- Complete an assessment of vertical jump height.
    - You will perform 3 jumps, touching the highest possible mark each time. You will be given a 1minute recovery period between each jump.
- **Collect a blood and muscle sample:**

    - <u>Blood draw:</u>
        - You will then donate about 15 milliliters (3 teaspoons) of venous blood from a vein in your arm. Blood samples will be obtained using standard/sterile procedures using a needle inserted into a vein in your arm. This will take about 10 minutes to complete.

    - <u>Muscle biopsy:</u>
        - After the blood draw, you will then be prepared for the muscle biopsy. The biopsy location will be identified on the thigh of your leg. The biopsy area will be cleaned with rubbing alcohol. In addition, the biopsy site will be further cleansed by swabbing the area with Betadine (fluid antiseptic). A small area of the cleaned skin approximately 2 cm in diameter will be anesthetized with a 1.5 ml subcutaneous injection of the topical anesthetic Lidocaine. Once the local anesthesia has taken effect (approximately 2-3 minutes) the biopsy procedure will only take 15-20 seconds. Once anesthetized, a small puncture will be made in your skin and a biopsy needle introduced 1 cm into the muscle. The puncture is so small that it will be simply covered with an adhesive bandage (Band-Aid). Due to the localized effects of the anesthetic, you should feel no pain during this process; however, you should feel a pressure sensation. After the anesthetic wears off within 2-3 hours, the sensation at the biopsy site is comparable to that of a bruise and

may persist for up to 24 hours after the procedure.

VISIT 3

Visit 3 will take roughly 3 hours to complete the dehydration procedures. Following this visit you will be given a volume of water to drink before returning the following morning. This visit will take place no sooner than 2 days following Visit 2.

At this visit, we will ask you to do the following procedures:

- Measure your vital signs: blood pressure, temperature, heart, breathing rates.
- Measure your weight and ingest a small sensor to monitor your internal temperature.
- Complete dehydration procedure.
    - Dehydration Procedure
        - You will be weighed wearing the swimsuit to be worn during session. You will then sit in a hot water bath and will be removed from the bath and weighed every 30 minutes until you lose 3.5% of your body mass. During the hydrated trial (either Visit 2 or Visit 4), you will receive water to drink following each time you weigh. Following both trials (Visit 2 and Visit 4), you will be given a volume of water to drink before returning the following morning.

VISIT 4

Visit 4 will take roughly 90 minutes to complete the resistance training. You will be required to return the morning following visit 3. All procedures will be identical to visit 2.

At this visit, we will ask you to do the following procedures:

- Measure your vital signs: blood pressure, temperature, heart, breathing rates
- Measure your height, weight, DEXA, and total body water
- Provide a urine sample. This sample will be used to measure your hydration status only.
- Provide estimations for perceived exertion, sleep, thirst, and recovery.
- Perform a resistance exercise session consisting of bench press, and leg press
    - Description of procedure detailed in visit 2
- Complete an assessment of vertical jump height.
    - Description of procedure detailed in visit 2
- Blood draw
    - Description of procedure detailed in visit 2
- Muscle biopsy
    - Description of procedure detailed in visit 2

What are the risks of taking part in this research study?

You will be asked on the medical history questionnaire to report all nutritional supplements, medically prescribed drugs, and non-medically prescribed drugs that you may be presently taking.

You will also be asked to report if you have any known allergies to local anesthetics and if you have had any prior allergic reactions to topical anesthetics. By a medical history questionnaire, you will be asked to report whether you have any additional medical problems that would prevent you from participating in this study. You must report all changes in medical status, nutritional and/or pharmacological agents (drugs) that you take during the course of the investigation to Darryn Willoughby, Ph.D. (254-710-3504). If you experience any unexpected problems or adverse events from participating in this study you may be referred to discuss the problem with your primary care physician, or if you are a Baylor student, the student health center to determine whether any medical treatment is needed and/or whether you can continue in the study.

Dehydration risks

Dehydration and heat exposure may disrupt sleep patterns and possibly result in other side effects such as headaches or nausea. To attempt to minimize these risks, core temperature will be monitored continuously throughout dehydration procedures. If core temperature reaches or exceeds 38.9ºC (102 degrees Fahrenheit) or if you experience negative subjective feelings (e.g. nausea) you will be removed from the hot bath and allowed to cool.

Blood draw risks

Risks of having blood drawn are soreness and/or a black and blue mark at the site from where the blood is drawn. Sometimes, people feel uncomfortable at the time of the blood draw. Occasionally people feel lightheaded or faint. There is also a small risk of infection whenever blood is drawn. On 4 separate occasions during this study (visits 1-4), you will have approximately four teaspoons (20 milliliters) of blood drawn from a forearm vein using standard procedures. All blood sampling will be performed by an experienced phlebotomist. This procedure may cause a small amount of pain when the needle is inserted into as well as some bleeding and bruising. You may also experience some dizziness, nausea, and/or faint if unaccustomed to having blood drawn.

Muscle biopsy risks

On 4 separate occasions during this study (visits 2 and 4), you will undergo a muscle biopsy in which a small sample of muscle will be obtained from the thigh of your exercised leg. Darryn Willoughby, Ph.D., Joshua Gann, M.S., or Thomas Andre, M.S. will perform all blood draws and muscle biopsies and that a local anesthetic (lidocaine) will be injected into the skin of your thigh prior to the biopsy, which will help prevent any pain and discomfort during the procedure. A small puncture will be made in your

skin and a biopsy needle introduced 1 cm into the muscle. The puncture is so small that it will be simply covered with an adhesive bandage (Band-Aid). After the anesthetic wears off within 2-3 hours, the sensation at the biopsy site is comparable to that of a bruise and may persist for up to 24 hours after the procedure. Although rare, there is a potential risk for striking a nerve during the biopsy procedure which can increase post procedure soreness and temporary numbness to the area. You are required to inform study personnel if you have had any prior allergic reactions to anesthesia (e.g. While in the hospital or during a dental visit). You are required to inform study personnel if you have had any prior allergic reactions to anesthesia (e.g. While in the hospital or during a dental visit). You are advised to refrain from vigorous physical activity with your leg during the first 24 hours post-biopsy. If you feel it necessary, you may take a non-prescription analgesic medication such as Tylenol to relieve pain if needed and that some soreness of the area may occur for about 24 hours after the biopsy. You are advised to avoid such medications such as aspirin, Ibuprofen, Advil, Bufferin, or Nuprin, as they may lead to bruising at the biopsy site.

Risks from radiation

Procedures such as DXA scans, CT scans, and/or X-Rays will be used during this research study. The cumulative radiation exposure from these tests is considered small and is not likely to adversely affect you. However, the effects of radiation add up over a lifetime. It is possible that having several of these tests may add to your risk of injury or disease. When deciding to enter this study, think about your past and future contact with radiation. Examples of contact with radiation include x-rays taken for any reason or radiation therapy for cancer treatment. Due to the risks associated with radiation and pregnancy, if you are pregnant, you will not be allowed to participate. If you become pregnant during the course of the study, you are required to inform study personnel.

Risks of completing resistance training

Muscle strains/pulls resulting from exercise testing and resistance training. It is possible that each exercise bout will make your muscles fatigued and possibly sore for the 24 to 48 hours after you complete the exercise bout. The soreness you may experience is normal and as an experienced resistance trainer, a sensation in which you should be familiar.

Loss of confidentiality

A risk of taking part in this study is the possibility of a loss of confidentiality. Loss of confidentiality includes having your personal information shared with someone who is not on the study team and was not supposed to see or know about your information. The researcher plans to protect your confidentiality. Their plans for keeping your information private are described later in this consent form.

Incidental findings

Although the procedures you will undergo in this study are being undertaken for research purposes only, it is possible that researchers may notice something that could be important to your health. If so, we will contact you to explain what was noticed. If you do not have a private physician, we will refer you to an appropriate clinic for follow-up. It will be your choice whether to proceed with additional tests and/or treatments to evaluate what we observed, and you or your insurer will be responsible for these costs.

Are there any benefits from being in this research study?

Possible benefits

The primary benefit obtained from participating in this study is to gain insight about your health and fitness status from the body composition, exercise, and nutritional assessments to be performed. In addition, you may also gain insight into how your body responds to resistance exercise in a hypohydrated state and possible ways to improve your hydration practices.

What alternatives are available?

You may choose not to take part in this research study.

Storing study information for future use

We would like to store your study information for future research related to exercise. We will label all your study information with a code instead of your name. The key to the code connects your name to your study information. The researcher will keep the code in a password-protected computer/locked file.

Future use of study information is required for this study. If you do not want your information to be used for future research, you should not be in this study.

Storing samples and health information for future use

We would like to store some of your samples and health information for future research related to dehydration and resistance training. We will label your samples and health information with a code instead of your name. The key to the code connects your name to your samples and health information. The study doctor will keep the key to the code in a locked file.

Your samples will be stored in an ultralow freezer located in MMG 120. Your sample will be kept for 3 years. After that time, the sample will be destroyed by methods in accordance with laboratory or institution procedures.

Future use of samples and health information is required for this study**.** If you do not want your samples and health information to be used for future research, you should not be in this study.

How will you keep my study records confidential?

We will keep the records of this study confidential: any information obtained about you in this research, including medical history, laboratory findings, or physical examination will be kept confidential to the extent permitted by law. However, in order to ensure that FDA regulations are being followed, it may be necessary for a representative of the FDA to review your records from this study, which may include medical history, laboratory findings/reports, statistical data, and/or notes taken about your participation in this study. In addition, records of this research may be subpoenaed by court order or may be inspected by federal regulatory authorities. The data derived from your participation in this study may be used in reports, presentations, and publications. However, you will not be individually identified unless your consent is granted in writing. Additionally, confidentiality will be maintained by assigning code numbers to your files, limiting access to data to research assistants, locking cabinets that store data, and providing passwords to limit access to computer files to authorized personnel only. Once blood and muscle samples are analyzed, they will be discarded. We will make every effort to keep your records confidential. However, there are times when federal or state law requires the disclosure of your records.

The following people or groups may review your study records for purposes such as quality control or safety:

- The researcher and members of Dr. Darryn Willoughby's research team (Josh Gann and Tom Andre).
- Authorized members of Baylor university who may need to see your information, such as administrative staff members from the office of the vice provost for research and members of the institutional review board (a committee which is responsible for the ethical oversight of the study).
- The sponsor or funding agency for this study.
- Federal and state agencies that oversee or review research (such as the HHS office of human research protection or the food and drug administration).

The study data will be stored in lock cabinets in the office of Dr. Darryn Willoughby in the exercise, biochemistry, and nutrition lab (EBNL).

The results of this study may also be used for teaching, publications, or presentations at professional meetings. If your individual results are discussed, your identity will be protected by using a code number or pseudonym rather than your name or other identifying information.

Study participation and early withdrawal

Taking part in this study is your choice. You are free not to take part or to withdraw at any time for any reason. No matter what you decide, there will be no penalty or loss of benefit to which you are entitled. If you decide to withdraw from this study, the information that you have already provided will be kept confidential. You cannot withdraw information collected prior to your withdrawal.

If you are a student, staff or faculty of Baylor, stopping your participation in this study will not class standing or grades, or your job status. You will not be offered or receive any special consideration if you take part in this research study.

The researcher may take you out of this study without your permission. This may happen because:

- The researcher thinks it is in your best interest.
- You can't make the required study visits.
- Other administrative reasons.

Will I get paid for taking part in this research study?

If you complete all 4 visits, we will pay you a total of $200. If you do not complete the entire study, we will pay you at the following visits that you complete ($50 for completing visit 2). Your Social Security number will be required to receive payment when you complete your final visit.

What will it cost me to take part in this research study?

There are no costs to you for taking part in this research study.

What happens if I am injured as a result of participating in this research study?

If you become ill or injured as a result of your participation in the study, you should seek medical treatment from your doctor or treatment center of choice. You should promptly tell the researcher about any illness or injury.

There are no plans for Baylor University to pay you or give you other compensation for your injury or illness. You do not give up any of your legal rights to seek compensation by signing this form.

What if I have any questions or concerns about this research study?

You can call us with any concerns or questions about the research. Our telephone numbers are listed below:

Darryn Willoughby, Ph.D. (principal investigator, Professor Department of Health,

Human Performance & Recreation, 120 Marrs-Mclean Gymnasium, Baylor University, phone: 254-710-3504)
E-mail: Darryn_Willoughby@baylor.edu

Joshua Gann, M.S. (graduate research assistant, Department of Health, Human Performance & Recreation, 120 Marrs-Mclean Gymnasium, Baylor University 254-710-4012*)*
E-mail: Joshua_Gann@baylor.edu

Tom Andre, M.S. (graduate research assistant, Department of Health, Human Performance & Recreation, 120 Marrs-Mclean Gymnasium, Baylor University 254-710-4012*)*
E-mail: Thomas_Andre@baylor.edu

If you want to speak with someone not directly involved in this research study, you may contact the Baylor University IRB through the office of the vice provost for research at 254-710-1438. You can talk to them about:

- Your rights as a research subject.
- Your concerns about the research.
- A complaint about the research.

Future contact

We may like to contact you in the future either to follow-up to this study or to see if you are interested in other studies taking place at Baylor University.

Do you agree to let us contact you in the future?

_____Yes _____No _____Initials

Statement of consent

Indicate your decision to participate in this study based on the information discussed in this form by signing below.

Signature of Participant:

I have read the information in this consent form including risks and possible benefits. I have been given the chance to ask questions. My questions have been answered to my satisfaction, and I agree to participate in the study.

_____ _____
Signature of Participant Date

Signature of Person Obtaining Consent:

I have explained the research to the participant and answered all of her questions. I will give a copy of the signed consent form to the participant.

_____ _____

Signature of person obtaining consent Date

APPENDIX B

IRB Proposal

The Effects of Hypohydration on Muscular Performance and Markers of Catabolism in Females

PRINCIPAL INVESTIGATOR:

Name: **Darryn S. Willoughby, PhD.**
Address: Baylor University, Department of HHPR

Phone #: 710- 3504
Fax #: 710-3527
Email: Darryn_Willoughby@baylor.edu

CO- or SUB-INVESTIGATORS:

Name: **Josh Gann, M.S.**
Institution(s): Baylor University
Phone #: 710-4012
Email: Joshua_Gann@baylor.edu

Name: **Tom Andre, M.S.**
Institution(s): Baylor University
Phone #: 710-4012
Email: Thomas_Andre@baylor.edu

PROTOCOL VERSION: 09/21/16

Synopsis

Title	The Effects of Hypohydration on Muscular Performance and Markers of Catabolism in Females
Protocol Date	September 21, 2016
Study Duration	One year
Study location(s)	BLEST (MMGYM 127) and EBNL (MMGYM 120) at Baylor University
Objectives	The specific aims of this study are to: 1. examine the impact of hypohydration on muscle performance. 2. examine the impact of hypohydration on serum cortisol concentration. 3. Examine the impact of hypohydration on glucocorticoid receptor/DNA binding activity. 4. Examine the impact of hypohydration on the expression of various muscle proteolytic genes affected by glucocorticoid receptor/DNA binding activity.
Number of Subjects	10
Main Inclusion/Exclusion Criteria	Apparently healthy 18-30 year old females

TABLE OF CONTENTS

1.0 BACKGROUND AND RATIONALE

The effects of hypohydration on aerobic performance have been well established (American College of Sports Medicine et al., 2007). However, less is known about the effects on anaerobic performance (e.g. high intensity, short duration performance such as sprinting and weight lifting). Many modes have been utilized to assess the impact on anaerobic performance including repeated sprints (J. J. Gann, Green, O'Neal, Renfroe, & Andre, 2015), resistance exercise (Judelson et al., 2008; Kraft et al., 2010), and isokinetic exercise (Hayes & Morse, 2010). In a review, Kraft et al. (Kraft et al., 2012) found that the effects of hypohydration on anaerobic exercise may not be seen until 3% body mass loss. Additionally, a meta-analysis found that when hypohydration was achieved ≥ 8 hr prior to exercise testing with an overnight sleep, residual effects of heat exposure and exercise were nullified (Savoie, Kenefick, Ely, Cheuvront, & Goulet, 2015). Hypohydration is a common problem among athletes and recreational exercisers. Many of these individuals fail to rehydrate adequately between exercise sessions (Maresh et al., 2004).

Both men and women have been shown to underestimate sweat loss during exercise (O'Neal et al., 2012; Thigpen, Green, & O'Neal, 2014). This underestimation could lead to insufficient fluid intake post-exercise and subsequent hypohydration. This suggests that females are at the same risk as males to suffering from progressive dehydration. However, to date, virtually all research focusing on hypohydration's effects on anaerobic exercise performance has been performed on male participants. A potential reason for the lack of research focusing on the effects of hypohydration on anaerobic exercise in females could be in part due to the menstrual cycle. Ovarian hormones fluctuate predictably over 23–28 days, on average. The menstrual cycle is divided into two phases: the follicular phase and luteal phase (X. A. K. Janse de Jonge, 2003). While fluid balance between phases could be a concern when performing hypohydration research with female participants, recent research from our lab (McKinley, 2005) has shown no difference in total body water between follicular and luteal phases. Additionally, menstrual cycle phase does not seem to effect exercise performance or perceptual response to exercise (Marsh & Jenkins, 2002) or replacement of fluid losses following exercise (Maughan, McArthur, & Shirreffs, 1996). Further, thermoregulatory differences (Kaciuba-Uscilko & Grucza, 2001) between males and females warrants investigation into the effects of hypohydration on females during anaerobic exercise.

Much of the previous literature has resulted in equivocal results in regards to hypohydration and catabolic responses to exercise. Previously Judelson et al. (Judelson et al., 2008) examined hypohydration's impact at 5% of body mass reduction demonstrating an increase in levels of cortisol following resistance exercise (e.g. catabolic state). However, this level of hypohydration is unrealistic and typically limited to weight-cutting sports. Yamamoto et al.(Yamamoto et al., 2008) examined the impact of hypohydration (2.5% and 5% body mass reduction)

on muscle damage utilizing blood markers of damage (myoglobin and creatine kinase) following lower-body anaerobic exercise, and found no statistical difference between all trials. Though it should be noted these markers can be poor indicators as they only provide indirect evidence indicative of myofibril damage and potential protein degradation. Hayes et al. (Hayes & Morse, 2010) examined progressive dehydration and found a dose-dependent relationship for maximal power output, demonstrating a lower force production for isometric leg extension at only a 1% reduction of body mass via dehydration. However, no hormonal data was collected for this study.

The glucocorticoid, cortisol, is elevated in the circulation during stress situations including nutritional deficits. Cortisol operates through its intracellular glucocorticoid receptor (GR) and acts as an inhibitor of muscle protein synthesis (MPS) through varied mechanisms. Firstly, cortisol decreases the rate of MPS (limits amino acid transport into the muscle), thereby increasing the rate of muscle protein breakdown. This leaves an individual in a state of catabolism which, over the course of time, can equate to muscle atrophy (Löfberg et al., 2002). Secondly, cortisol inhibits the stimulatory effects of insulin, insulin-like growth factor 1 (IGF-1), the amino acid, L-leucine, and the phosphorylation of two key controlling factors (4E-BP1 and S6K1) for translation initiation and subsequent MPS (Zhenqi Liu, Li, Kimball, Jahn, & Barrett, 2004). Additional evidence suggests that the cortisol-induced up-regulation on muscle proteolysis also occurs with activation of the ubiquitin protease system (UPS), the lysomal system (autophagy), and the calcium-dependent system (calpains) (Tiao et al., 1996). In regards to the PI3K/Akt pathway (mediator for the anabolic actions of insulin and IGF-1), evidence suggests that cortisol exerts its catabolic actions by causing a decreased expression of insulin receptor substrate 1 (IRS-1) protein, which operates upstream in the PI3K/Akt pathway (Nakao et al., 2009). The result of this inhibition on the PI3K/Akt pathway is an inhibition on mRNA translation and subsequent MPS (potentially leaving an individual in a state of catabolism).

Currently, it appears that no published data are available investigating the catabolic hormonal response to an acute bout of anaerobic exercise at moderate hypohydration (3% body mass lost) in females. Therefore, the purpose of this study is to compare the impact moderate hypohydration has on the catabolic hormonal and molecular responses to resistance exercise. The specific aims of the proposed study are to examine the impact moderate hypohydration has on: 1) muscle performance (strength, power, and endurance), 2) serum hormonal marker of catabolism (cortisol), 3) expression of muscle-specific genes involved in proteolysis, and 4) the protein content of the GR and extent of GR-DNA binding in skeletal muscle.

2.0 STUDY OBJECTIVES

Independent and Dependent Variables

The independent variables will be hydration status (hypohydrated and euhydrated). The dependent variables are muscle performance, serum cortisol hormones, and glucocorticoid receptor (GR) content, GR/DNA binding activity, and proteolytic gene expression in skeletal muscle.

3.0 SUBJECT SELECTION & RECRUITMENT

3.1 INCLUSION CRITERIA

Participants

Ten apparently healthy females between the ages of 18-30 and a body mass index between 18.5-30 kg/m2 will complete the randomized cross-over study. Enrollment will be open to women of all ethnicities. Only participants considered as low risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM) will be allowed to participate. All women must be eumenorrheic and have had regular periods for at least 6 months prior to the study. All participants will provide written informed consent and be cleared for participation by passing a mandatory medical screening. All eligible subjects will sign university-approved informed consent documents and approval will be granted by the Institutional Review Board for Human Subjects. A female research assistant will be present for initial screening of each participant and when possible, for all other testing sessions. Additionally, all experimental procedures involved in the study will conform to the ethical consideration of the Declaration of Helsinki.

Randomization Procedure

At visit 1, eligible participants will be randomly assigned to complete either a euhydrated trial or hypohydrated trial first using a random number generator (www.random.org).

Recruitment

Recruitment flyers (see appendix) will be posted on campus, at area fitness centers, on the Baylor Department of Health, Human Performance, and Recreation (HHPR) webpage, on the internet (https://www.facebook.com/groups/BaylorHHPR/), and distributed in various classes by HHPR faculty.

Consent Form Process

Informed consent forms may be emailed to the potential participant for them to read over and become familiar with the study to help them decide if they want to participate. However, they will be instructed not to sign the form. Upon reporting to the lab for visit 1, participants will be familiarized to the study protocol via a verbal and written explanation outlining the study design, and will then read (or re-read) and sign the university-approved informed consent form. Upon signing the consent form, participants will complete a medical history questionnaire and undergo a general physical examination to determine whether they meet eligibility criteria.

Subject Withdrawals

The participants are free not to take part in this study, or to withdraw from the study at any time, for any reason. No matter what they decide, there will be no penalty or loss of benefit to which they are entitled. If they decide to withdraw from this study, the information that they have already provided will be kept confidential. Participants cannot withdraw information collected prior to their withdrawal. The researcher may take the participant out of this study without their permission. This may happen because: 1) the researcher thinks it is in the best interest of the participant; 2) the participant cannot comply to the resistance training and/or hydration protocol; 3) the participant cannot attend the required study visits; and 4) if the researchers find physical problems that, in their opionion, make completing the experimental procedures risky.

3.2 EXCLUSION CRITERIA

Participants will not be allowed to participate in the second study if they:

1. Have not been involved in a habitual resistance training program which utilizes the entire body (minimum of 3 hours/week for at least 6 months).

2. Have any known metabolic disorder including heart disease, arrhythmias, diabetes, thyroid disease, or hypogonadism.

3. Have a known allergy to topical anesthetics.

4. Are not eumenorrheic.

5. Are pregnant.

6. Have orthopedic limitations that would limit participation in exercise.

7. Have a history of pulmonary disease, hypertension, hepatorenal disease,

musculoskeletal disorders, neuromuscular/neurological diseases, autoimmune disease, cancer, peptic ulcers, or anemia.

8. Are taking any heart, pulmonary, thyroid, anti-hyperlipidemic, hypoglycemic, anti-hypertensive, endocrinologic (e.g, thyroid, insulin, etc), psychotropic, neuromuscular/neurological, or androgenic medications.

9. Have a known bleeding disorder and/or are taking a blood thinning medication such as Warfarin.

10. Have any absolute or relative contraindication for exercise testing or prescription as outlined by the American College of Sports Medicine.
11. Report any unusual adverse events associated with this study that in consultation with the supervising physician recommends removal from the study.

4.0 RESEARCH METHODS & PROCEDURES

Experimental Approach

In a randomized, cross-over design, participants will visit the laboratory on 4 separate occasions over a 2 week period in the following manner: Visit 1 = dehydration with or without fluid replacement, Visit 2 = resistance training testing, vertical jump testing, blood draw, muscle biopsy, urine specific gravity, and perceptual measures, Visit 3 = dehydration with or without fluid replacement, Visit 4 = resistance training testing, vertical jump testing, blood draw, muscle biopsy, urine specific gravity, and perceptual measures. Participants will begin testing sessions upon cessation of menses. At visit 1, participants will be randomly determined using a random number generator (www.random.org) to complete the dehydration procedures with or without fluid replacement first. The entire duration of the study will be approximately one year.

Muscle Strength and Endurance Assessment

In order to determine possible effects of the hypohydration on muscular strength, participants will perform 1 repetition maximum (1-RM) tests on the free-weight bench press and angled leg press exercises at visit 2 and 4. Participants will warm up by completing 5 to 10 repetitions at approximately 50% of the estimated 1-RM. The participant will rest for 1 minute, and then complete 3 to 5 repetitions at approximately 70% of the estimated 1-RM. The weight will then be increased conservatively, and the participant will attempt to lift the weight for one repetition. If the lift is successful, the participant will rest for 2 minutes before attempting the next weight increment. This procedure will be continued until the participant fails to complete the lift. The 1-RM will be recorded as the maximum weight that the participant is able to lift for 1 repetition. In order to assess muscle endurance, using the bench press and angled leg press exercises, participants will

perform as many repetitions as possible with 75% of their 1-RM for 5 sets. A recovery period of 2 minutes will be given between each set.

Vertical Jump

Vertical jump height will be assessed using a Vertec (Sports Imports, Columbus OH) height measurement system. Participants will perform 3 jumps. The highest attempt will be recorded. A recovery period of 1 minute will be given between attempts.

Dehydration procedures

Prior to arrival, participants will be encouraged to aggressively hydrate during the day and consume a minimum of 500 ml of fluid 1 hour before arriving. Upon arrival, participants will have their pre-treatment weight recorded wearing the swimming suit they will be wearing during treatment. Participants will then be submerged in a hot water bath (~40º C). Core temperature and water temperature will be continuously monitored. If core temperature reaches or exceeds 38.9º C, participants will be removed from the water and allowed to cool before returning to the water. This safety temperature is the temperature used in previous investigations. Also, if participants experience any adverse feelings (nausea, light headedness etc.) they will be removed from the water. Participants will exit the water and will towel off and weigh every 30 minutes, before returning to the water. For the euhydrated/heat exposed trial, participants will consume water equal to 75% of the fluid they lost during the next 30 min submersion interval. This continues until their total sweat loss minus the fluid replacement equals a loss of ~3.5% body mass. Upon completion, participants will be given an amount of fluid to consume overnight to equal 125% of total fluid lost minus fluid given during treatment. Participants must consume this water before returning, but are allowed/encouraged to drink extra as the purpose of this trial is to have participants exposed to heat but not be dehydrated. Dehydration procedures for the hypohydrated trial will be performed in the same manner as described above minus the fluid replacement. Participants will be given one 500 ml bottle of water to consume before returning.

Body composition assessment

Body composition assessment will be performed in line with our previous studies (24,25) at Visit 1. Total body mass (kg) will be determined on a standard dual beam balance scale (Detecto Bridgeview, IL). Percent body fat, fat mass, and fat-free mass will be determined using DEXA (Hologic Discovery Series W, Waltham, MA). Quality control calibration procedures will be performed on a spine phantom (Hologic X-CALIBER Model DPA/QDR-1 anthropometric spine phantom) and a density step calibration phantom prior to each testing session. The DEXA scans will be segmented into regions (right & left arm, right & left leg, and trunk). Each of these segments will be analyzed for fat mass and fat-free

mass. Based on previous studies in our laboratory, the accuracy of the DEXA for body composition assessment is ± 3.7% as assessed by direct comparison with hydrodensitometry and scale weight. Total body water will be determined by bioelectric impedance analysis (Tanita Inc., Arlington Heights, IL). Total body water will be determined with bioelectrical spectroscopy [(BIS) ImpediMed Ltd., Australia] using a low energy, high frequency current (500 micro amps at a frequency of 50 kHz).

Venous Blood Sampling

At Visit 2 and 4, venous blood samples will be obtained from the antecubital vein into one 10 ml serum collection tube and one 5 ml whole blood collection tube using a standard vacutainer apparatus. The serum tubes will be allowed to stand at room temperature for 10 min, and then centrifuged at 2,000 rpm. The serum will be removed and frozen at -80°C for later analysis. For each exercise session, blood samples will be obtained immediately pre-exercise and 1 hour post-exercise.

Percutaneous Muscle Biopsies

At Visit 2 and 4, percutaneous muscle biopsies (20-30 mg) will be obtained from the middle portion of the right vastus lateralis muscle at the midpoint between the patella and the greater trochanter of the femur at an approximate depth between 1 and 2 cm using a 14-gauge fine needle aspiration biopsy instrument (Tru-Core I Biopsy Instrument, Medical Device Technologies, Gainesville, FL) based on our previously-published procedures [Schwarz et al., 2013]. A small area of the skin (cleaned with alcohol and Betadine) will be anesthetized with a subcutaneous injection of the topical anesthetic 1% Lidocaine. (Supervising physician is Dr. Ronald Wilson) After the initial biopsy, the next biopsy attempts will extract tissue from approximately the same location as the initial biopsy by using the pre-biopsy markings and depth markings on the needle. After removal, adipose tissue will be removed and muscle specimens will be immediately frozen in liquid nitrogen and then stored at -80°C for subsequent analysis. For each exercise session, muscle samples will be obtained immediately pre-exercise and 1 hour post-exercise.

Urine Specific Gravity Assessment

Participants will be asked to provide a urine sample prior to treatment in order to determine urine specific gravity (USG) (the concentration of particles in the urine) to assess pre-treatment hydration status using an automated urine analyzer (Clinitek Status +, Siemens, Malvern, PA). Upon completion of USG analysis, the urine sample will be safely discarded in an appropriately-marked biohazard waste container.

Perceptual Measures

Following each set during the resistance training protocol, participants will be asked to estimate ratings of perceived exertion (RPE) a 10 point omni scale. Participants will be shown the scale for reference. Ten minutes following completion of each resistance training session participants will asked to estimate their session RPE (sRPE) for the entire session using the 10 point omni scale. Prior to resistance training sessions, participants will also estimate subjective feelings for thirst, and previous night's sleep quality using a 10cm visual analog scale as well. Perceive recovery status (PRS) will be estimated prior to resistance exercise testing using a scale developed by Laurent et al. (2011).

5.0 STUDY VISITS (if applicable)

Muscle Strength and Endurance Assessment (Visit 2 & 4)

At visits 2 & 4 participants will complete testing to determine their muscular strength and muscular endurance. Participants will perform 1 repetition maximum (1-RM) tests on the free-weight bench press and angled leg press exercises. Participants will warm up by completing 5 to 10 repetitions at approximately 50% of the estimated 1-RM. The participant will rest for 1 minute, and then complete 3 to 5 repetitions at approximately 70% of the estimated 1-RM. The weight will then be increased conservatively, and the participant will attempt to lift the weight for one repetition. If the lift is successful, the participant will rest for 2 minutes before attempting the next weight increment. This procedure will be continued until the participant fails to complete the lift. The 1-RM will be recorded as the maximum weight that the participant is able to lift for 1 repetition. In order to assess muscular endurance, using the bench press and angled leg press exercises, participants will perform as many repetitions as possible with 75% of their 1-RM for 5 sets with two minutes of recovery between each set.

Vertical Jump (Visits 2 & 4)

Participants will complete three maximal vertical jumps to assess lower body power. The highest attempt will be recorded and a one minute recovery period will be given between each attempt.

Dehydration Session (Visit 1 and 3)

Participants will be weighed wearing the swimsuit to be worn during session. Participants will then be submerged in a hot water bath (~40º C) and will be removed from the bath and weighed at 30 minute intervals until 3.5% mass loss has occurred. For euhydrated trial 75% of fluid lost at each interval will be given to the participant to be consumed. Following completion fluid equal to 125% fluid lost will be given to the participant to be consumed prior to returning the following morning. For hypohydrated trial, no fluid will be replaced during the

session and a 500ml bottle of water will be given to consume before returning the following morning. Core temperature will be monitored throughout each session via ingestible sensors (Schwarz et al., 2013).

Body Composition Testing (Visit 2 and 4)

At the initial visit, total body mass (kg) will be determined on a standard dual beam balance scale (Detecto). Total body water (total, intracellular, and extracellular) will be determined with bioelectrical impedance [(BIA) Xitron 4200, San Diego, CA]. Percent body fat, fat mass, and fat-free mass, will be determined using a calibrated Hologic 4500W dual-energy x-ray absorptiometry (DEXA). The DEXA will segment regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments.

Venous Blood Sampling (Visit 2 and 4)

Venous blood samples will be obtained from the antecubital vein into a 10 ml and 5 ml collection tube using a standard vacutainer apparatus based on our standard laboratory protocol [Willoughby & Leutholtz, 2013; Willoughby et al., 2014]. Blood samples will be obtained at the same time in which muscle biopsies are obtained and will be allowed to stand at room temperature for 10 min, and then centrifuged. Whole blood will be used immediately to determine complete blood counts. However, the serum will be removed and frozen at -80°C for later analysis. For each exercise session, muscle samples will be obtained immediately pre-exercise and 1 hour post-exercise. For all blood draws, nitrile gloves will be used.

Muscle Biopsies (Visit 2 and 4)

Using a 14-gauge fine needle aspiration procedure, percutaneous muscle biopsies (~30 mg) will be obtained from the middle portion of the vastus lateralis muscle of the leg at the midpoint between the patella and the greater trochanter of the femur at a depth between 1 and 2 cm under local anesthesia with 1% Lidocaine based on our standard laboratory protocol [Schwarz et al., 2013]. Prior to puncture the area will be cleaned with alcohol and Betadine. (Supervising physician is Dr. Ronald Wilson) The leg used for the initial biopsy at Visit 2 will be randomly selected using a web-based random number generator (www.random.org). The next biopsy at Visit 3 will involve the opposite leg. For both biopsies on each leg, attempts will be made to extract the tissue from the same location by using the pre-biopsy scar, depth markings on the needle, and a successive puncture that will be made approximately 0.5 cm to the former from medial to lateral. Following removal, muscle samples will be immediately frozen in liquid nitrogen and stored at -80°C for later analysis. For each exercise session, muscle samples will be obtained immediately pre-exercise and 1hour post-exercise. For all biopsies, nitrile gloves will be used.

Hydration status (Visits 2 & 3)

Hydration status will be assessed through a urine sample provided prior to each treatment session. Participants will be provided with a sterile collection cup. Upon entering the restroom, they will be asked to void a small amount of urine into the toilet and then to collect a small amount of urine into the collection cup. The urine samples will be measured for urine specific gravity [USG (the concentration of particles in the urine)] to assess pre-resistance exercise session hydration determined by a urine analyzer (Clinitek Status+ Analyzer, Siemens, Tarrytown, NY, USA). Adequate hydration will be established if USG is (≤ 1.02). If participants are classified as dehydrated (USG >1.02) participants will ingest water until necessary hydration status is met. Upon completion of USG analysis, the urine sample will be safely discarded in a marked biohazard waste receptacle.

Perceptual Measures (Visit 2 and 4)

At visit 2 and 4 RPE will be assessed following each set of resistance exercise and RPE will be assessed 10 minutes after completion of each resistance testing session using a 10 point Omni scale. Subjective measures of sleep and thirst will be assessed prior to beginning each resistance testing session using a 10 cm visual analog scale. PRS will be estimated prior to beginning each resistance testing session.

Assessment of Skeletal Muscle Total Protein Content

From the muscle tissue samples obtained at the two testing sessions, myofibrillar protein will be further isolated from the skeletal muscle cellular extracts with repeated incubations in 0.1% SDS at 50˚C and separated by centrifugation, and protein content will be determined spectrophotometrically based on the Bradford method at a wavelength of 595 nm (Bradford, 1976). A standard curve will be generated using bovine serum albumin (Bio-Rad, Hercules, CA, USA), and based on our previous approach, myofibrillar protein content will be expressed relative to muscle wet-weight [Shelmadine et al., 2009; Spillane et al., 2011].

Skeletal Muscle Cellular Extraction

Based on our previous approach [Shelmadine et al., 2009; Spillane et al., 2011], approximately 20 mg of each muscle sample will be weighed and subsequently homogenized using a commercial cell extraction buffer (Biosource, Camarillo, CA) and a tissue homogenizer. The cell extraction buffer will be supplemented with 1mM phenylmethanesulphonylfluoride (PMSF) and a protease inhibitor cocktail (Sigma Chemical Company, St. Louis, MO) with broad specificity for the inhibition of serine, cysteine, and metallo-proteases.

Assessment of Glucocorticoid Receptor

The GR protein content will be determined in nuclear-extracted muscle samples by a commercially-available nuclear receptor ELISA kit (Active Motif, Carlsbad, CA). The kit uses a specific polyclonal antibody which recognizes the N-terminal, ligand-independent, domain of the GR. The assay is 100% specific and has a sensitivity of 0.6 µg of GR in nuclear-extracted LNCaP cells. All samples will be ran in duplicate and in one run to avoid batch effects. The absorbance's will be determined at a wavelength of 450 nm using a microplate reader (iMark, Bio-Rad, Hercules, CA) and expressed relative to total protein concentration.

Assessment of Glucocorticoid/DNA Binding

The extent of GR-DNA binding will be determined in nuclear extracts by a commercially-available ELISA kits (Assay Bio Tech, Inc., Sunnyvale, CA, USA). This kit uses a consensus DNA oligonucleotide (AGAACA) representing the GRE to first bind the GR, after which a specific polyclonal antibody interacts with the N-terminal domain of the GR. The assay is 100% specific and has a sensitivity of 0.3 µg of GR-DNA binding in nuclear-extracted MCF7. All samples will be ran in duplicate and in one run to avoid batch effects. The absorbances will be determined at a wavelength of 450 nm using a microplate reader (iMark, Bio-Rad, Hercules, CA) and expressed relative to total protein concentration.

Assessment of Serum Cortisol

From the blood samples obtained at the three testing sessions, testosterone and cortisol levels will be determined using a commercially available enzyme-linked immunoabsorbent assay (ELISA) kit (Alpha Diagnostic Laboratories, San Antonio, TX). All samples will be run in duplicate and the assays will be performed at 450 nm wavelength with a microplate reader (iMark, Bio-Rad, Hercules, CA). Hormone concentrations will be determined using data reduction software (Microplate Manager, Bio-Rad, Hercules, CA).

6.0 RISKS & BENEFITS

Potential Risks

Participants who meet eligibility criteria will be subjected to strength testing sessions involving various exercises (described above). They will likely experience muscle soreness for up to 24 to 48 hours after exercise. This soreness is normal and as experienced resistance trainers, a sensation in which they should be familiar. Muscle strains/pulls resulting from exercise testing are possible. During the familiarization session, participants will be informed of the exercise protocol and correct technique for the exercise protocol. Therefore, potential injury due to exercise will be minimized since all participants will be instructed

on how to adhere to correct lifting technique. In addition, only Darryn Willoughby, Ph.D., Joshua Gann, M.S. and Tom Andre, M.S., all with expertise in resistance training, will conduct the testing and exercise procedures.

Dehydration and heat exposure as in the proposed study may also disrupt sleep patterns and possibly result in other side effects such as headaches or nausea. To attempt to minimize these risks, core temperature will be monitored continuously throughout dehydration procedures. If core temperature reaches or exceeds 38.9°C or the participant experiences negative subjective feelings (e.g. nausea) the participant will be removed from the hot bath and allowed to cool. When removed from the water to be weighed, participants will be removed from the bath slowly and will be allowed to sit upon exiting in an attempt to avoid dizziness and nausea.

Body composition/bone density will then be determined using a calibrated Hologic 4500W dual energy x-ray absorptiometry (DEXA) by study personnel who have all received training on radiation safety from Baylor's Department of Risk Management. In addition, Dr. Willoughby has a certification in limited x-ray technology training for DEXA. The DEXA body composition test will involve having the participant lie down on their back in a standardized position in a pair of shorts and t-shirt. A low dose of radiation will then scan their entire body for approximately six minutes. The DEXA segments regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments for determination of fat, soft tissue (muscle), and bone mass. Radiation exposure from DEXA for the whole body scan is approximately 1.5 mR per scan. This is similar to the amount of natural background radiation a person would receive in one month while living in Waco, TX. The maximal permissible x-ray dose for non-occupational exposure is 500 mR per year. Total radiation dose will be less than 6 mR for the entire study.

Participants will donate about approximately 15 milliliters of venous blood and approximately 20-30 mg of muscle a total of 4 times during the study. Blood samples will be obtained by an experienced phlebotomist using standard procedures and sterile techniques. In addition, Dr. Willoughby is a certified phlebotomy technician. These procedures may cause a small amount of pain when the needle is inserted into the vein as well as some bleeding and bruising. However, proper pressure will be applied upon removal to reduce bruising. The participant may also experience some dizziness, nausea, and/or faint if they are unaccustomed to having blood drawn.

The area of the biopsy will be cleaned with alcohol and Betadine. However, there is a risk of infection if the participant does not adequately cleanse the area for approximately 24 hours post-biopsy. Participants will be instructed to remove the band-aid and cleanse the biopsy puncture site with soap and water, pat the area dry, and reapply a fresh adhesive band-aid. The participant will be instructed to leave the band-aid on for 24 hours. The participant will be further advised to

refrain from vigorous physical activity with the affected leg for 24 hours after the biopsy. Although rare, there is a potential risk for striking a nerve during the biopsy procedure increasing post procedure soreness and possibly temporary numbness to the area. There is a potential risk of an allergic reaction to the Lidocaine. All participants will be asked if they have known allergies to local anesthetics (e.g. Lidocaine, Xylocaine, etc.) that they may have been previously given during dental or hospital visits. Participants with known allergies to anesthesia medications will not be allowed to participate in the study. This procedure may cause a small amount of pain when the needle is inserted to subcutaneously inject the Lidocaine and participants may also experience some dizziness, nausea, and/or faint if unaccustomed to needles. However, due to the localized effects of the anesthetic, the participant should feel no pain during this process. In addition, the biopsy procedure may cause some bleeding and bruising. If needed, the subject may take non-prescription analgesic medication such as acetominophen to relieve pain if needed. However, medications such as aspirin, Advil, Nuprin, Bufferin, or Ibuprophen will be discouraged as these medications may lead to ecchymosis at the biopsy site. Soreness of the area may occur for about 24 hours post-biopsy. Darryn Willoughby, Ph.D., Joshua Gann M.S., and Thomas Andre, M.S. are trained in muscle biopsy techniques and will be the only study personnel performing all blood sampling and muscle biopsies.

Trained, non-physician exercise physiologists certified in CPR will supervise participants undergoing testing and assessments. An automated electronic defibrillator (AED) is located in the BLEST, Marrs McLean Gym, room 127 and two doors down from the EBNL (Marrs McLean Gym, room 120). A telephone is in both laboratories in case of any emergencies, and there will be no less than two researchers working with each participant during testing sessions. In the event of any unlikely emergency one researcher will check for vital signs and begin any necessary interventions while the other researcher contacts Baylor's campus police at extension 2222. Instructions for emergencies are posted above the phones in the event that any other research investigators are available for assistance. Participants will be informed to report any unexpected problems or adverse events they may encounter during the course of the study to Darryn S. Willoughby, Ph.D. If clinically significant side effects are reported, the participants will be referred to their physician for medical follow-up. New findings and/or medical referrals of unexpected problems and/or adverse events will be documented, placed in the participants research file, and reported to the Baylor IRB committee.

Incidental Findings

Although the procedures the participants will undergo in this study are being undertaken for research purposes only, it is possible that researchers may notice something that could be important to their health. If so, we will contact them to explain what was noticed. If they do not have a private physician, we will refer

them to an appropriate clinic for follow-up. It will be their choice whether to proceed with additional tests and/or treatments to evaluate what we observed, and them or their insurer will be responsible for these costs.

Potential Benefits

The main benefit that participants may obtain from this study is a better understanding of how various hydration states and resistance training can impact the physiological response that occurs. However, even if no individual benefit is obtained, participating in this study will help to determine the impact of hydration on resistance training. This information will be helpful to athletes, non-athletes and coaches alike who employ different types of resistance training with the intent of increasing hypertrophic conditions to know how hydration status impacts resistance training.

7.0 STATISTICAL ANALYSIS

Statistical analysis

Data will be analyzed with separate 3 (trial) x 1 (session) analysis of variance (ANOVA) using SPSS for Windows Version 20.0 software (SPSS, Chicago, IL). Significant differences among groups will be identified by a Tukey HSD post-hoc test. However, to protect against Type I error, the conservative Hunyh-Feldt Epsilon correction factor will be used to evaluate observed within-group F-ratios. For statistical procedures, a probability level of ≤ 0.05 will be adopted throughout the study.

Data Management

Participants in this study will not be individually identified unless they give their written consent. All participants will have a number to identify their results. Only the study personnel will know the subject numbers. Only study personnel will have access to the data. All data will be stored in a locked cabinet in the Exercise and Biochemistry Laboratory and only Darryn Willoughby, Ph.D. will have access to the key.

8.0 DATA MANAGEMENT & PRIVACY/CONFIDENTIALITY

Confidentiality

We will keep the records of this study confidential by assigning each participant with an ID code that will also include the experimental condition code and an identifier for the particular sample. Hard copy records will be kept in filing cabinets that are housed in our lab and locked at all times. Electronic data is stored on password-protected computers housed in our lab. Data collected on these computers will be transferred to Dr. Willoughby's password-protected

computer and backed-up on a password-protected external hard drive. The researcher's computer will be maintained in MMG 123 (Dr. Willoughby's office), and the external hard drive locked in a combination-protected, fire-proof safe, also located in MMG 123. The office is locked in Dr. Willoughby's absence. The researchers will not discuss participant information with anyone other than them or the members of the research team (Darryn Willoughby, Ph.D., Joshua Gann, M.S., and Thomas Andre, M.S.).

We will make every effort to keep the participants' records confidential. However, there are times when federal or state law requires the disclosure of their records. The following people or groups may review the study records for purposes such as quality control or safety: The researcher and any member of his research team; authorized members of Baylor University who may need to see study information, such as administrative staff members from the Office of the Vice Provost for Research and members of the Institutional Review Board (a committee which is responsible for the ethical oversight of the study), and; federal and state agencies that oversee or review research (such as the HHS Office of Human Research Protection or the Food and Drug Administration).

The study data will be stored in our lab (MMG 120) for the duration of the study and for three years following the conclusion of the study.

Future Use of Study Information

We would like to store the study information – including blood and muscle samples - for future research related to the relationship between hypohydration, catabolism, and muscle performance. We will label all of the participant's study information with a code instead of their name. The key to the code connects their name to their study information. The researcher will keep the code in a password-protected computer-locked file.

The samples will be stored in an ultralow freezer located in MMG 120. Each sample will be kept for three years. After that time, the sample will be destroyed by methods in accordance with laboratory or institution procedures.

Future use of study information is optional for this study. If the participant does not want their information to be used for future research, they can still be in the study. The participant will indicate their decision at the end of the informed consent form.

Research Team

Darryn S. Willoughby, PhD, FACSM, FISSN, FACN. Dr. Willoughby will serve as the principal investigator. He is a Professor of Exercise and Muscle Physiology and Biochemistry in the Department of Health, Human Performance, & Recreation at Baylor University. He is also an Associate Professor of Baylor's

Biomedical Science Institute. Dr. Willoughby is an internationally recognized exercise biochemist and molecular physiologist. He has conducted a vast amount of research focusing on the biochemical and molecular regulatory mechanisms regarding exercise performance and nutrition. He will perform blood sampling and oversee all aspects of the study and perform the majority of the biochemical and clinical chemistry assays involved in the project.

Joshua Gann, M.S. Mr. Gann is an exercise physiologist pursuing his Ph.D. in Exercise, Nutrition, and Preventative Health and serves as a research assistant in the EBNL. He may assist in any aspect of the project, particularly recruiting participants, muscle biopsies and blood draws, and data analysis.

Thomas Andre, M.S. Mr. Andre is an exercise physiologist pursuing his Ph.D. in Exercise, Nutrition, and Preventative Health and serves as a research assistant in the EBNL. He will assist in all aspects of the project, particularly recruiting participants, muscle biopsies and blood draws, and data analysis.

9.0 DATA & SAFETY MONITORING

Adverse Events

Trained, non-physician exercise physiologists certified in CPR will supervise participants undergoing testing and assessments. An automated electronic defibrillator (AED) is located in the BLEST, Marrs McLean Gym, room 127 and two doors down from the EBNL (Marrs McLean Gym, room 120). A telephone is in both laboratories in case of any emergencies, and there will be no less than two researchers working with each participant during testing sessions. In the event of any unlikely emergency one researcher will check for vital signs and begin any necessary interventions while the other researcher contacts Baylor's campus police at extension 2222. Instructions for emergencies are posted above the phones in the event that any other research investigators are available for assistance. Participants will be informed to report any unexpected problems or adverse events they may encounter during the course of the study to Darryn S. Willoughby, Ph.D. If clinically significant side effects are reported, the participants will be referred to their physician for medical follow-up. New findings and/or medical referrals of unexpected problems and/or adverse events will be documented, placed in the participants research file, and reported to the Baylor IRB committee. There will not be any independent monitoring of the source data.

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APPENDIX C

Data Collection Form

Dehydration Trial

ID# _____ Age _____ Date _____

Height _____ Weight_____ Bod Fat% _____

Pre-Weight _____ Target Weight _____

Weight at 30 mins _____ 60 mins_____ 90 mins _____ 120_____ 150_____ 180_____

Body temp/Water Temp at 30 mins _____/_____ 60 mins_____/_____ 90 mins

_____/_____ 120 _____/_____ 150 _____/_____ 180_____/_____

Time in hot bath _____

Pre-RT Wt _____ Pre- RT Wt _____ Avg Wt. _____

Pre-RT USG _____ Pre- RT USG _____ Avg USG _____

How thirsty are you?

Not thirsty at all _____ **Extremely thirsty**

How well did you sleep?

Very Poorly _____ **Very Well**

DT

PRS _____ Perceived Readiness_____ Session RPE _____

Bench Press 1RM _____

	Bench Press	**#Reps Set 1**	**# Reps Set 2**	**#Reps Set 3**	**Total Reps**	**RPE**
1.	_____	_____	_____	_____	_____	
	_____					
		HR: _____	_____	_____		
		#Reps Set 4	**# Reps Set 5**			
		_____	_____			
		HR: _____	_____			

Leg Press 1RM _____

	Leg Press	**#Reps Set 1**	**# Reps Set 2**	**#Reps Set 3**	**Total Reps**	**RPE**
2.	_____	_____	_____	_____	_____	
	_____					
		HR: _____	_____	_____		
		#Reps Set 4	**# Reps Set 5**			
		_____	_____			
		HR: _____	_____			

Vertical Jump **_____ _____ _____**

Hydration Trial

ID# _____ Age _____ Date _____

Height _____ Weight_____ Bod Fat% _____

Pre-Weight _____ Target Weight _____

Weight at 30 mins _____ 60 mins_____ 90 mins _____ 120_____ 150_____ 180_____

Body temp/Water Temp at 30 mins _____/_____ 60 mins_____/_____ 90 mins

_____/_____ 120 _____/_____ 150 _____/_____ 180_____/_____

Time in hot bath _____

Pre-RT Wt _____ Pre- RT Wt _____ Avg Wt. _____

Pre-RT USG _____ Pre- RT USG _____ Avg USG _____

How thirsty are you?

Not thirsty at all _____ **Extremely thirsty**

How well did you sleep?

Very Poorly _____ **Very Well**

HT

PRS _____ Perceived Readiness_____ Session RPE _____

Bench Press 1RM _____

	Bench Press	#Reps Set 1	# Reps Set 2	#Reps Set 3	Total Reps	RPE
1.	_____	_____	_____	_____	_____	_____
	HR:	_____	_____	_____		
		#Reps Set 4	**# Reps Set 5**			
		_____	_____			
	HR:	_____	_____			

Leg Press 1RM _____

	Leg Press	#Reps Set 1	# Reps Set 2	#Reps Set 3	Total Reps	RPE
2.	_____	_____	_____	_____	_____	_____
	HR:	_____	_____	_____		
		#Reps Set 4	**# Reps Set 5**			
		_____	_____			
	HR:	_____	_____			

Vertical Jump **_____ _____ _____**

APPENDIX D

Medical History Questionnaire

BAYLOR UNIVERSITY
EBNL

MEDICAL HISTORY INVENTORY

Directions. The purpose of this questionnaire is to enable the staff of the exercise and sport sciences laboratory to evaluate your health and fitness status. Please answer the following questions to the best of your knowledge. All information given is **confidential** as described in the **informed consent statement.**

Name: _____ Age: ____Date of birth: _____

Name and address of your physician (if known):

_____

_____

Medical history

Do you have or have you ever had any of the following conditions? (Please write the date when you had the condition in blank).

___ Heart murmur, clicks, or other cardiac findings?
___ Frequent, extra, skipped, or rapid heartbeats?
___ Chest pain (with or without exertion)?
___ High cholesterol?
___ Diagnosed high blood pressure?
___ Heart attack or any cardiac surgery?
___ Leg cramps (during exercise)?
___ Chronic swollen ankles?
___ Varicose veins?
___ Frequent dizziness/fainting?
___ Muscle or joint problems?
___ High blood sugar/diabetes?
___ Thyroid disease?
___ Bleeding disorder?
___ Topical anesthetic allergy?
___ Asthma/breathing difficulty?
___ Bronchitis/chest cold?
___ Melanoma/skin lesions?
___ Stroke or blood clots?
___ Emphysema/lung disease?
___ Epilepsy/seizures?
___ Rheumatic fever?
___ Scarlet fever?
___ Ulcers?
___ Pneumonia?
___ Anemias?
___ Liver or kidney disease?
___ Autoimmune disease?
___ Nerve disease?
___ Psychological disorders?

Do you have or have you been diagnosed with any other medical condition not listed?

_____

_____

_____

_____

_____

Please provide any additional comments/explanations of your current or past medical history.

_____

_____

_____

Please list any recent surgery (e.g., type, dates etc.).

_____

_____

_____

_____

List all prescribed/non-prescription medications and nutritional supplements you have taken in the last 3 months.

_____

_____

_____

_____

What was the date of your last complete medical exam? _____

_____

Are you pregnant, do you think you may be pregnant or are you planning on becoming pregnant over the course of the study? Yes _____ No _____

What was the date of your last period? _____

Have you had regular periods for the past 6 months? Yes _____ No _____

If you answered no to the question above, how many periods have you missed during the past 6 months?

_____

_____

Do you know of any medical problem that might make it dangerous or unwise for you to participate in this study (including strength and maximal exercise tests)? If yes, please explain.

Yes ____ No ____

_____
_____
_____
_____

Recommendation for participation

____ No exclusion criteria presented. Subject is ***cleared*** to participate in the study.

____ Exclusion criteria is/are present. Subject is ***not cleared*** to participate in the study.

Signed: _____ date: _____

APPENDIX E

Exercise History Questionnaire

PERSONAL INFORMATION

Name: _____

Address: _____

City: _____ State: _____ Zip code _____

Home phone: (___) _____ Work phone: (___)_____

Cellular (___) _____

Email address:_____

Birth date: _____ / _____ / _____ Age: _____ Height: _____ Weight: _____

Exercise & supplement history/activity questionnaire

1. Describe your typical occupational activities.

2. Describe your typical recreational activities

3. Describe any exercise training in which you routinely participate.

4. How many days per week do you exercise/participate in these activities?

5. How many hours per week do you exercise?

6. How long (years/months) have you been consistently exercising/training?

7. When was the last time you ingested any nutritional supplement?

8. If applicable, list the nutritional supplement(s) that you most recently ingested.

APPENDIX F

Recruitment Flyer

LADIES, WE NEED YOU!

PHYSICALLY-ACTIVE FEMALES NEEDED FOR A RESISTANCE TRAINING/HYDRATION STUDY

RESEARCHERS IN THE EXERCISE & BIOCHEMICAL NUTRITION LAB AT BAYLOR UNIVERSITY ARE RECRUITING 10 HEALTHY, PHYSICALLY-ACTIVE FEMALES BETWEEN THE AGES OF 18-30 TO PARTICIPATE IN A STUDY DESIGNED TO EVALUATE EFFECTS OF HYPOHYDRATION ON RESISTANCE EXERCISE PERFORMANCE AND MARKERS OF CATABOLISM. PARTICIPANTS WILL BE REQUIRED TO ENGAGE IN 4 VISITS TO THE LAB FOR TESTING. PARTICIPANTS WILL BE ALSO REQUIRED TO UNDERGO BODY COMPOSITION AND MUSCULAR STRENGTH/ENDURANCE TESTING, AND TO ALSO SUBMIT TO PROVIDING BLOOD SAMPLES AND MUSCLE BIOPSIES. ELIGIBLE SUBJECTS WILL RECEIVE $200 FOR COMPLETING THE STUDY AND FREE MUSCLE STRENGTH, AND BODY FAT TESTING.

FOR MORE INFORMATION CONTACT:

EXERCISE & BIOCHEMICAL NUTRITION LAB
DEPARTMENT OF HHPR
RENA MARRS MCLEAN GYMNASIUM ROOM 120

JOSHUA_GANN@BAYLOR.EDU

DARRYN_WILLOUGHBY@BAYLOR.EDU

Exercise • Biochemistry • Nutrition • Health
BU EBNL
BAYLOR UNIVERSITY
Exercise & Biochemical Nutrition Laboratory

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