EFFECT OF ACUTE ALCOHOL INGESTION ON RESISTANCE EXERCISE INDUCED mTORC1 SIGNALING IN HUMAN MUSCLE

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Dissertation Prepared for the Degree of

DOCTOR OF PHILOSOPHY

UNIVERSITY OF NORTH TEXAS

August 2015

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Duplanty, Anthony A. *Effect of acute alcohol ingestion on resistance exercise induced mTORC1 signaling in human muscle*. Doctor of Philosophy (Biology), August 2015, 84 pp, 2 tables, 7 figures, references, 46 titles.

The purpose of this project was to further elucidate the effects post-exercise alcohol ingestion. This project had many novel aspects including using a resistance exercise (RE) only exercise design and the inclusion of women. To our knowledge, we are the first to investigate the effect of post-RE alcohol ingestion in women. In the first chapter of this project, information on the prevalence of alcohol use and the importance of skeletal muscle as a dynamic and metabolic tissue was provided. In chapter two, the effects of post-RE alcohol ingestion in men and women are detailed. The major findings of this study was that although RE elicited similar mTORC1 signaling both in men and in women, alcohol ingestion appeared to only attenuate RE-induced phosphorylation of the mTORC1 signaling pathway in men. The third chapter focused on examining the effects of post-RE alcohol ingestion on acute testosterone bioavailability. The primary findings of this study was that alcohol substantially elevated serum total and free testosterone concentrations during recovery from a bout of resistance exercise. The fourth chapter detailed factors that contribute to bone density in men. The major findings of this study was that young adult male long-distance runners who participated in resistance training at least once per week had greater bone mineral density than their non-resistance trained and non-exercise trained peers.

ACKNOWLEDGMENTS

I would like to sincerely thank my major professor, Dr. Jakob L. Vingren, for all the years of mentorship at the Applied Physiology Laboratory. Without your support and guidance I could not have become the scientist that I am today. The teachings that you have provided me, both in the classroom and in the lab, have enabled me to accomplish so much. I will always consider you a great mentor and friend.

I would also like to express my gratitude for the contributions of my committee. The donation of your time and expertise was an essential component for the completion of this dissertation.

I would also like to thank all of the students that I have worked with in the Applied Physiology Laboratory over the years: Ron Budnar, Danielle Levitt, Adam Venable, Ying Luk Hui, Alex Fernandez, Travis Layman, and Daniel Fancher. Without your valuable assistance, I could not have completed the many weeks of data collection for this study. We had the best science adventures and I will miss you all.

Finally, I would like to give a very special thanks to my dear wife, Shaye. You have stood by me through my years of grad school with so much patience and encouragement. You are my sunshine, my love, and my best friend.

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CHAPTER 1

IMPLICATIONS OF ALCOHOL ON RESISTANCE EXERCISE-INDUCED mTORC1 SIGNALING

1.1 Introduction

Alcohol (ethanol) is consumed worldwide and represents the most commonly used and abused recreational drug. Alcohol has toxic effects on most organs and tissues in the body, and chronic use can lead to dependence. Acute heavy episodic drinking (i.e., binging), defined as ingesting six or more standard drinks on one occasion, is associated with detrimental consequences even if an individual's average level of alcohol consumption is relatively low (WHO, 2014). An estimated 16% of drinkers worldwide aged 15 years and above are thought to engage in binge drinking (WHO, 2014). In the USA, over 70% of adults reported having consumed alcohol in the previous year, 29% reported excessive drinking, and 27% reported binge drinking in the previous month (Esser et al., 2014). In college athletes in the USA, over 83% report drinking alcohol during the past 12 months, and approximately 49% report binge drinking (NCAA, 2012). Alcohol consumption has been found to be higher in individuals who engage in regular physical activity than in sedentary individuals (Moore et al., 2008). Furthermore, among exercisers, those who exercise vigorously are more likely to engage in heavy drinking (French et al. 2009).

Skeletal muscle is an important and dynamic tissue, comprising of ~40% of a human's total mass, and it contains approximately 50-75% of all body proteins (Frontera et al., 2014). Skeletal muscle plays a key role in whole body metabolism and is essential for mobility, disease prevention, and quality of life (Lynch, 2004; Srikanthan

and Karlamangla, 2011). Muscle growth (hypertrophy) occurs when there is a net increase in the rate of protein synthesis, while reduction in muscle mass (atrophy) occurs when there is a net decrease in the rate of protein synthesis (Goodman, 2014). A major regulator of the rate of skeletal muscle protein synthesis in mammalian systems is the mechanistic target of rapamycin complex 1 (mTORC1), which has been extensively researched over the past decade [for extensive reviews see (Sarbassov et al., 2005; Caron et al., 2010; Ge and Chen, 2012; Laplante et al., 2013)].

Resistance exercise (RE) has been shown to be a potent stimulator for mTORC1 mediated protein synthesis (Barr and Esser, 1999; Bodine et al., 2001; Spiering et al., 2008). Conversely, alcohol consumption has been shown to inhibit signal transduction of mTORC1 (Lang et al., 2003, 2004, 2007, 2009). The possible interaction between RE and alcohol consumption has received limited attention. The purpose of this brief review is to: 1) provide an overview of the mechanisms of mTORC1 signaling and its effects on protein synthesis in skeletal muscle; 2) present current evidence on RE-induced stimulation of mTORC1 signaling; 3) present current evidence on the effect of acute alcohol ingestion on mTORC1 signaling; and 4) present what is known of the effects of acute alcohol ingestion on RE-induced mTORC1 signaling.

1.2 mTORC1 Signaling

Rapamycin is an immunosuppressive and anti-tumor drug that was discovered in 1965 in soil samples on the island of Rapa Nui (Sehgal et al., 1975). Research on rapamycin lead to the 1991 discovery of its target, an atypical serine/threonine kinase, aptly named target of rapamycin (TOR) found in yeast (Saccharomyces cerevisiae)

(Heitman et al. 1991). After several name changes, including mammalian TOR, the HUGO Gene Nomenclature Committee standardized the name of the enzyme to mechanistic target of rapamycin (mTOR), to make it more applicable to non-mammal species (Hall, 2013). mTOR is a 289 kDa protein Ser-Thr kinase that resides within two heteromeric complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 is largely involved in mediating mRNA translation and, thus, protein synthesis, while mTORC2 regulates actin cytoskeleton organization and cell survival. Moreover, mTORC1 consists of four proteins: mTOR, raptor, mLST8, and PRAS40, with the latter three facilitating increased mTOR activity (Corradetti and Guan, 2006; Fonseca et al., 2007). For the purposes of this review, further mention of mTOR will only reflect mTORC1 signaling.

mTORC1 mediates mRNA translation through its activation (phosphorylation) of two primary downstream targets: ribosomal S6 kinase 1 (S6K1) and the eukaryotic initiation factor 4E binding protein 1 (4E-BP1). When activated by mTORC1, S6K1 phosphorylates the S6 ribosomal subunit 40S, which in turn, increases translation of mRNAs (Terada et al., 1994). 4E-BP1 inhibits mRNA translation by binding to, and preventing the action of, eukaryotic translation initiation factor 4E (eIF4E). When mTORC1 hyper-phosphorylates 4E-BP1, it unbinds from eIF4E, allowing the formation of the translation initiation complex eIF4F, thus increasing translation (Kimball et al., 2002). Thus combined mTORC1 signaling enhances ribosomal translation efficiency. Normal mTORC1 functioning is essential for anabolic processes, including the muscle hypertrophy that occurs consequent to chronic performance of RE (i.e., training);

whereas, aberrant mTORC1 functioning has been associated with human diseases such as diabetes, cancer, and obesity (Laplante and Sabatini, 2012).

In summary, mTORC1-mediated phosphorylation of S6K1 and 4E-BP1 stimulates protein synthesis by increasing the rate of translation initiation and ribosomal translation efficiency. Following sections in this review will focus on the stimulatory effects of RE on mTORC1 signaling, the inhibitory effects of alcohol ingestion on mTORC1 signaling, and the effect of acute alcohol ingestion on RE-induced mTORC1 signaling.

1.3 Resistance Exercise-Induced mTORC1 Signaling

RE is a potent stimulus for increasing strength and skeletal muscle mass (Tesch et al., 1988) and has been shown to increase the phosphorylation of mTORC1 and its downstream targets S6K1 and 4E-BP1 in human and animal studies (Barr and Esser, 1999; Bodine et al., 2001; Dryer et al., 2006). In the first study to investigate the role of muscle contraction on mTORC1 signaling, Barr and Esser (1999) found that S6K1 phosphorylation was increased in response to electrode-produced high intensity contractions in rats. The contraction-induced S6K1 phosphorylation was associated with increased translation initiation, thus providing evidence for the first time that high intensity muscle contraction is associated with increased mTORC1 signaling and protein synthesis (Barr and Esser, 1999). Subsequent rodent studies have reported similar increased mTORC1 signaling in response to acute muscle contraction (Atherton et al., 2005; Parkington et al., 2003; Thompson et al., 2008). The importance of mTORC1 signaling for muscle contraction-induced muscle growth (i.e., training-induced

hypertrophy) was shown in an early study conducted by Bodine and colleagues (2001). Following rapamycin treatment to selectively block mTOR signaling, rat muscles were overloaded and compared to overloaded non-rapamycin treated muscle (Bodine et al., 2001). In the rapamycin treated rats, S6K1 phosphorylation was reduced and 4E-BP1 binding to eIF4E was increased, leading to attenuated protein synthesis. These findings provide evidence that the increase of mTORC1 signaling is crucial to RE-induced skeletal muscle growth.

Activation of mTORC1 signaling by RE has also been shown in human studies to be an important stimulator of skeletal muscle protein synthesis (Drummond et al., 2008). Acute RE and acute endurance exercise in men has been shown to independently increase mTOR phosphorylation in untrained human muscles, with only RE resulting in increased S6K1 phosphorylation and myofibrillar protein synthesis post exercise. Additionally, after 10 weeks of unilaterally training each leg with either RE or cycling, only the RE trained leg had increased mTOR phosphorylation and protein synthesis at rest and post exercise (Wilkinson et al., 2008). While amino acid intake (leucine) has been shown to be a regulator of mTORC1 signaling, S6K1 phosphorylation can still be increased in men in a fasted state after a single bout of RE; however, S6K1 is significantly greater when in the fed state versus a fasted state (Glover et al., 2008). While most human studies have investigated RE-induced mTORC1 signaling in men, Dreyer et al (2010) established that RE increases mTORC1 signaling and muscle protein synthesis independent of sex. For both genders, mTOR and S6K1 phosphorylation increased 2 hours after RE and muscle protein synthesis was increased by 52% in men and 47% in women (post-RE (Dreyer et al., 2010).

In summary, RE has an important influence on muscle training adaptations (i.e., hypertrophy) through its stimulus on mTORC1 signaling and muscle protein synthesis. The RE-induced stimulus on mTORC1 signaling has been implicated as a necessary component of muscle protein synthesis and any effectors that may negatively influence normal mTORC1 signaling represent a potentially potent detriment for muscle training adaptations.

1.4 The Effects of Alcohol on mTORC1 Signaling

It has been long understood that acute and chronic alcohol consumption contributes to skeletal muscle atrophy (Fahlgren et al., 1957). Several physiological mechanisms including hormonal disruption, increased proteolysis, and reduced protein synthesis have been identified as contributors to this alcohol-induced myopathy (Preedy et al., 1994; Reilly et al., 1997). Acute alcohol consumption leads to aberrant mTORC1 signaling in a time and dose-dependent manner resulting in reduced ribosomal translation efficiency (Lang et al., 2001). It appears that the effect of acute alcohol ingestion on this signaling pathway is at the level of mTORC1 (Lang et al 2009). In support of this, Kumar et al. (2002) found that phosphorylation of mTOR, but not of AKT (PKB) (an upstream regulator of mTORC1) was inhibited by acute alcohol ingestion in rats. Furthermore, administration of an intoxicating dose of alcohol did not affect the abundance of mRNA in skeletal muscle (Lang et al.,2000; Reilly et al., 1997), emphasizing that alcohol-induced reduction in protein synthesis is likely due to reduced translation. This inhibition of mTORC1 signaling appears to primarily affect signal transduction (phosphorylation) and is independent of changes in total content of

mTORC1 proteins, alcohol metabolism, glucocorticoids, and proinflammatory cytokines (Frost et al., 2005; Kozrick et al., 2013; Lang et al., 2004; Preedy et al., 1991).

A series of rodent model studies conducted by Lang et al. (2003, 2004, 2007, 2009, 2010) collectively showed that, at rest, alcohol intoxication decreases phosphorylation of mTOR and its downstream targets S6K1 and 4E-BP1 and decreases protein synthesis. In muscle cell culture investigations using human and mouse myocytes, alcohol incubation *in vitro* was found to directly inhibit mTORC1 activity and protein synthesis (Hong-Brown et al., 2001, 2012). Alcohol has been shown to decrease the distribution of the 40S ribosomal subunit, which is activated by S6K1, representing an interference in ribosomal initiation and elongation (Lang et al., 1999). Acute alcohol administration greatly increases the amount of 4E-BP1 bound to eIF4E, which prevents formation of the eIF4F complex (Lang et al., 2000). The eIF4F complex is a rate limiting and major regulatory component of translation initiation in muscle. A decrease in 4E-BP1 phosphorylation was found in both young and mature rats, providing evidence that the inhibitory effect of alcohol is independent of age (Lang et al., 2009).

Thus, the effects of alcohol on 4E-BP1 and S6K1 signaling and the resultant decrease in ribosomal translation might, at least in part, explain resultant decreased protein synthesis. While the exact mechanisms underlying alcohol-induced aberrant mTORC1 signaling have yet to be fully elucidated, findings suggests that the loci of these impairments are at the level of mTORC1 and its downstream targets, rather than upstream of mTORC1.

In summary, alcohol ingestion can be considered myotoxic as it decreases mTORC1 signaling and rates of protein synthesis. There is currently a lack of human

studies available investigating the effect of alcohol on mTORC1 signaling at rest. Only two studies to date, discussed in the following section, appear to have investigated the effects of acute alcohol ingestion on mTORC1 signaling in humans in vivo.

1.5 Effects of Acute Alcohol Ingestion on Resistance Exercise-Induced mTORC1 Signaling

Independently, alcohol consumption and RE have opposite effects on mTORC1 signaling and muscle protein synthesis (See Figure 1.1) but the combined effects from alcohol consumption and RE on mTORC1 are complex and have not yet been fully established. Only a few studies have investigated alcohol ingestion in the context of exercise and the results are divergent. This divergence might arise from differences in model (rodent vs. human), exercise mode, alcohol dose and dosing pattern, and time points of muscle sampling between studies. The inhibitory effects of alcohol consumption on mTORC1 signaling post-muscle contraction can persist for greater than 12 hours after acute administration in mice (Steiner and Lang, 2014). Muscle contraction induced increase of S6K1 and 4E-BP1 was blunted by alcohol intoxication and rates of protein synthesis were decreased for up to 12 hours even after alcohol clearance via metabolism and return of baseline mTOR phosphorylation (Steiner and Lang, 2014).

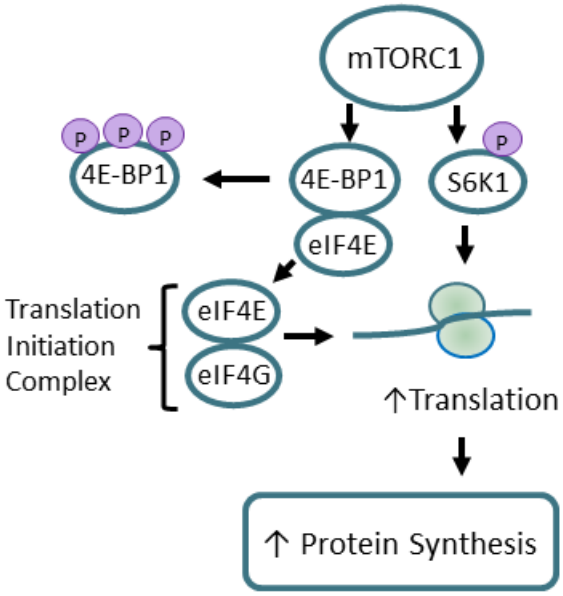


Figure 1.1. mTORC1 Signaling Pathway. Effects of alcohol and resistance exercise on mTORC1 mediated phosphorylation of 4E-BP1 and S6K1. Phosphorylation of S6K1 increases mRNA translation. Phosphorylation of 4E-BP1 allows eIF4E to bind with eIF4G to form a translation initiation complex, thus increasing translation.

In the first study to examine the effects of acute alcohol ingestion on exercise-induced mTORC1 signaling in men, Parr et al. (2014) found that alcohol attenuated the stimulatory effect of combined endurance and resistance exercise and protein supplementation on mTOR phosphorylation and protein synthesis, but not on S6K1 phosphorylation 2 hours after exercise. In contrast, 4E-BP1 was not affected by exercise and protein ingestion with or without alcohol ingestion. It is noteworthy that, during recovery from exercise, Parr et al. (2014) fed participants two large boluses of whey protein which is rich in leucine. Due to the stimulatory effect of leucine on S6K1 phosphorylation, potential negative effects of alcohol on S6K1 phosphorylation might have been overwhelmed. This notion is supported by the lack of an increase in S6K1 phosphorylation post-exercise when carbohydrate and alcohol was ingested instead of protein and alcohol (Parr et al., 2014). Recently, Duplanty et al. (unpublished data)

investigated the effect of alcohol ingestion on RE-induced mTORC1 signaling in men and women. Alcohol ingestion affected RE-mediated phosphorylation of the mTORC1 pathway in men but not in women. In men, phosphorylation of mTOR and S6K1 was increased during the recovery phase (at 3 hours after RE) for the placebo condition, but alcohol ingestion prevented this increase in phosphorylation of mTOR and S6K1. It is important to note, that while alcohol ingestion prevented the RE-induced increase of mTOR phosphorylation, the RE bout might have prevented an alcohol-induced decrease in mTOR phosphorylation. In women, mTOR phosphorylation was increased during the recovery phase (at 3 hours after RE), but this increase was not affected by alcohol ingestion; no changes from baseline were found for S6K1 phosphorylation following exercise with or without alcohol ingestion. Despite the increases in mTOR phosphorylation, no changes from baseline were observed for 4E-BP1 for men or women regardless of drink condition. Thus, it appears that alcohol ingestion affects RE-mediated phosphorylation of the mTORC1 signaling in men but not in women 3 hours after exercise.

In summary, these findings suggest that alcohol attenuates RE-induced mTORC1 signaling in men but they also suggest that RE could be used as a countermeasure for the negative effects of alcohol ingestion on mTORC1 signaling. Since only one study appears to have investigated potential effects of alcohol consumption post exercise in women, future investigations are needed to further elucidate possible sexual dimorphism.

1.6 Conclusion

Protein synthesis, mediated by mTORC1, provides the basis for adaptations (e.g. muscle growth and recovery) to resistance exercise (RE) training. Independently, resistance exercise and alcohol have opposite effects on mTORC1 signaling; when alcohol and RE are combined, the limited evidence suggests that these effects on mTORC1 signaling cancel out each other in men but that for women the mTORC1 signaling response to RE is not affected by alcohol. While extensive investigations on mTOR and its upstream regulators and downstream targets have been conducted over the previous 10 years, the precise mechanisms by which alcohol inhibits mTORC1 signaling are still unknown and future studies are warranted.

CHAPTER 2

EFFECT OF ACUTE ALCOHOL INGESTION ON RESISTANCE EXERCISE-INDUCED mTORC1 SIGNALING IN HUMAN MUSCLE

2.1 Introduction

Excessive alcohol (ethanol) ingestion is a major health risk and can lead to detrimental effects on most physiological systems in the body. Individuals who engage in regular physical activity have higher rates of alcohol use than sedentary individuals (Moore et al., 2008); this association also exists between engagement in vigorous physical activity and alcohol use (French et al., 2009). Furthermore, college athletes are at a greater risk for binge drinking than non-athletes (Ford, 2007) and over 54 percent report drinking alcohol during both their competitive and off seasons with approximately 49 percent drinking five or more drinks in one sitting (i.e., binging) (NCAA, 2012). Although it is common for athletes to consume alcohol directly after training or events, almost 60% believe that their use of alcohol does not affect performance or overall health (NCAA, 2012). However, post-exercise alcohol ingestion negatively influences recovery from muscle damage and accentuates loss of dynamic and static strength (Barnes et al. 2010a; Barnes et al. 2010b). The underlying mechanism for this negative effect of alcohol on recovery has not been elucidated but research on alcohol ingestion in the absence of exercise suggests that reduced protein synthesis might be involved.

Protein synthesis provides the basis for adaptations (e.g. muscle growth and recovery) to resistance exercise (RE) training. The mammalian (or mechanistic) target of rapamycin complex 1 (mTORC1), a protein Ser-Thr kinase, mediates an essential pathway for regulating protein synthesis in skeletal muscle (Bodine et al. 2001). When

activated, mTORC1 mediates phosphorylation of its downstream targets S6K1 and 4E-BP1, enhancing the translational efficiency of ribosomes, thus increasing protein synthesis (Hara et al., 1998). S6K1 is responsible for phosphorylation of the S6 subunit of the 40S ribosomal protein (Terada et al., 1994) resulting in stimulation of mRNA translation initiation. In this signaling pathway, mTORC1 also influences protein synthesis by removing the inhibitory effect of the translational regulator 4E-BP1. When 4E-BP1 is phosphorylated, it unbinds from eukaryotic translation initiation factor 4E (eIF4E), resulting in the formation of the translation initiation complex eIF4F (Kimball et al., 2002), allowing for greater translation initiation.

The mechanical deformation of muscle fibers from RE is a potent stimulator for activation (phosphorylation) of the mTORC1 pathway (Spiering et al., 2008). A direct relationship between increased mTORC1 signaling and RE has been shown in human and animal studies. Barr and Esser (1999) conducted one of the first studies to report evidence of the role of muscle contraction in activating mTORC1 signaling. In that study, rats were attached with stimulating electrodes to produce high-resistance contractions and it was found that S6K1 phosphorylation was increased at 3 and 6 hours post contractions. Similarly, Atherton et al. (2005) found mTORC1 and S6K1 phosphorylation was increased in rats 3 hours following high frequency stimulations that were designed to mimic resistance exercise. In men and women, it has been found that mTORC1 phosphorylation is increased at 1 and 2 hours post RE; whereas, S6K1 is elevated at 2 hours post RE (Dreyer et al., 2006, 2010). The effect of resistance exercise on 4E-BP1 phosphorylation is less clear as decreases, no changes, and increases in phosphorylation have been found during the first hour after RE (Bolster et

al. 2003, Dryer et al., 2006). The collective effects of mTORC1, S6K1, and 4E-BP1 phosphorylation resulting from muscle contraction stimulates a central pathway for increased protein synthesis that is important for adaptations to RE.

Similar to RE, alcohol also affects the signal transduction of the mTORC1 pathway. In a series of studies, Lang and colleagues (Lang et al., 2003, 2004, 2007, 2009) investigated the effects of acute alcohol ingestion on mTORC1 signaling in rats. Collectively, these studies showed that, at rest, alcohol ingestion induced a decrease in protein synthesis and a reduction in mTORC1 pathway activation including reduced mTORC1, S6K1, and 4E-BP1 phosphorylation. A reduction in mTORC1 pathway activation reduces ribosomal translation initiation which could, at least in part, explain the reduced protein synthesis found with alcohol ingestion. Only a single study (Parr et al., 2014) appears to have investigated the effects of acute alcohol ingestion on mTORC1 signaling in the context of exercise in humans. In that study, Parr et al. (2014) found that ingestion of alcohol attenuated protein synthesis and mTORC1 phosphorylation, but not S6K1 phosphorylation, from the collective stimulatory effects of combined endurance and resistance exercise and protein supplementation in men. The effect of acute alcohol ingestion on mTORC1 signaling has not been investigated with heavy resistance exercise or with women.

Independently, resistance exercise and alcohol have opposite effects on mTORC1 signaling. Despite the potential negative implications of acute alcohol ingestion on the physiological response to heavy resistance exercise, no study has examined the mTORC1 signaling in this context. Therefore, the purpose of this study

was to investigate the effect of alcohol ingestion on resistance exercise induced mTORC1 signaling in men and women.

2.2 Methods

Experimental Approach to the Problem

To examine the acute effect of alcohol ingestion following resistance exercise on mTORC1, S6K1, and 4E-BP1 activation (phosphorylation), a within subjects repeated measures design was utilized. Ten resistance trained men and nine resistance trained women completed two identical acute heavy resistance exercise trials (AHRET) as previously described (Vingren et al., 2013). The AHRET sessions were separated by approximately 28 days (to allow for standardization of menstrual phase for the women). From 10-20 minutes post exercise participants consumed either alcohol (alcohol condition) or no alcohol (placebo condition) diluted in an artificially sweetened and calorie free beverage. All participants completed both conditions; conditions were assigned using randomization and were counter-balanced within each gender. Prior to exercise (PRE) and three (+3h) and five (+5h) hours post exercise, muscle tissue samples were obtained from the vastus lateralis by biopsies. Muscle samples were analyzed for phosphorylated mTORC1, S6K1, and 4E-BP1.

Participants

Ten men (mean ± SD: 25.1 ± 3.1 yr, 83.8 ± 15.7 kg, 177 ± 7 cm, 14.8 ± 8.5 % body fat) and nine women (22.4 ± 1.7 yr, 60.1 ± 6.0 kg, 161 ± 4 cm, 26.8 ± 2.9 % body fat) who were recreationally resistance trained (at least 2 sessions per week including the back squat for the past 6 months) participated in this study. Participants were

screened for relevant exclusion criteria including: medical concerns, musculoskeletal problems, use of hormonal substances or medications such as androgenic-anabolic steroids, growth hormone, or glucocorticoids, or adherence to atypical diets that could confound the results of this study. In addition, female participants had to be eumenorrheic and not trying to become pregnant. To be included in the study, participants had to be 21 to 34 years of age and low-moderate consumers of alcohol. Participants completed the "Young Adult Alcohol Problems Screening Test" (Hurlbut et al. 1992) and the "Alcohol Use Disorders Identification Test" (Babor et al. 2001)-written questionnaires on current and past alcohol use to screen for signs of alcohol abuse. Finally, to be included, participants could not have ingestion-induced metabolic intolerance nor be naïve to alcohol ingestion. The study was approved by the University Institutional Review Board and the participants provided written informed consent to participate; all procedures were conducted in accordance with the Declaration of Helsinki.

Procedures

Session 1: Anthropometric Measurements, Familiarization, and 1-Repetition Maximum

Approximately one week before the first exercise and alcohol/placebo session participants reported to the laboratory for anthropometric measurements, familiarization and 1-repetition maximum (1-RM) determination. Body composition was measured using Dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy, GE Healthcare, Fairfield, CT). Participants wore only light athletic clothes and no shoes or metals for measurements of height, body mass, and body composition. Participants then performed a standardized warm-up consisting of light dynamic stretches (heel kicks,

lunges, high knees, high kicks), and unweighted body squats. Following warm-up, participants were familiarized with the proper technique for performing the squat exercise using a Smith machine. The Smith machine allows only vertical translation of the bar. Linear bearings attached to either side of the bar allow it to slide up and down two steel shafts with minimal friction. Once participants have demonstrated proper technique in the Smith machine squat exercise, their 1-RM strength was measured (Kraemer and Fry, 1995). Briefly, participants performed squats for 8-10 repetitions at ~50% of their estimated 1-RM followed by another set of 2-5 repetitions at ~85% of estimated 1-RM. Subsequently, 4-5 one-repetition trials were used to determine the 1-RM.

Sessions 2 & 3: Experimental Treatments

Each participant completed both experimental treatment conditions (alcohol and placebo) and thereby served as his/her own control. To account for hormonal variations during the menstrual cycle, women were “phased” so that they completed the treatment sessions during their early follicular phase (days 2-7 after the start of menses). Therefore, the treatments were performed approximately 28 days apart and administered in a balanced, randomized, crossover design. In order to prevent participants from anticipating treatment conditions, participants were blinded to the drink conditions and informed that they could potentially receive the same treatment on both treatment sessions. Participants were asked to record their dietary intake for the morning of the first treatment session (alcohol or placebo session) and to replicate that diet prior to the second treatment session.

Upon arrival to treatment sessions, participants confirmed that they had complied with the following pre-session instructions; refrain from: 1) eating or drinking anything (except for water) during the 2 hours leading up to the treatment session, 2) any ingestion of alcohol for 84 hours prior to each treatment session, 3) consuming large amounts of caffeine (no more than 1 cup of coffee or equivalent allowed on the morning of the treatment session), 4) performing any resistance exercise or intense aerobic exercise for 96 hours prior to the session, and 5) donating blood within 8 weeks or plasma within 96 hours of the session. All sessions began at the same time of day for each participant (1000-1200h arrival time for all participants) to avoid circadian influences.

Figure 2.1. contains an overview of the experimental treatment session timeline. Upon arrival at the laboratory on each treatment day, participants were screened for the presence of blood alcohol with a breathalyzer and were queried regarding compliance with study guidelines regarding diet, caffeine, alcohol, drugs and exercise. Participants were asked to report to the laboratory in a euhydrated state. Upon arrival at the laboratory, hydration status was measured using urine refractometry; if participants present with urine specific gravity ≥1.020 they were provided water to drink. After the hydration status was addressed, body mass was measured. Sixty five minutes prior to the AHRET, participants consumed a standardized meal replacement drink (Ensure Plus®) containing 8 kcal per kg body mass.

Figure 2.1. Timeline for Experimental Treatment Session.

Fifteen minutes before performing the AHRET (50 min after the meal), participants completed the standardized warm-up described above. Participants then completed 10 warm-up squats at 50% of 1-RM followed by the AHRET. Briefly, the AHRET consisted of 6 sets of 10 repetitions of Smith machine squats starting at 80% of 1-RM with 2 min of rest between sets. If participants were not able to complete 10 repetitions on their own, they were assisted by the researchers to do so and then the load was reduced for subsequent sets. The same load for each set was used for both treatment sessions to standardize total work performed in each session.

After the completion of the AHRET, participants rested in a comfortable and reclining chair for the remainder of the session (next 5 hours) and were not allowed to sleep. Participants were permitted to read, write, or use a laptop/tablet during the rest period.

Alcohol Ingestion

From 10-20 minutes post-AHRET, participants consumed either alcohol (alcohol condition) or no alcohol (placebo condition) in an artificially sweetened and calorie free beverage, similar to procedures previously described (Vingren et al. 2013). For the alcohol condition, a dose of 1.09 g of alcohol (Vodka, Smirnoff Co., Norwalk, CT, 40%

v/v alcohol) per kg fat free body mass was diluted to a concentration of 15 % v/v absolute alcohol. The placebo condition substituted alcohol for an equal volume of water. The participants consumed 1/10 of the drink each minute during the 10-minute ingestion period. To reduce the ability of the participants to differentiate between the drinks (i.e., taste of alcohol) the rim of the glass was smeared with a small amount of alcohol for both conditions.

Muscle Biopsies

Muscle samples were obtained at PRE and three and five hours post exercise by biopsies. Three separate sites 3 cm apart on the same thigh (vastus lateralis) were used to obtain the three biopsies during a treatment session; the opposite thigh was used for the other session- three separate sites were used to avoid confounding influences of local immune/inflammatory responses. Order of thigh biopsy was assigned in a randomized, crossover design. Each muscle sample was obtained from the superficial portion of the vastus lateralis using microbiopsy procedures. Briefly, the skin over the muscle was cleaned using betadine and a local anesthetic (1% lidocaine w/o epinephrine) was injected under the skin and into the muscle. Then, a Pro-Mag 14 gauge microbiopsy needle (Angiotech, Gainesville, FL) was introduced past the fascia and the trigger mechanism was engaged. This advanced an inner cannula 22mm to cut the tissue. The needle puncture site was covered with sterile gauze and compression was applied to prevent bleeding. The site was then closed with a small adhesive bandage (i.e., Band-Aid); no suture was used. Muscle samples were immediately flash frozen in liquid nitrogen and stored at -80ºC until later analysis.

Biochemical analysis

Muscle homogenization

Muscle samples were homogenized following procedures similar to previously described (Vingren et al. 2008). Briefly, muscle samples were homogenized on ice in a buffer (Tissue Extraction Reagent I, Invitrogen, Carlsbad, CA) containing protease and phosphatase inhibitor cocktails (Sigma-Aldrich, St. Louis, MO) (10 µl buffer per mg muscle) using a mini glass tissue grinder (Kontes, Vineland, NJ).

Western Blotting

Eighty µg of homogenized protein was heated (95ºC) for 5min with an equal volume of loading buffer. The sample was loaded into a 4-20% tris-gycine gel (BioRad, Hercules, CA) (200V for 45 min at room temperature). All samples for each participant's biopsies were loaded onto the same gel. Samples were then electrophoretically transferred onto a PVDF membrane (BioRad, Hercules, CA) (70V for 2 h at 4ºC). The membrane was blocked in milk (1 h, 5% dry milk) and incubated (overnight at 4ºC, 5% BSA) with rabbit antibodies (Cell Signaling Technology, Danvers, MA) for phosphorylated: mTORC1 at Ser2448 (#5536), S6K1 at Thr389 (#9234), and 4E-BP1 at Thr37/Thr46 (#2855) and subsequently incubated with an anti-rabbit horseradish peroxidase-linked secondary antibody (#7074) (1 h, 5% BSA). The membrane was then visualized using enhanced chemiluminescence (C-Digit® Blot Scanner, Li-Cor, Lincoln, NE) Phosphorylated concentrations were expressed on a per protein content basis (arbitrary units) using α-tubulin (#3873, Cell Signaling, Danvers, MA) as a reference protein for each time point on the same membrane.

Statistical Analysis

Data for each variable was analyzed for each gender using a two-way ANOVA (treatment x time point) with repeated measures on both factors (IBM SPSS Statistics version 20, Chicago, IL). Fisher's least significant difference post hoc test was used to determine pairwise differences. The alpha-level of significance was set at $p \leq 0.05$ and data are displayed as mean ± SE unless otherwise indicated.

2.3 Results

mTORC1

For men, there was a significant interaction (treatment × time point) effect for mTORC1 phosphorylation ($F_{(2,18)} = 6.713$, $p = 0.007$). At +3h, mTORC1 phosphorylation was higher for placebo than for alcohol. For placebo, mTORC1 phosphorylation was significantly higher at +3h than at to PRE. For women, there was a significant main effect for time ($F_{(2,16)} = 5.685$, $p = 0.014$). mTORC1 phosphorylation was higher at +3h than at PRE and at +5h.

S6K1

For men, there was a significant interaction (treatment × time point) effect for S6K1 phosphorylation ($F_{(2,18)} = 3.756$, $p = 0.043$). For placebo, S6K1 phosphorylation was significantly higher at +3h compared to PRE and +5h. For women, a trend ($p = 0.052$) for time was found.

4E-BP1

There were no significant effects found for 4E-BP1 phosphorylation in men or women. For men, a trend ($p = 0.064$) for an interaction (treatment × time point) effect was found.

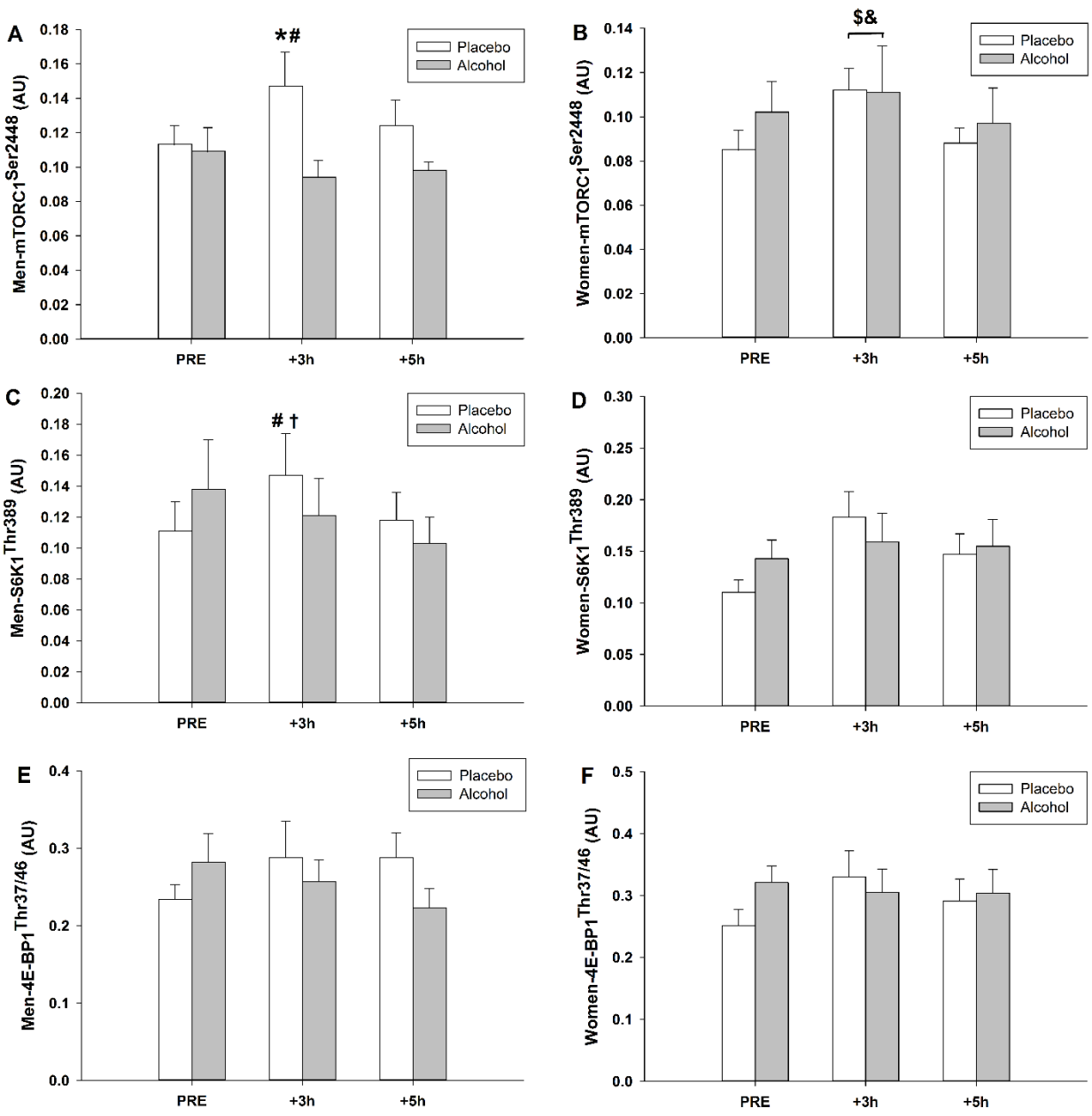


Figure 2.2. Phosphorylation of mTORC1, S6K1, and 4E-BP1. Before resistance exercise (Pre) and 3 (+3h) and 5 hours (+5h) after exercise for men and women. Data are displayed as relative to α-tubulin and presented in arbitrary units (AU). *Significantly different $p \leq 0.05$ from Alcohol condition for corresponding time point. #Significantly different for corresponding Pre. †Significantly different from corresponding +5h. $Significantly different (main effect of time) for corresponding Pre. &Significantly different (main effect of time) for corresponding +5h. Values are Mean ± SE.

2.4 Discussion

The major findings of this study was that alcohol ingestion affected RE-mediated phosphorylation of the mTORC1 pathway in men but not in women. Considering the high rate of alcohol usage among athletes and other individuals who engage in regular recreational physical activity, the findings from this study provide important physiological insight regarding alcohol's effect on processes involved in protein synthesis during recovery from resistance exercise. The mTORC1 signaling pathway mediates protein synthesis in skeletal muscle (Bodine et al. 2001) and RE increases phosphorylation of mTORC1 and its downstream targets (Bodine, 2006). In contrast, alcohol ingestion (in the absence of exercise) reduces mTORC1 signaling and protein synthesis (Lang et al., 2003, 2004, 2009) but the collective effects of RE and alcohol has not been elucidated. It has previously been found that ingestion of alcohol attenuated protein synthesis and mTORC1 phosphorylation, but not S6K1 phosphorylation, from the collective stimulatory effects of combined endurance and resistance exercise and protein supplementation in men. To our knowledge, this is the first study to investigate the effects of post-heavy resistance exercise alcohol ingestion (post-RE/Alc) on mTORC1 signaling in men and women.

mTORC1 is a protein Ser-Thr kinase that plays key roles in signaling pathways for cell growth and proliferation through its effects on ribosomal translation (Caron et al. 2010; Harris and Lawrence, 2003). A novel finding of this study was that post-RE/Alc prevented a resistance exercise-induced increase in mTORC1 phosphorylation at +3h post exercise in men. As expected, based on previous findings for men (Dreyer et al., 2006, 2010), for the placebo condition mTORC1 phosphorylation was increased during

the recovery phase (i.e., +3h). An interference of alcohol with mTORC1 phosphorylation from RE was also expected since, in the absence of exercise, acute alcohol ingestion reduces mTORC1 phosphorylation in male rats (Lang et al., 2003, 2009). The alcohol-induced reduction of protein synthesis signal transduction appears to be independent of the kinase directly upstream of mTORC1. Phosphorylation of AKT, which leads to activation of mTORC1 (Bodine et al. 2001), is not affected by alcohol treatment in male rats, while at the same time, mTORC1 phosphorylation is suppressed by the alcohol treatment (Kumar et al., 2002). Therefore, it is important to note, that while alcohol ingestion in the present study prevented the RE-induced increase of mTORC1 phosphorylation, the RE bout might have prevented an alcohol-induced decrease in mTORC1 phosphorylation. The findings of the present study are similar to those of Parr et al. (2014), who recently found that alcohol ingestion attenuated mTORC1 phosphorylation at 2 hours after combined endurance and resistance exercise and protein ingestion in men (Parr et al., 2014); however, in that study mTORC1 phosphorylation at 2 hours was increased compared to baseline in the alcohol condition. Thus, it appears that the ingestion of alcohol prevents or lessens the RE-induced increase in mTORC1 phosphorylation in men. This effect could have important implications for the signal transduction of the mTORC1 pathway and thus initiation of ribosomal translation and could, at least in part, help explain the reduced protein synthesis previously found with alcohol ingestion.

The present study presents, for the first time, data on the effect of alcohol on exercise-induced mTORC1 phosphorylation in women. In women, mTORC1 phosphorylation was increased at +3h, but in contrast to the findings for men, mTORC1

phosphorylation was not affected by alcohol ingestion. Although the effect of alcohol ingestion on mTORC1 does not appear to have been previously investigated in females, the lack of an effect of alcohol ingestion on RE-induced mTORC1 phosphorylation in the current study was a surprising finding. Acute alcohol ingestion has been found to reduce signaling downstream of mTORC1 (e.g., phosphorylation of ribosomal protein S6 and 4E-BP1) in female rats (Lang et al., 2007). Although only observed with chronic alcohol feeding, a sexual dimorphism in signaling downstream of mTORC1 appears to exist; female rats did not exhibit the decrease in signaling found in their male counterparts (Lang et al., 2007). Thus, alcohol ingestion immediately following heavy RE appears to have a greater influence on mTORC1 phosphorylation in men than in women.

S6K1, a downstream target of mTORC1, mediates protein synthesis as a regulator of the translation of mRNAs encoding ribosomal proteins (Avruch et al. 2001). The kinase activity of S6K1 is an important contributor to translation initiation, an essential rate-limiting step in protein synthesis (Baar and Esser, 1999). In the present study, alcohol prevented a RE-induced increase in S6K1 phosphorylation at +3h in men. Resistance exercise is a potent stimulus for S6K1 phosphorylation in men (Bodine et al., 2001; Dreyer et al., 2006, 2010) thus the increase found in the placebo condition is in agreement with previous findings. As hypothesized, alcohol ingestion impeded RE-induced S6K1 phosphorylation. Alcohol treatment has previously been found to reduce S6K1 phosphorylation in male rats in the absence of exercise (Lang et al., 2003, 2009). As in the present study, these previous findings of impaired S6K1 phosphorylation with alcohol ingestion were associated with reduced mTORC1 phosphorylation. Since mTORC1 an upstream regulator of S6K1 phosphorylation the observed effect of alcohol

on S6K1 phosphorylation in men might simply be due to reduced activation by mTORC1. As with mTORC1, it is important to note, that while alcohol ingestion in the present study prevented the RE-induced increase of S6K1 phosphorylation, the RE bout might have prevented an alcohol-induced decrease in S6K1 phosphorylation.

The present findings for S6K1 are in contrast to those of Parr et al. (2014) who reported no inhibitory effect of alcohol on S6K1 phosphorylation from combined endurance and resistance exercise and protein ingestion in men. The amino acid leucine is a potent stimulator of mTORC1. During recovery from exercise, Parr et al. (2014) feed participants two large boluses of whey protein which is rich in leucine, thus the stimulatory effect of leucine on S6K1 phosphorylation might have overwhelmed any negative effects of alcohol. This notion is supported by the lack of an increase in S6K1 phosphorylation from combined endurance and resistance exercise when carbohydrate and alcohol was ingested instead of protein and alcohol (Parr et al., 2014). In the present study, a trend (p = .052) for a main effect for time was found for increased S6K1 phosphorylation at +3h in women, but no effect of alcohol was evident. Previously, Dreyer et al. (2006, 2010) have found RE induced an increase in S6K1 phosphorylation in women at 2 hours post exercise. This increase was accompanied by an increase in mTORC1 phosphorylation at 1 and 2 hours post exercise. Thus, similar as to for men, changes in S6K1 phosphorylation in women could simply due to an increase in phosphorylation of its upstream of regulator mTORC1. Regardless, alcohol ingestion in women did not appear to affect S6K1 phosphorylation.

When phosphorylated by mTORC1, 4E-BP1 releases from eIF4E allowing for greater translation initiation. Despite the increases observed in 4E-BP1's upstream

activator mTORC1, there were no change from baseline found for 4E-BP1 phosphorylation at +3h or +5h for men or women regardless of drink condition. Consistent with these findings, prior studies have observed no RE-induced changes in 4E-BP1 phosphorylation at 1 and 2 hours after exercise in men and women (Dreyer et al., 2006, 2010). In contrast to the lack of an effect observed with RE, acute alcohol ingestion independently reduces 4E-BP1 phosphorylation (Lang et al., 2003, 2007) in the hours after ingestion in males and females, an effect that could be attributed to the reduced mTORC1 activation caused by alcohol. Similar to the present study, Parr et al. (2014) found no change in 4E-BP1 phosphorylation 2h after combined endurance and resistance exercise with and without alcohol ingestion in men. Although RE does not have a stimulatory effect on 4E-BP1 activation during the 1-5 hours after exercise, RE might be able to prevent an alcohol-induced reduction in 4E-BP1 activation.

This study appears to be the first to investigate mTORC1 signaling at both +3h and +5h after resistance exercise. In absence of alcohol ingestion (i.e., placebo) the present study found that in men, resistance exercise increased mTORC1 and S6K1 phosphorylation at +3h post exercise compared to at PRE and that by +5h phosphorylation had returned to baseline levels; there was no change in 4E-BP1 phosphorylation across time points. In women, the same pattern was found for mTORC1 and 4E-BP1 but not S6K1 phosphorylation; as only a statistical trend (main effect of time: p=0.052) for an increase in S6K1 phosphorylation was observed. These findings follow that of previous studies showing that mTORC1 signaling at 1 and 2 hours after exercise (in the absence of alcohol) is independent of sex (Dreyer et al., 2006,

2010). Combined these findings provides important and novel information on the time-dependent changes in RE-induced mTORC1 signaling at these time points.

In conclusion, the major findings of this study was that although RE elicited similar mTORC1 signaling both in men and in women, alcohol ingestion appeared to only attenuate RE-induced phosphorylation of the mTORC1 signaling pathway in men. These findings in men are important because they suggest a potential mechanism underlying the attenuated RE-induced protein synthesis from alcohol ingestion previously observed for men. Furthermore, the findings suggest that RE could be used as a countermeasure for alcohol induced myopathy or to counter act the negative effects of alcohol ingestion on mTORC1 signaling. Future studies should further investigate the effect of alcohol ingestion on RE-induced signaling involved in regulating protein synthesis and exercise training adaptations, especially in women, since prior research has found that alcohol reduces protein synthesis in women despite the lack of changes in mTORC1 signaling found in the current study.

CHAPTER 3

POST-RESISTANCE EXERCISE ETHANOL INGESTION AND ACUTE TESTOSTERONE BIOAVAILABILITY*

3.1 Introduction

Ethanol use and abuse is at least as prevalent among athletes (especially men) as it is among non-athletes (Bodine et al., 2001). Furthermore, the vast majority of athletes in the U. S. (~80%) have begun drinking ethanol by the time they reach college/university (Izquierdo et al., 2001). Although the number of National Collegiate Athletic Association athletes who use ethanol declined from 1997 to 2005, the incidence of binge drinking (more than 5 drinks in a sitting) increased significantly during the same time period (Izquierdo et al., 2001). In certain recreational sports settings, it is common for athletes to consume ethanol directly after or in some cases even during these events. Similarly, for the collegiate athletes who consume ethanol, ~50% reported doing so after competitions (Jensen et al., 1991), and almost 60% believe that their use of ethanol does not affect their athletic performance or overall health (Izquierdo et al., 2001).

Testosterone stimulates intramuscular uptake of amino acids and synthesis of muscle protein (Mendelson, Mello, & Ellingboe, 1977) and serves a vital role for the adaptations to resistance training (Noble, Borg, Jacobs, Ceci, & Kaiser, 1983; Overman & Terry, 1991). Acutely, heavy resistance exercise causes an elevation in serum total testosterone (TT) (Mahoney, Dempsey, & Blenis, 2009; Nakano et al., 1994) and free

* This chapter is reproduced from Vingren, J. L., Hill, D. W., Buddhadev, H., & Duplanty, A. A. (2013). Post-resistance exercise ethanol ingestion and acute tetosterone bioavailability. Med Sci Sports Exerc., Mar 6. [Epub ahead of print].

testosterone (FT) (Mahoney et al., 2009; Nakano et al., 1994) concentrations. Resistance exercise does not appear to change serum sex hormone binding globulin (SHBG) concentration acutely unless combined with protein-carbohydrate supplementation (Lang, Kimball, Frost, & Vary, 2001). The testosterone binding proteins, especially SHBG, regulate the biological activity of circulating testosterone. Approximately 44-60% of TT is normally bound to SHBG (Spiering et al., 2008), while the remainder is either bound loosely to other binding proteins or unbound (free); less than 2% of TT is free (Mahoney et al., 2009; Spiering et al., 2008). SHBG effectively inhibits testosterone action, as only the testosterone not bound to SHBG is biologically active (Barnes, Mündel, & Stannard, 2010) with FT being the most active; thus concentrations of SHBG and biologically active testosterone are inversely related.

At rest, ethanol intoxication in men acutely can suppress serum TT concentration for up to several hours following the ethanol ingestion (Kapoor, Luttrell, & Williams, 1993b; Koziris, Kraemer, Gordon, Incledon, & Knuttgen, 2000; Kraemer et al., 1998; Kraemer et al., 1999). The effect of acute ethanol intoxication on SHBG is unclear; however, serum SHBG concentration is elevated in men who are alcoholics (Ford, 2007). Two different physiological effects of acute ethanol intoxication may be responsible for the reduction in TT. 1) Ethanol has an adverse acute effect on Leydig cell function (Kraemer et al., 1990), thus reducing testosterone production in the testes (Kraemer, Volek, Bush, Putukian, & Sebastianelli, 1998; Kvorning, Andersen, Brixen, & Madsen, 2006). 2) Ethanol increases the clearance of testosterone by conversion to dihydrotestosterone and estradiol by aromatase in the liver (Linnoila, Prinz, Wonsowicz, & Leppaluoto, 1980). The reduction in serum testosterone seen in men following

ethanol intoxication may, therefore, be due to the combined effect of a lower synthesis rate and a higher clearance rate. Recently, several studies have found that acute high-dose ethanol ingestion (1 g ethanol·kg^{-1} body mass) disrupts muscle recovery (i.e., force production capability) from a bout of strenuous eccentric resistance exercise (Baldari et al., 2009; Barnes, Mundel, & Stannard, 2010). Ethanol ingestion following a bout of resistance exercise could disrupt recovery by reducing the anabolic milieu (e.g., reduced testosterone bioavailability) which would lead to compromised adaptations from that exercise bout and if repeated over time to compromised training outcomes. Heikkonen and colleagues (Kim et al., 2002) found that ethanol ingestion (1.5 g ethanol·kg^{-1} body mass) after exhaustive cycle ergometer exercise had no apparent effect on the testosterone or cortisol response to exercise during the first 10.5 h post exercise, but prolonged the depressant effect of alcohol on testosterone secretion (at ~21.5 hours post exercise). In contrast to these findings, Koziris et al. (Lang, Pruznak, Nystrom, & Vary, 2009) found that post-exercise ethanol consumption (0.83 g ethanol·kg^{-1} body mass) resulted in higher total testosterone and cortisol concentrations at 60-120 minutes into recovery from a bout of heavy circuit resistance exercise compared to when no ethanol was ingested.

Independently, resistance exercise and ethanol have opposite effects on circulating testosterone concentrations and its bioavailability. Despite the potentially important implications of acute alcohol ingestion for individuals involved in a physical conditioning program, only a single study (Lang et al., 2009) has examined the anabolic endocrine response to ethanol ingestion during the recovery from resistance exercise, and no investigations have examined the response on the bioavailable fraction of

testosterone in this context. Thus, the purpose of this study was to examine the testosterone bioavailability and the anabolic endocrine milieu in response to acute ethanol ingestion following a bout of heavy resistance exercise.

3.2 Materials and Methods

Overview

To examine the effect of ethanol ingestion following resistance exercise on testosterone bioavailability, a within subjects repeated measures design was used. Eight resistance trained men completed two identical acute heavy resistance exercise tests (AHRET) separated by one week. From 10-20 minutes post-AHRET participants consumed either grain ethanol (EtOH condition) or no ethanol (Placebo condition) diluted in an artificially sweetened and calorie free beverage. Blood was collected before (PRE) and immediately after the AHRET (IP; before ethanol/placebo ingestion); and then every 20 min for five hours. Blood collected after ethanol ingestion was pooled into 3 batches (phases: 20-40 min, 60-120 min, and 140-300 min post-exercise) for biochemical analysis. PRE, IP, and pooled phases samples were analyzed for serum TT, FT, SHBG, cortisol and estradiol concentrations; in addition, percent of TT which was free (%FTT), free androgen index (FAI), and testosterone to cortisol ratio (T:C) were calculated.

Participants

Eight men (21-34 years; mean ± SD: 25.3 ± 3.2 years, 87.7 ± 15.1 kg, 177 ± 7 cm, 15.1 ± 4.1 %fat) who were recreationally resistance trained (including the back squat) participated in this study. Participants were screened for any medical concerns

that could confound the results of the study or place the participants at an elevated risk during the study. The participants were found to be free of relevant orthopedic and pathological conditions. To be included in the study participants could not have used drugs such as glucocorticoids or anabolic-androgenic steroids within one year prior to the start of the study.

Participants were screened for clinical signs of ethanol abuse using several questionnaires concerning their current and historic use of ethanol. To be considered for the study the participants had to be low-to-moderate ethanol consumers (NCAA Research Staff, 2012). Based on the screening, participants were not ethanol dependent, did not have consumption-induced metabolic tolerance to ethanol, and were capable of tolerating the amount of ethanol ingestion required in this study without being affected in any extreme manner such as by nausea or flushing. After screening fat free body mass was measured using skin folds from seven sites (Parkington, Siebert, LeBrasseur, & Fielding, 2003). The study was approved by the University Institutional Review Board and the volunteers provided written informed consent to participate.

Experimental treatments

Each participant completed both experimental treatments (EtOH and Placebo) and thereby served as his own control. The treatments were performed one week apart and administered in a balanced, randomized, crossover design. The participants were kept blind to the experimental conditions selected for that treatment day. In order to prevent participants from anticipating a particular treatment, they were informed that they could potentially receive the same treatment on both experimental treatments visits. Participants were required to consume the same diet for the two days before and

the morning of each treatment visit (EtOH or Placebo visit). Participants completed a diet record prior to the first treatment visit and were instructed to follow the same diet prior to the second treatment visit. All participants attested that they had complied with the diet instructions for their 2nd visit.

Treatment days

Participants refrained from: 1) eating or drinking anything (except for water and non-caffeinated diet drinks) during the 2 hours leading up to each treatment visit, 2) any ingestion ethanol for 84 hours prior to each treatment visit, 3) consuming large amounts of caffeine on test day (no more than 1 cup of coffee allowed), 4) engaging in sexual activity for 24 hours prior to each treatment period, and 5) performing any resistance exercise or intense aerobic exercise for 96 hours prior to each visit. It was also required of the participants that they had not donated blood within 8 weeks or plasma within 96 hours of the laboratory visits. The participants attested that they had adhered to all instructions and that their records were accurate. All treatment visits for a participant began at the same time of day for each participant (1100h arrival time for all participants) to avoid circadian influences.

An overview of the timeline for a treatment day is provided in Figure 3.1. Upon arrival at the laboratory on each treatment day participants were screened for the presence of blood ethanol with a breathalyzer and were queried regarding compliance with study guidelines regarding diet, caffeine, sexual activity, ethanol, drugs and exercise. Participants were asked to report to the laboratory in a euhydrated state. Upon arrival at the laboratory, hydration status was measured using urine refractometry; if participants presented with urine specific gravity ≥1.020 they were provided with cold

water to drink. After the hydration status had been addressed, body mass was measured. Sixty five minutes prior to the AHRET participants consumed a standardized meal replacement drink (Ensure Plus®) containing 8 kcal per kg body mass. After the meal replacement drink was ingested a cannula was inserted in a superficial vein of the forearm.

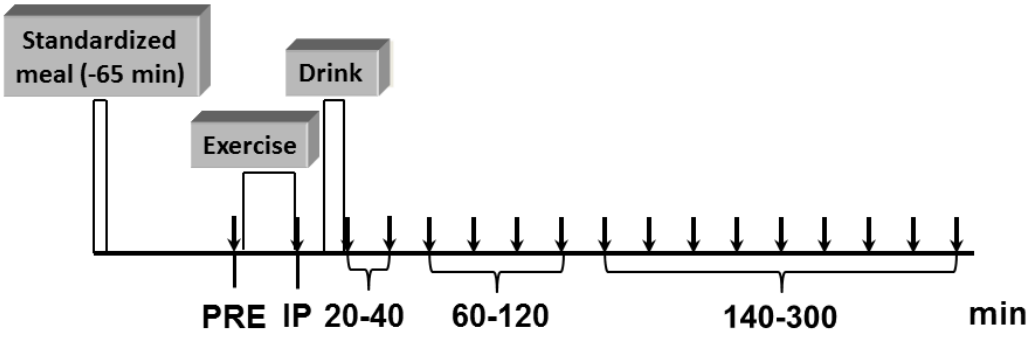


Figure 3.1. Timeline for a Treatment Day. ↓ Represents individual blood draws. ↔ Represents time phases for individual blood draws pooled for analysis.

Twenty five minutes before the AHRET (40 min after the meal) participants completed a standardized warm-up which consisted of 5 minutes of ergometer cycling at a low intensity followed by dynamic stretches (10 lunges, 20 heel kicks, 20 high knees, 10 body weight moving squats). 15 minutes before the AHRET participants completed balance test and power tests (bench press throw and high pull at 30% of 1RM, maximal vertical jump) which were part of a different aspect of this project. Participants then completed 10 warm-up squats at 50% of 1RM followed by the AHRET (Vingren et al., 2008, 2009). The AHRET consisted of 6 sets of 10 repetitions of Smith machine squats starting at 80% of 1RM and 2 min of rest between sets. If participants were not able to complete 10 repetitions on their own, they were assisted by the

researchers to do so and the load was reduced for subsequent sets. The same load for each set was used for both AHRETs. After the completion of the AHRET, the participants sat quietly in a chair for the remainder of the test period and were not allowed to sleep. Blood ethanol concentration (BEC) was determined and blood samples drawn according to the timeline presented in Figure 3.1. To prevent anxiety and boredom the participants were permitted to read, write, or watch TV/movies during periods in which they sat quietly. They were asked not to become involved in any reading, writing, or TV/movie watching that would cause them to experience any form of stress, anxiety, or other strong emotions, either positive or negative.

Blood ethanol concentration

The BEC was estimated from breath ethanol with an Alco-sensor IV (Inoximeters, St. Louis, MO). Standard gas was used to calibrate the instrument before and at regular intervals during each visit following manufacture instructions. BEC was measured upon arrival at the laboratory, every 10 minutes from 40 to 100 min post-AHRET (measurement of BEC from breath immediately after ethanol consumption is not meaningful), and every 20 minutes from 100 to 300 min post AHRET for both the EtOH and Placebo condition. At no time were the participants allowed to know their BEC or whether they had received any ethanol during their visit. However, due to the obvious physiological and psychological effects of ethanol ingestion, participants were generally aware of when they had received ethanol, but could not easily discern when they had not received ethanol.

Ethanol ingestion

From 10-20 minutes post-AHRET, participants consumed either grain ethanol (EtOH condition) or no ethanol (Placebo condition) in an artificially sweetened and calorie free beverage. For the EtOH condition, a dose of 1.09 g of ethanol (Everclear grain alcohol, 95% v/v ethanol, Luxco, St. Louis, MO) per kg fat free body mass (mean: 0.94 ± 0.05 $g \cdot kg^{-1}$ body mass; range: 0.84-1.01g $g \cdot kg^{-1}$ body mass, 82-122 ml of 95% v/v ethanol) was diluted to a concentration of 19 % v/v absolute ethanol; for the Placebo condition the ethanol was substituted with an equal volume of water. The participants drank 1/10 of the drink each minute during the 10-minute ingestion period. To reduce the ability of the participants to differentiate between the drinks (i.e., taste of EtOH) participants wore a nose-clip during each drink ingestion..

Heart rate and rating of perceived exertion

Upon arrival at the laboratory, immediately after each set of squats during the AHRET, and at each blood draw during recovery from exercise, heart rate was measured using a standard heart rate monitor with telemetry (Polar, Lake Success, NY). Rating of perceived exertion (RPE) was assessed at PRE and after each set of squats during the AHRET using the category-ratio (CR-10) scale of perceived exertion (Thomson, Fick, & Gordon, 2008).

Blood collection

On treatment days, a Teflon coated cannula (Vascular Access, Becton-Dickerson, Sandy, UT) was inserted in a superficial vein of the forearm while the participants were seated. Cannula patency was maintained with sterile saline (0.9% sodium chloride inj., USP, Hospira Inc. Lake Forest, IL), and blood was drawn while participants were seated. Blood was collected 5 minutes before the AHRET (PRE),

immediately after the AHRET (IP), and every 20 minutes for the 300 minutes following the AHRET (Figure 3.1).

Blood processing

Blood was allowed to clot at room temperature (~21 °C) and subsequently was centrifuged at 1,500 *g* at 4 °C for 15 minutes. The resultant serum was stored in several aliquots at -80°C until analysis. Samples for PRE and IP were analyzed individually. The remaining samples were pooled into 3 batches (phases: 20-40 min, 60-120 min, and 140-300 min post-exercise) following the methods of Koziris and colleagues (Lang et al., 2009) for later hormone and binding protein analysis.

Biochemical analysis

A small portion of whole blood obtained at PRE, IP, and 20, 40, 60, 120, 180, 240, and 300 min post exercise was analyzed immediately for lactate, hematocrit and hemoglobin. Blood lactate was determined using an automated Lactate Plus analyzer (Sports Resource Group Inc., Hawthorne, NY) in order to characterize the metabolic demands of the exercise; the Lactate Plus analyzer has been validated against a standard bench top method (Hong-Brown, Brown, Huber, & Lang, 2006). Hematocrit was measured by standard microcapillary technique and hemoglobin concentration was determined using an automated analyzer (Hb201, HemoCue AB, Angelholm, Sweden). Plasma volume change (percent) was calculated using the hematocrit and hemoglobin values (Vingren et al., 2008). Circulating hormone and binding protein concentrations were not corrected for plasma volume changes due to the molar exposure at the tissue level. The TT, FT, SHBG, cortisol, and estradiol concentrations were analyzed using commercially available enzyme-linked immunosorbent assays (Alpco, Salem, NH). The

intra-assay variances (CV) were as follows: TT: 8.8%; FT: 8.5%; SHBG: 4.8%; cortisol: 5.7%; estradiol: 11.3%. The samples were not decoded until after the analysis was completed (blinded analysis). The free androgen index (FAI) (ratio of TT to SHBG: 100 × [TT] ÷ [SHBG]) and the TT to cortisol ratio (T:C) ([TT] ÷ [cortisol]) were calculated for all time point/phases examined. All samples for a particular time point/phase were analyzed in duplicate within the same assay batch to eliminate potential inter-assay variance for a particular variable.

Statistical analysis

Data for HR and RPE were each averaged across the six sets and the average value used for further analysis. Data for FAI and estradiol were log10 transformed prior to analysis. For each variable data were analyzed using a two-way ANOVA (treatment x time phase/point) with repeated measures on both factors. Where appropriate, Fisher's least Significant Difference *post hoc* test was used for pairwise comparisons. The α-level of significance was set at $p \leq 0.05$. The Statistica software package (StatSoft, Inc., Tulsa, OK) was used for all statistical analysis. Data are presented as mean ± standard deviation (SD).

3.3 Results

For all participants, BEC peaked (0.088 ± 0.015 $g \cdot dl^{-1}$) 60-90 min post-exercise on ethanol ingestion days and gradually dropped to 0.033 ± 0.009 $g \cdot dl^{-1}$ at 300 min post exercise. BEC was 0.000 $g \cdot dl^{-1}$ at PRE for EtOH and at all time points for Placebo. Significant main effects of time ($p \leq 0.05$) were found for markers of metabolic demand (blood lactate, HR, and RPE). Blood lactate concentration, HR, and RPE increased significantly during the AHRET, peaking at IP (Lactate: EtOH 12.7 ± 1.6 $mmol \cdot l^{-1}$,

Placebo 12.9 ± 1.5 mmol·l^{-1}; HR: EtOH 174 ± 13 bpm, Placebo 179 ± 8 bpm; RPE: EtOH 7.8 ± 2.3, Placebo 7.4 ± 2.0) and then gradually decreased during recovery until reaching a plateau (at or below PRE values) 120 min post-AHRET (RPE) and 180 post-AHRET (HR and lactate). There was no difference for the lactate, HR, or RPE response between conditions. Plasma volume was significantly reduced at IP (EtOH -11.5 ± 4.9 %, Placebo -12.6 ± 7.1 %) and returned to baseline at 20 min post-AHRET; there were no differences between conditions for plasma volume change throughout recovery.

A significant interaction effect (treatment x time phase/point) was found for TT (Figure 3.2). Compared to PRE, serum TT was significantly elevated at IP for both conditions and at 140-300 min for EtOH; furthermore, compared to PRE TT was significantly decreased at 60-120 min and 140-300 min for Placebo. Serum TT was significantly higher for EtOH than for Placebo at 60-120 min and 140-300 min. A significant interaction effect was also found for FT (Figure 3.2). Compared to PRE, serum FT was significantly elevated at IP and 20-40 min for both conditions and at 60-120 min and 140-300 min for EtOH. Serum FT was significantly higher for EtOH than for Placebo at 60-120 min and 140-300 min. A significant main effect of time was found for %FTT (Table 3.1). Compared to PRE, %FTT was significantly higher at 140-300 min. No differences between conditions were noted for %FTT.

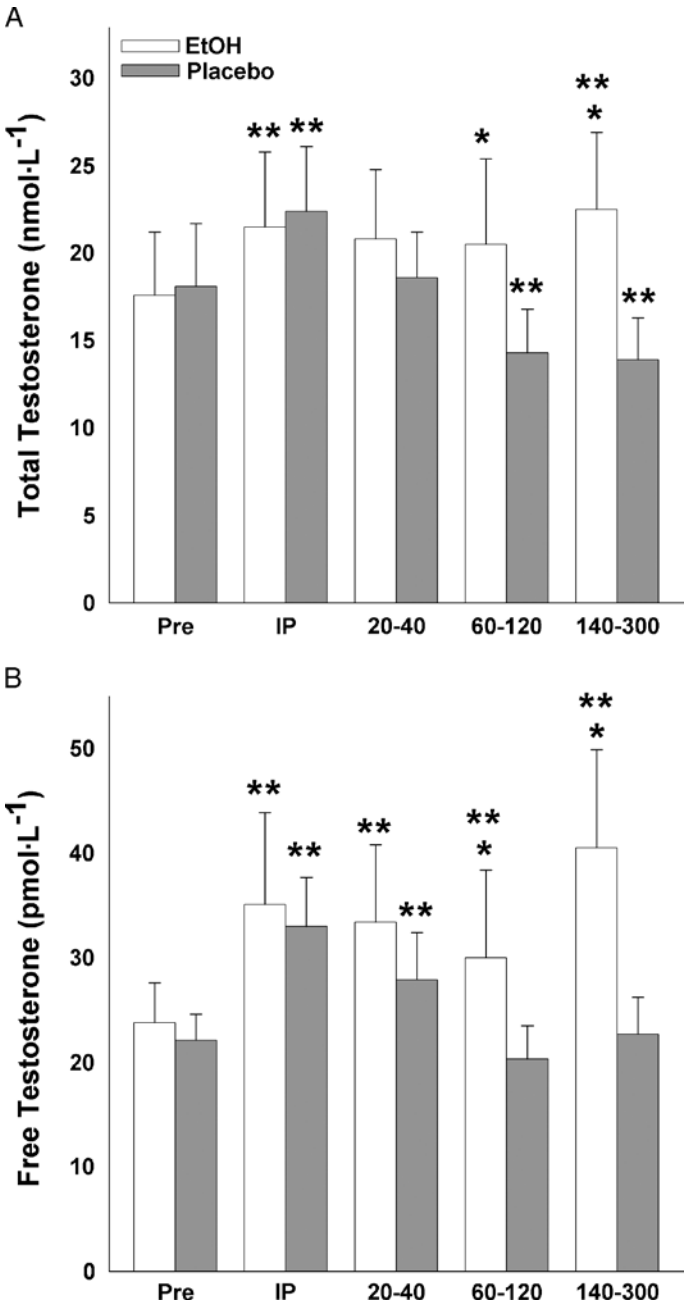


Figure 3.2. Serum A) Total Testosterone Concentrations and B) Free Testosterone Before (PRE) and Immediately (IP), 20-40, 60-120 and 140-300 Min Post-exercise. *Significantly different ($p \leq 0.05$) from Placebo for corresponding time phase. #Significantly different from corresponding PRE. Mean ± SE.

A significant main effect of time was found for SHBG (Table 3.1). Compared to PRE, SHBG was significantly elevated at IP and significantly decreased at 60-120 min.

No differences between conditions were noted for SHBG. A significant interaction effect was observed for FAI (Table 3.1). Compared to PRE, the FAI was significantly higher at 20-40 min, 60-120 min, and 140-300 min for EtOH and there was a trend (p=0.06) for a higher FAI at IP compared to PRE for Placebo. The FAI was also significantly higher for EtOH than for Placebo at 140-300 min.

A significant main effect for time was found for cortisol (Table 3.1). Cortisol was significantly elevated at IP, 20-40 min, and 60-120 min and significantly decreased at 140-300 min compared to PRE. Cortisol concentrations were not affected by ethanol ingestion during recovery. A significant interaction effect was found for the T:C ratio (Table 3.1). Compared to PRE, the T:C ratio was significantly lower at 20-40 min for both conditions and at 60-120 min for Placebo; furthermore, compared to PRE the T:C ratio was significantly higher at 140-300 min for EtOH. In addition, the T:C ratio was significantly greater for EtOH compared to Placebo at 140-300 min and there was a trend (p=0.08) for a greater T:C ratio for EtOH compared to Placebo at 60-120 min. A significant main effect of time was found for estradiol (Table 3.1). Estradiol was significantly elevated at IP and 20-40 min compared to PRE. Estradiol concentrations were not affected by ethanol ingestion during recovery.

	Pre	IP	20-40	60-120	140-300
%FTT (%)					
EtOH	0.14 ± 0.05	0.16 ± 0.04	0.15 ± 0.05	0.15 ± 0.05	0.17 ± 0.05$^{\#}$
Placebo	0.15 ± 0.05	0.16 ± 0.04	0.16 ± 0.05	0.15 ± 0.04	0.18 ± 0.04$^{\#}$
FAI					
EtOH	0.53 ± 0.44	0.57 ± 0.43	0.66 ± 0.61*	0.62 ± 0.50*	0.70 ± 0.54*^
Placebo	0.48 ± 0.28	0.59 ± 0.44	0.52 ± 0.22	0.42 ± 0.14	0.40 ± 0.13
T:C ratio					
EtOH	0.03 ± 0.02	0.03 ± 0.01	0.02 ± 0.01*	0.03 ± 0.02	0.06 ± 0.02*^
Placebo	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01*	0.02 ± 0.01*	0.03 ± 0.01
Estradiol (pmol·l^{-1})					
EtOH	564 ± 414	687 ± 549$^{\$}$	709 ± 539$^{\$}$	668 ± 516	665 ± 570
Placebo	669 ± 586	719 ± 575$^{\$}$	652 ± 562$^{\$}$	605 ± 469	520 ± 295

Table 3.1. Percent free to total testosterone (%FTT), free androgen index (FAI), testosterone to cortisol ratio (T:C ratio), and estradiol concentrations before exercise (PRE), immediately post-exercise (IP) and for the time phases 20-40, 60-120, and 140-300 min post exercise. Values with different superscript letters are significantly (p<0.05) different. *Significantly different (interaction effect) from corresponding PRE. ^Significantly different (interaction effect) from corresponding Placebo. $^{\#}$Significantly different (main effect of time) from all other time points/phases. $^{\$}$Significantly different (main effect of time) from PRE and 140-300 min. Data are presented as Mean ± SD.

3.4 Discussion

This is the first study to examine the effect of post-resistance exercise ethanol ingestion on testosterone bioavailability in men. The findings from this investigation provide unique physiological insight regarding ethanol's effect on the anabolic endocrine milieu during recovery from resistance exercise. The major finding of this study was that

ethanol substantially elevated serum TT or FT concentrations during recovery from a bout of resistance exercise. Contrary to what was expected based on findings for ethanol ingestion in the absence of exercise, TT and FT concentrations were significantly higher for EtOH compared to Placebo from 60 to 300 min post AHRET (40 to 280 minutes following ethanol ingestion). Several confounding factors such as hydration state were controlled in this investigation to allow for the findings to be attributed to the ethanol ingested. It had been hypothesized that post-exercise ethanol ingestion would significantly decrease TT and FT below the values observed for Placebo at corresponding time points/phases due to the suppressive effect of ethanol on testosterone production in the Leydig cells of the testis (Kraemer et al., 1998; Kraemer et al., 2006; Kvorning et al., 2006) and the increased clearance of testosterone by aromatase in the liver (Linnoila et al., 1980). It appears that ethanol ingestion following heavy resistance exercise results in a testosterone response pattern that differs markedly from the pattern found when ethanol is ingested in the absence of prior resistance exercise.

Consistent with previously findings for TT and FT responses to an acute bout of heavy resistance exercise involving the lower body (Mahoney et al., 2009; Vingren et al., 2009), serum TT and FT were elevated immediately following the AHRET (before drink ingestion). The elevation in TT and FT at IP was not accompanied by an elevation in the %FTT. This suggests that the acute bout of resistance exercise caused a similar relative elevation in the concentration of total (i.e., TT) and the most bioactive portion of testosterone (i.e., FT) at IP. Following ingestion of the placebo drink, TT and FT returned to or below PRE by 60-120 min and remained as such at 140-300 min. The

return to or below baseline concentrations within 1 hour post-exercise is consistent with the TT and FT response observed previously following the AHRET and similar heavy resistance exercise protocols (Mahoney et al., 2009; Vingren et al., 2009). For the EtOH condition, TT and FT were elevated at 60-120 min and 140-300 min compared to corresponding PRE and time phase for Placebo. Our findings are in contrast to those of Heikkonen et al. (1996) who found that consuming a large dose of ethanol (1.5 g ethanol·kg^{-1} body mass, ingested over three hours) after exhaustive cycle ergometer exercise did not affect TT during the first 10.5 hours post exercise (measured every 2 hours starting 30 min post-exercise). In what appears to be the only previous study on the neuroendocrine response to post resistance exercise alcohol consumption Koziris and colleagues (Lang et al., 2009) found that ethanol ingestion (0.83 g·kg^{-1} body mass, a dose slightly less than that used in the present study) prevented a drop below baseline (rebound low) in TT at 60-120 min following exercise compared to a no-exercise and placebo drink condition; FT was not investigated. Since a similar ethanol dose was used, the difference between the current findings for TT and those of Koziris et al. (Lang et al., 2009) are potentially due to the difference in the exercise protocols used by the two studies. Koziris et al. (Lang et al., 2009) used a circuit protocol that did not produce the large acute elevation in TT which is usually found following heavy resistance exercise in men (as it was in the current study).

The mechanism for this prolonged elevated testosterone concentration following post-exercise ethanol ingestion is not clear and thus remain speculative. The elevation in TT and FT found for EtOH could involve 1) increased testosterone production and release, 2) decreased testosterone clearance by liver and/or 3) decreased uptake by

muscle tissues. At rest ethanol ingestion or injection of ≤1 g·kg^{-1} body mass can result in either no change or an elevation in TT for men, whereas doses >1 g·kg^{-1} body mass result in a reduction in TT (DeHass, ; Kapoor, Luttrell, & Williams, 1993a; National Center for Health Statistics, ; Spiering et al., 2009). It is not known how lower doses of ethanol (≤1 g·kg^{-1} body mass) might cause an elevation in circulating testosterone but large doses are known to suppress testosterone production in the Leydig cells of the testis (Kraemer et al., 1998; Kvorning et al., 2006). The current investigation involved ethanol ingestion of 0.84-1.01 g·kg^{-1} body mass over a short time period (10 min). It is possible that the dose of ethanol used in this study was too low for an ethanol-induced decline in bioavailable testosterone to occur. Although the dose of ethanol ingested in the present study (~5.3 drinks in 10 minutes for a 70 kg man with 15% body fat) was an appreciable amount, it was less than that often experienced in real life settings (Vingren, Hill, Buddhadev, & Duplanty, 2013). Future investigations should examine testosterone bioavailability after post-exercise ingestion of larger doses (>1 g·kg^{-1} body mass) of ethanol.

Ethanol is known to increase the conversion of testosterone to estradiol by aromatase in the liver (Linnoila et al., 1980) and thus to increase testosterone clearance from circulation; a suppressive effect of ethanol on aromatase has not been demonstrated. In the current study there were no differences in estradiol concentrations between conditions, although it could appear visually that the estradiol concentration was higher for EtOH in the latter phases of recovery. Thus, the elevation in testosterone likely is not due to a reduction in hepatic clearance. In the absence of ethanol ingestion, muscle androgen receptor content is elevated during later parts of recovery (60 minutes

post-exercise) from resistance exercise compared to immediately post-exercise in men (Hurlbut & Sher, 1992; Mahoney et al., 2009). Consistent with these findings (Hurlbut & Sher, 1992; Mahoney et al., 2009), the reduction in TT for the Placebo condition at 60-120 and 140-300 min post-exercise could be caused by an increased testosterone uptake due to an increase in muscle androgen receptor content and potentially receptor affinity for testosterone. It has previously been found that chronic ethanol ingestion (~6 weeks) in male rats reduce the androgen receptor content of the predominantly type II muscle fiber rectus femoris, and prevents a training induced increase in the androgen receptor content of the predominantly type I muscle fiber soleus (Laplante & Sabatini, 2013). It seems that ethanol interferes with the androgen receptor expression in muscle fibers. Therefore, the prolonged elevated concentrations of TT and FT following post-exercise ethanol ingestion might be due to an acute ethanol-induced reduction in the muscle androgen receptor content compared to that following resistance exercise alone (i.e., Placebo). Regardless of the mechanism involved, it appears that post-exercise ethanol ingestion can cause an elevation in TT and FT during the later parts of recovery (60-300 min) from resistance exercise. Future research should examine potential mechanisms for this augmenting effect of ethanol ingestion on the testosterone response during recovery from heavy resistance exercise.

Ethanol ingestion had an effect on only one (i.e., FAI) of the two indirect measures of testosterone bioavailability (%FFT and FAI). Since SHBG binds to and prevents bioactivity of testosterone, the FAI has been used in lieu of FT as a measure of bioavailable testosterone, although the validity of FAI has long been questioned (Frost, Nystrom, Burrows, & Lang, 2005). Compared to PRE, FAI was higher at all time

phases following alcohol ingestion; no changes over time were found for Placebo. Despite this divergent response pattern, FAI was higher for EtOH compared to Placebo only at 140-300 min. Since SHBG concentration and %FTT did not differ between conditions, the elevation found for measures of bioavailable testosterone (FT and FAI) with EtOH appears to be largely due an elevation in TT.

Cortisol was elevated during the first 2 hours following the AHRET (i.e., at IP, 20-40 min, and 60-120 min) but no difference was noted between conditions. This cortisol response follows the pattern commonly found after acute heavy lower-body resistance exercise (Vingren et al., 2009). The lack of difference between conditions is in contrast to the findings by Koziris et al (Lang et al., 2009) who found that cortisol was elevated longer during recovery following post-exercise ethanol ingestion; however, in that study the cortisol concentration returned to or below baseline by 60-120 min for the placebo condition and by 140-300 min for the ethanol condition. In the current study, cortisol returned to (below) baseline by 140-300 min for both conditions and thus there is no difference between the two studies for the duration of the elevation in cortisol for the ethanol ingestion conditions. The T:C ratio is a crude measure of the anabolic:catabolic endocrine milieu. This ratio largely followed the response pattern of TT, with a greater T:C ratio for EtOH compared to Placebo at 140-300 min post-exercise. Although the T:C is a crude measure, it response shows that the elevated in testosterone in the latter stages of recovery following ethanol ingestion is not associated with a corresponding elevation in cortisol, a hormone with catabolic and anti-anabolic physiological effects. The results for the T:C ratio suggest that a more anabolic milieu exists during the latter stages of recovery from resistance exercise following ethanol ingestion. To date no

study has investigated the effect of post-exercise ethanol ingestion on protein accretion but negative effects of ethanol on muscle strength recovery have been found (Baldari et al., 2009; Barnes et al., 2010); unfortunately, in those studies the hormonal response was not investigated. It remains unclear how the elevated testosterone bioavailability and anabolic milieu found with ethanol ingestion in the current study affects muscle adaptations to resistance exercise.

In conclusion, post-exercise ethanol ingestion changed the total concentration and bioavailability of testosterone. The post-exercise ingestion of ethanol elevated TT and FT concentrations at 60-300 min post exercise compared to ingestion of the placebo drink. The increase in testosterone bioavailability did not appear to be caused by changes in %FTT, SHBG, or hepatic clearance of testosterone as there were no differences between conditions for these variables at any time phase following ethanol ingestion. It follows that either testosterone release was increased by post-exercise ethanol ingestion, or that muscle uptake was reduced by post-exercise ethanol ingestion (or both).

Thus, the primary finding of this study, that total and bioavailable testosterone concentrations were elevated acutely following post-resistance exercise ethanol ingestion, should be interpreted with care. If testosterone release is increased this could be beneficial; however, if muscle uptake is reduced this could be detrimental to the desired adaptations. Acute ethanol ingestion has adverse consequences on muscle strength recovery from exercise (Baldari et al., 2009; Barnes et al., 2010) and on other aspects of the endocrine response (e.g., elevated acute cortisol) (Lang et al., 2009). Long term ethanol use has negative effect on muscle tissue (e.g., preventing an

increase in muscle androgen receptor content from resistance training) (Laplante & Sabatini, 2013). Therefore, the findings of in the present study should not be seen by coaches and athletes as evidence that ethanol ingestion following resistance exercise is beneficial to their conditioning program.

CHAPTER 4

FACTORS THAT CONTRIBUTE TO BONE DENSITY IN MEN

4.1 Introduction

Increases in bone mineral density (BMD) are stimulated by mechanical loading, including weight bearing physical activities (Frost 1987). Running is a weight-bearing exercise and as such should provide potent stimulus for bone growth and maintenance. Surprisingly, numerous studies report low BMD in both male and female long distance runners (Bilanin et al., 1989; Burrows et al., 2003; Hetland et al., 1993; Hind et al., 2006). The underlying causes of low BMD in female runners are well established and associated with a syndrome termed the Female Athlete Triad (Otis et al., 1997). The Female Athlete Triad includes three factors, with the first two contributing to the third: low energy availability (sometimes due to eating disorders), amenorrhea or oligomenorrhea (caused by hormonal imbalance), and osteoporosis/osteopenia (Otis et al., 1997). The Female Athlete Triad is usually found in females participating in sports were athletes might have, or are often perceived to have, a competitive advantage by being very lean, such as gymnastics, ballet, swimming, and long distance running. However, the presence of the Female Athlete Triad can cause decreased physical performance and increased morbidity and mortality (Otis et al., 1997). It appears that male runners can encounter factors similar to those involved in the Female Athlete Triad: low energy availability (Zanker et al., 2000), low BMD, especially in the lumbar vertebrae (Bilanin et al., 1989; Hetland et al., 1993; Hind et al., 2006), and hypothalamic-pituitary-gonadal axis hormone deficiency (MaConnie et al., 1986). In

contrast to females, the mechanisms resulting in low BMD in male long distance runners are not well understood.

Several studies report low resting free and total testosterone concentrations in male distance runners (Hackney et al., 1988; MacKelvie et al., 2000; Wheeler et al., 1984, 1991). A comparison of the reproductive hormonal profiles of endurance trained and untrained men suggested that chronic endurance training suppresses testicular function, resulting in decreased resting testosterone concentrations (Hackney et al., 1988). Low concentrations of androgens (including testosterone) can lead to decreased bone mass by increasing the rate of bone reabsorption and by reducing the rate of bone formation (Vanderschueren et al., 2000). This negative bone remodeling balance due to low circulating testosterone can ultimately result in osteoporosis (Kelepouris et al., 1995). Therefore, the low resting testosterone concentrations, which are induced by endurance training, may contribute to the low BMD found in male long distance runners.

The nature of the impact of mechanical loading also appears to be an important factor for understanding the apparent BMD paradox in male long distance runners. Cyclists, who have only limited loading and impact of the spine during exercise, are up to seven times more likely than runners to have osteopenia of the lumbar spine (Rector et al., 2008). In contrast, power athletes (sprinters, jumpers, weight lifters, etc.) have greater BMD compared with long distance runners who do not participate in those types of high impact and/or high load exercises (Bennell et al., 1997; Heinrich et al., 1990; Hamdy et al., 1994). Furthermore, it has been reported that among masters runners (ages 40-64 years), those who participate in training regimens that elicit higher

magnitudes of impact (e.g. speed-power training) had higher BMD than those who only train in distance running (Nowak et al., 2010).

Resistance exercise is a high load activity and a potent stimulus for bone growth and maintenance and, therefore, is often prescribed to those with osteopenia/osteoporosis (Layne and Nelson, 1999; Braith et al., 1996). The magnitude of the mechanical loading is important factor for bone formation and resistance exercise elicits a magnitude of strain that exceeds the threshold required for increased bone modelling (Frost 1987). Considering the positive effects of resistance exercise on bone growth and the potential negative effects of long distance running on testosterone and BMD in men, an investigation on the relationship between long distance running and participation in regular resistance exercise is warranted.

Currently, there is a paucity of data on the effects of resistance exercise training on BMD in male long distance runners. Identifying the factors that lead to low BMD in male runners is important in the prevention of developing osteopenia/osteoporosis. While amenorrhea in females is an apparent sign of a hormonal imbalance; men experience no such warning sign. Unless injured, osteopenia/osteoporosis is generally painless and without obvious symptoms. It is possible that the inclusion of resistance training can attenuate the detrimental effects on BMD in endurance runners. Therefore, the purpose of this study is to investigate the relation between testosterone concentrations and BMD in young adult male distance runners and how resistance training is associated with these factors.

4.2 Methods

Experimental Approach to the Problem

To investigate the relation between hormonal status and BMD in young adult male distance runners and how resistance training is associated with these factors, a between subjects design was used. Apparently healthy Caucasian men who reported belonging to one of the following three groups based on exercise engagement over the previous three years were enrolled in the study: non-exercise trained controls (CON), non-resistance trained distance runners (NRT), and resistance trained distance runners (RT). Participants completed medical and exercise training history questionnaires. Body composition including BMD was assessed using dual energy X-ray absorptiometry (DXA). Fasted blood samples were collected and analyzed for serum total testosterone (TT) and free (FT) testosterone concentrations.

Participants

Twenty five men (23-32 y; mean ± SD: 25.9 ± 2.9 y; 1.77 ± 0.04 m, 75.4 ± 8.5 kg) participated in this study. Participants were assigned to one of the following three groups based on exercise engagement over the previous three years: Non-exercise trained control (CON; n=8) participated in less than 1 hour of exercise per week; non-resistance trained runners (NRT; n=8) ran at least 32 km per week and did not engage in resistance training; and resistance trained runners (RT; n=9) ran at least 32 km per week and engaged in at least one resistance training session per week. NRT and RT did not participate in any regular aerobic cross training (biking, swimming, etc.) over the previous three years.

To be considered for the study, the participants had to be free of diagnosed medical conditions (e.g., hyperthyroidism, hypogonadism, hypergonadism) or long-term use of substances (e.g., corticosteroids, anabolic steroids, human growth hormone, tobacco, or excessive alcohol) that might influenced bone formation or testosterone concentrations. Only participants of Caucasian descent were recruited because bone structure, size, and density are known to vary by ethnicity and because of insufficient reference data (Gilsanz et al., 1998). The study was approved by the University Institutional Review Board, and the volunteers provided written informed consent to participate.

Dual Energy X-ray Absorptiometry

Total body BMD, regional BMD, and soft tissue composition (fat and lean mass), were measured using DXA (Lunar Prodigy, GE Healthcare, Fairfield, CT) following manufacturer instructions. Regional BMD measurements were obtained for total proximal femur, femoral neck, trochanteric region, and lumbar spine (L1-L4).

Blood Collection and Analysis

Resting and fasting venous blood samples (~20 ml) were obtained via venipuncture in the early morning (0700-0900h) after a 12 hours fast and 48 hours without exercise. Samples were centrifuged at 1,500 *g* and the resultant serum was aliquoted and stored at –80 °C until analysis. Samples were analyzed for TT and FT using commercially available enzyme-linked immunosorbent assays (Alpco, Salem, NH). The intra-assay variances (CV) were 3.5% for TT and 10.3% for FT. All samples were analyzed in duplicate within the same assay batch to eliminate potential inter-assay variance.

Statistical Analyses

Data were analyzed using a one-way analysis of variance. Pairwise differences were determined using Fisher's LSD *post hoc* test (IBM SPSS Statistics version 20, Chicago, IL). Alpha level of the study was set at $P < 0.05$ and data are displayed as mean ± SE unless otherwise indicated.

4.3 Results

Age, height, and total mass did not differ between groups. RT and NRT had significantly greater lean body mass and lower body fat % than CON ($P < 0.05$). See Table 4.1 for data. NRT ran further distances per week than RE ($P < 0.05$), with individual values ranging from 35.8 to 96.6 km in NRT and 32.2 to 68.4 km in RT.

	CON	NRT	RT
Age (y)	27 ± 2.7	26.1 ± 3.5	24.8 ± 2.3
Height (cm)	176.8 ± 3.9	179.1 ± 3.7	176.2 ± 5.0
Mass (kg)	74.8 ± 8.3	73.2 ± 7.0	77.8 ± 10.1
Lean mass (kg)	53.8 ± 3.2	59.5 ± 4.4*	62.7 ± 5.8*

Table 4.1. Descriptive data for control participants (CON), runners who engage in no resistance training (NRT), and runners who engage in resistance training (RT). Values are means ± SD. *Significantly different ($P \leq 0.05$) from CON. #Significantly different from RT.

Bone Mineral Density

At each measured site, BMD was significantly greater for RT than for NRT and CON. NRT and CON did not differ in BMD at any measured site. BMD data are shown in Figure 4.1.

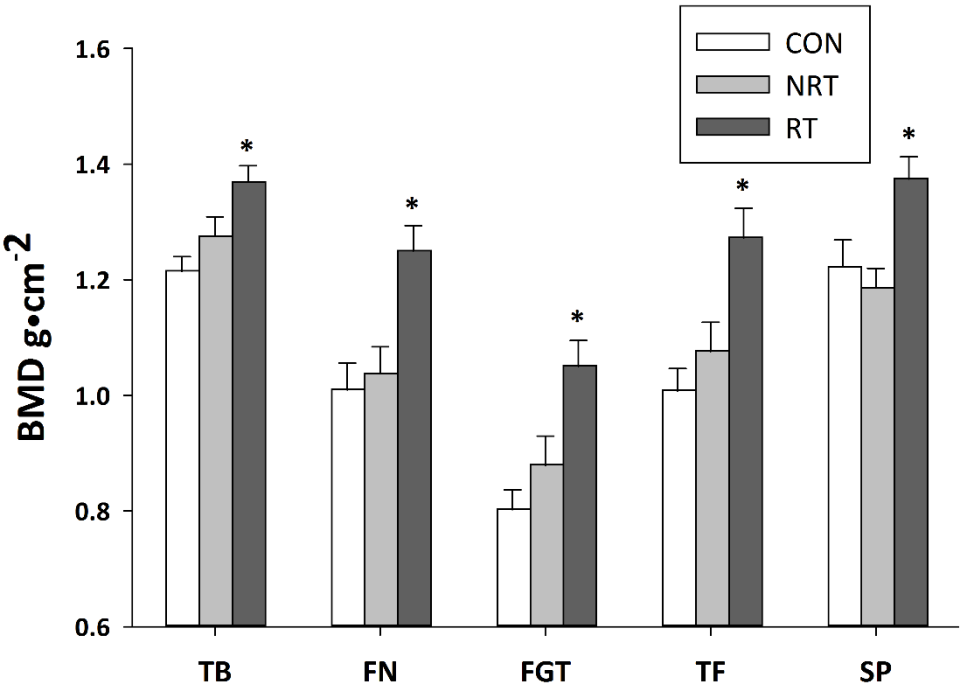
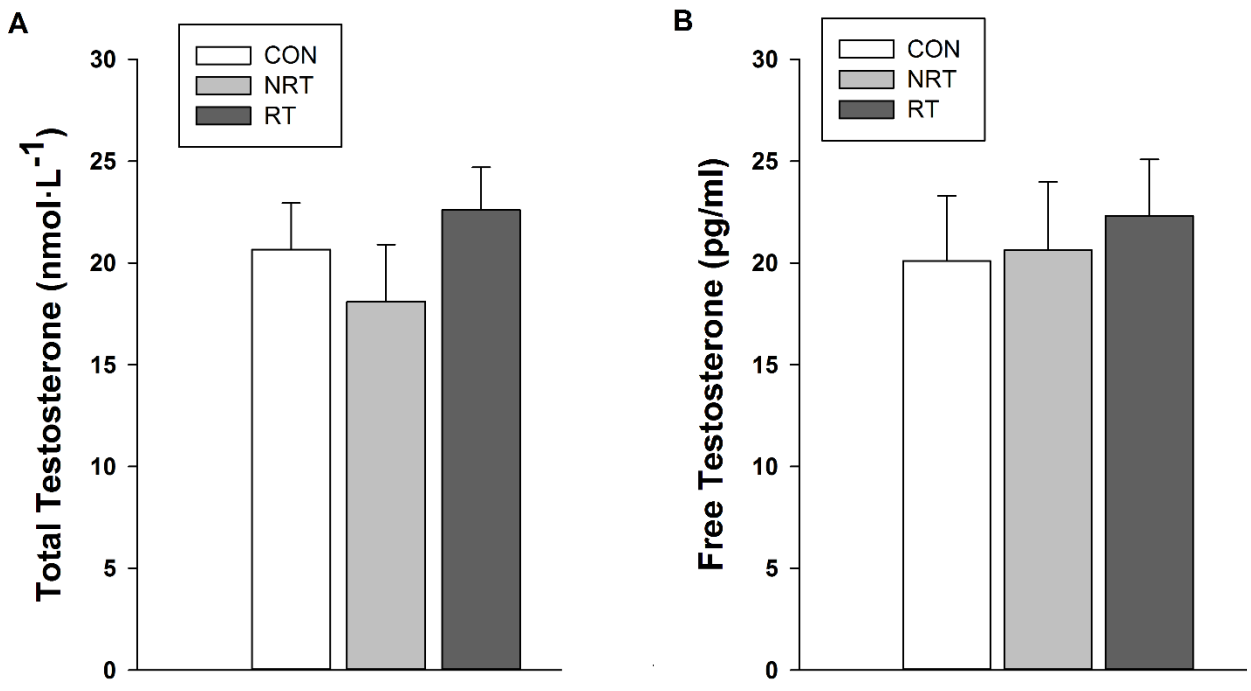


Figure 4.1. BMD for control participants (CON), runners who engage in no resistance training (NRT), and runners who engage in resistance training (RT). BMD Sites: TB- Total body, FN- Femoral neck region, FGT- Femoral greater trochanteric region, TF- Total femur, SP- L1-L4 spine. Values are means ± SE. *Significantly different ($P \leq 0.05$) from other groups.

Testosterone

TT and FT concentrations for the three groups are shown in Figure 4.2. There were no significant differences between groups for TT or FT concentrations.



Figures 4.2. (A) TT Concentrations and (B) FT Concentrations for Control Prticipants (CON), runners who engage in no resistance training (NRT), and runners who engage in resistance training (RT). Values are means ± SE.

4.4 Discussion

In this study, we compared characteristics of two groups of male runners (resistance trained and non-resistance trained) and a non-exercise trained control group that were similar to age and body mass as the runner groups. This is the first study to examine the relation between hormonal status and BMD in young adult male distance runners and how resistance training is associated with these factors. The major findings of this study were that resistance trained runners had greater BMD than non-resistance trained runners and controls, while, despite the weight bearing and repeated impact

nature of running, the BMD of non-resistance trained runners did not differ from their non-exercise trained peers. TT and FT concentrations were not different between groups, suggesting that the greater BMD of the RT group can be attributed to participation in resistance training and not deficiency of testosterone.

Performance of RE or power/plyometric training (e.g. track and field training) is associated with a higher magnitude of bone strain, which more effective for generating bone mass than distance running (Bennell et al., 1997; Layne and Nelson, 1999; Nowak et al., 2010). A novel finding of the present study was that runners who participated in regular resistance training had higher BMD than runners who did not engage in resistance training. These results are comparable to those of a 12 month longitudinal study by Bennell et al. (1997) that compared BMD of young adult male track power athletes (track and field) and endurance runners and found that power athletes had higher lumbar spine BMD than endurance runners. Similarly, among masters track and field athletes (aged 40-64 years) who reported to have participated in their sport before the age or 30, BMD across a range of measurement sites was greater in speed-power than in endurance athletes or an age and body mass matched control group (Nowak et al., 2010). The endurance athlete and control groups did not differ in BMD. As in the present study, total and free testosterone concentration were not different between groups, suggesting that the higher BMD of the speed-power athletes could be contributed to the nature of speed-power training, which elicits a higher magnitude of impact. The investigation conducted by Nowak et al. provides evidence that despite the age-associated decline in BMD (Kelepouris et al., 1995), runners who participate in high impact/loading exercise can achieve a greater BMD than runners who don't. The high

magnitude of bone strain that is provided by RE is an effective stimulus for generating higher bone mass (Layne and Nelson, 1999).

Testosterone, the major gonadal androgen in males, is a potent anabolic hormone that plays an important role in regulating bone remodeling (Vanderschueren et al., 2004). Low resting concentrations of testosterone in male distance runners have been previously reported (Hackney et al., 1988; MacKelvie et al., 2000; Wheeler et al., 1984, 1991). A study that investigated male marathon runners reported that highly trained male athletes, like their female counterparts, have a deficiency of gonadotropin-releasing hormone (GnRH) (MacConnie et al., 1986). Deficiency of GnRH can lead to low levels of luteinizing hormone (LH), which could possibly explain the lower circulating testosterone concentration in distance runners. Also, endurance running can still lead to decreased concentrations of total and free testosterone, even if LH levels remain the same or are elevated (Hackney et al., 1988; Wheeler et al., 1991). In the present study, NRT did not have lower circulating concentrations of TT or FT than RT or CON. However, it can be postulated that when male distance runners participate in a running volume that is higher than that of the runners in the present study, decreases in resting testosterone could occur. MacKelvie et al. (2000) found a negative correlation between weekly running volume (ranging from 64 to 94 km per week) and total and free testosterone concentrations. While lower than control values, the reported testosterone concentrations found by MacKelvie et al. (2000) were still within the normal physiological range, with the highest volume runners presenting with the lowest levels.

It has been found that there is a complex relationship between BMD and weekly running distance. Studies report negative associations between running volume and

BMD. A study by MacDougall et al. (1992) investigated BMD in male runners categorized into different weekly running distance groups. They reported that BMD was higher in participants who ran 22 to 32 km/wk than in controls; but BMD was the same in participants who ran 96 to 120 km/wk as in controls. Their data showed that running volume >32 km per week (40-48 and 64-88 km/wk groups) did not cause further increase in BMD, as compared to the 22 to 32 km/wk group, and could start to decrease so that the 96-120 km/week group looked similar to the control group's lower leg BMD (MacDougall et al., 1992). In another study investigating the relationship of running volume and BMD, a group running 64-80 km/wk had higher femoral BMD than controls and a 95+ km/wk running group (MacKelvie et al., 2000). The 95+ km per week group did not differ in BMD from the control group for any site. These studies suggest that low to moderate running volume could lead to increases in BMD, while high running volume could lead to a decrease in BMD or a BMD that is comparable to controls. In the current study, the average weekly running volume for NRT (69.1 km/wk) was not associated with greater BMD as compared to controls. This is in contrast to the reports from MacDougall et al., (1992) and MacKelvie et al., (2000), who found greater BMD with moderate running volumes (22-88 km/wk and 64-80 km/wk, respectively). However, the age of participants may account for the differences in results between the current and previous studies. Mackelvie et al., (2000) investigated male runners aged 40-55 years and MacDougall et al., (1992) investigated male runners aged 20-45 years compared to 23-32 years in the current study. It is possible, then, that for middle age and older adults, running of low to moderate volume results in greater BMD compared to no running (sedentary controls). Since sedentary controls in that age range typically have

already experienced a decline in BMD (Kelepouris et al., 1995), the low to moderate running volume might have prevented or attenuated such an age associated decline in BMD.

In conclusion, the major findings of this study was that young adult male long-distance runners who participated in resistance training at least once per week had greater BMD than their non-resistance trained and non-exercise trained peers. Resistance training's effect on BMD has been well documented and is largely regarded as an effective method of building bone mass (Frost 1987) and, in the present study, is associated with higher BMD in runners who lift weights versus runners who do not. Long distance running alone, according to our results, does not appear to affect BMD in young adults. However, it is important to note that BMD generally declines with aging (Kelepouris et al., 1995) and that low to moderate volume of running might prevent or attenuate this decline; whereas, high running volume appears to not have such an protective effect on BMD (MacKelvie et al., 2000). Furthermore, testosterone declines with age (Tenover 1997) and this could further contribute to dysregulation of bone remodeling due to distance running. Thus, it appears that participation in regular resistance training, resulting in increased bone modelling, can be an effective method for the prevention of osteopenia/osteoporosis.

CHAPTER 5

DISCUSSION

The purpose of this project was to further elucidate the effects post-exercise alcohol ingestion. This project had many novel aspects including using a RE only exercise design and the inclusion of women. To our knowledge, we are the first to investigate the effect of post-RE alcohol ingestion in women.

In the first chapter of this project, information on the prevalence of alcohol use and the importance of skeletal muscle as a dynamic and metabolic tissue was provided. Skeletal muscle plays a key role in whole body metabolism and is essential for mobility, disease prevention, and quality of life (Lynch, 2004; Srikanthan and Karlamangla, 2011). Alcohol (ethanol) is consumed worldwide and represents the most commonly used and abused recreational drug. Alcohol has toxic effects on most organs and tissues in the body, and chronic use can lead to dependence. Acute heavy episodic drinking (i.e., binging), defined as ingesting six or more standard drinks on one occasion, is associated with detrimental consequences even if an individual's average level of alcohol consumption is relatively low (WHO, 2014). In the USA, over 70% of adults and 83% of college athletes reported having consumed alcohol in the previous year.

Muscle growth (hypertrophy) occurs when there is a net increase in the rate of protein synthesis, while reduction in muscle mass (atrophy) occurs when there is a net decrease in the rate of protein synthesis (Goodman, 2014). A major regulator of the rate of skeletal muscle protein synthesis in mammalian systems is the mechanistic target of rapamycin complex 1 (mTORC1), which has been extensively researched over the past

decade. The mTORC1 pathway mediates protein synthesis through the phosphorylation of its downstream targets S6K1 and 4E-BP1. These substrates, once phosphorylated stimulate protein synthesis by increasing the rate of translation initiation and ribosomal translation efficiency (Terada et al., 1994). Normal mTORC1 functioning is essential for anabolic processes, including the muscle hypertrophy that occurs consequent to chronic performance of RE (i.e., training); whereas, aberrant mTORC1 functioning has been associated with human diseases such as diabetes, cancer, and obesity (Laplante and Sabatini, 2012).

Resistance exercise is a potent stimulator of protein synthesis through its effects on mTORC1 signaling. The RE-induced stimulus on mTORC1 signaling has been implicated as a necessary component of muscle protein synthesis (Bodine et al., 2001) and any effectors that may negatively influence normal mTORC1 signaling represent a potentially potent detriment for muscle training adaptations. In contrast, alcohol ingestion (in the absence of exercise) reduces mTORC1 signaling and protein synthesis (Lang et al., 2003, 2004, 2009) but the collective effects of RE and alcohol has not been elucidated. It has previously been found that ingestion of alcohol attenuated protein synthesis and mTORC1 phosphorylation, but not S6K1 phosphorylation, from the collective stimulatory effects of combined endurance and resistance exercise and protein supplementation in men (Parr et al., 2014).

In chapter two, the effects of post-RE alcohol ingestion in men and women are detailed. Independently, resistance exercise and alcohol have opposite effects on mTORC1 signaling. Despite the potential negative implications of acute alcohol ingestion on the physiological response to heavy resistance exercise, no study has

examined the mTORC1 signaling in this context. The major findings of this study was that although RE elicited similar mTORC1 signaling both in men and in women, alcohol ingestion appeared to only attenuate RE-induced phosphorylation of the mTORC1 signaling pathway in men. Considering the high rate of alcohol usage among athletes and other individuals who engage in regular recreational physical activity, the findings from this study provide important physiological insight regarding alcohol's effect on processes involved in protein synthesis during recovery from resistance exercise. These findings in men are important because they suggest a potential mechanism underlying the attenuated RE-induced protein synthesis from alcohol ingestion previously observed for men. Furthermore, the findings suggest that RE could be used as a countermeasure for alcohol induced myopathy or to counter act the negative effects of alcohol ingestion on mTORC1 signaling.

The third chapter focused on examining the effects of post-RE alcohol ingestion on acute testosterone bioavailability. At rest, alcohol intoxication in men acutely can suppress serum TT concentration for up to several hours following the alcohol ingestion (Kapoor, Luttrell, & Williams, 1993b; Koziris, Kraemer, Gordon, Incledon, & Knuttgen, 2000; Kraemer et al., 1998; Kraemer et al., 1999). The primary findings of this study was that alcohol substantially elevated serum TT or FT concentrations during recovery from a bout of resistance exercise. Contrary to what was expected based on findings for alcohol ingestion in the absence of exercise, TT and FT concentrations were significantly higher for alcohol ingestion compared to a placebo drink from 60 to 300 min post RE (40 to 280 minutes following alcohol ingestion). It had been hypothesized that post-exercise alcohol ingestion would significantly decrease TT and FT below the

values observed for placebo at corresponding time points/phases due to the suppressive effect of alcohol on testosterone production in the Leydig cells of the testis (Kraemer, Volek, Bush, Putukian, & Sebastianelli, 1998; Kraemer et al., 2006; Kvorning, Andersen, Brixen, & Madsen, 2006) and the increased clearance of testosterone by aromatase in the liver (Linnoila, Prinz, Wonsowicz, & Leppaluoto, 1980). It appears that alcohol ingestion following heavy resistance exercise results in a testosterone response pattern that differs markedly from the pattern found when alcohol is ingested in the absence of prior resistance exercise.

The fourth chapter detailed factors that contribute to bone density in men. Increases in bone mineral density (BMD) are stimulated by mechanical loading, including weight bearing physical activities (Frost 1987). Running is a weight-bearing exercise and as such should provide potent stimulus for bone growth and maintenance. Surprisingly, numerous studies report low BMD in both male and female long distance runners (Bilanin et al., 1989; Burrows et al., 2003; Hetland et al., 1993; Hind et al., 2006).The major findings of this study was that young adult male long-distance runners who participated in resistance training at least once per week had greater BMD than their non-resistance trained and non-exercise trained peers. Resistance training's effect on BMD has been well documented and is largely regarded as an effective method of building bone mass (Frost 1987) and, in the present study, is associated with higher BMD in runners who lift weights versus runners who do not. Long distance running alone, according to our results, does not appear to affect BMD in young adults. However, it is important to note that BMD generally declines with aging (Kelepouris et al., 1995) and that low to moderate volume of running might prevent or attenuate this

decline; whereas, high running volume appears to not have such an protective effect on BMD (MacKelvie et al., 2000). Furthermore, testosterone declines with age (Tenover 1997) and this could further contribute to dysregulation of bone remodeling due to distance running. Thus, it appears that participation in regular resistance training, resulting in increased bone modelling, can be an effective method for the prevention of osteopenia/osteoporosis.

Resistance Exercise has an important influence on muscle training adaptations (i.e., hypertrophy) through its stimulus on mTORC1 signaling and muscle protein synthesis. The RE-induced stimulus on mTORC1 signaling has been implicated as a necessary component of muscle protein synthesis and any effectors that may negatively influence normal mTORC1 signaling represent a potentially potent detriment for muscle training adaptations. Participation in resistance exercise can also be a potent stimulator of acute testosterone availability and increases in bone mineral density. The first two data chapters in this project have investigated factors that might influence normal physiological responses to resistance exercise. In men, post-RE alcohol ingestion was demonstrated to decrease mTORC1 signaling and alter circulating androgen response. Additionally, the effects of post-RE alcohol ingestion in women has been demonstrated for the first time. Future studies should further investigate the effect of alcohol ingestion on RE-induced signaling involved in regulating protein synthesis and exercise training adaptations, especially in women, since prior research has found that alcohol reduces protein synthesis in women despite the lack of changes in mTORC1 signaling found in the current study.

APPENDIX A

IRB INFORMED CONSENT FOR

University of North Texas Institutional Review Board

Informed Consent Form

Before agreeing to participate in this research study, it is important that you read and understand the following explanation of the purpose, benefits and risks of the study and how it will be conducted.

Title of Study: The effect of alcohol ingestion on resistance exercise induced hormonal response and mTOR signaling.

Principal Investigator: Dr. Jakob Vingren, University of North Texas (UNT) Department of Kinesiology, Health Promotion and Recreation.

Purpose of the Study: You are being asked to participate in a research study which involves testing the effects of drinking alcohol after resistance exercise on hormonal and muscle responses.

Study Procedures:
Before you can be approved for participation you must complete a medical history questionnaire, a nutrition & physical activity questionnaire, and two alcohol use questionnaires (Young Adult Alcohol Problems Screening Test, and Alcohol Use Disorders Identification Test) to ensure that you meet all the inclusion and none of the exclusion criteria.

Inclusion/exclusion criteria*:*
Inclusion

To meet inclusion criteria you must be: 1) at least 21years old; 2) resistance exercise trained (at least 2 sessions per week including the back squat for at least 6 months); and 3) considered a low to moderate alcohol (no more than 14 drinks per week on average) consumer according to the US Department of Health and Human Services. In addition, women must be eumenorrheic (normal monthly menstrual cycle).

Exclusion

You will be excluded if you are pregnant or trying to become pregnant (Women only), or have a greater than moderate weekly alcohol intake.

You will also be excluded if you have preexisting heart conditions or anomalies, respiratory conditions, blood pressure problems, musculoskeletal problems, herniated inter-vertebral discs or previous orthopedic injuries that would limit the range of motion about the shoulder, elbow, hip, knee or ankle joint.

Potential volunteers will be excluded from participation in the study if they have recently taken (with the past year), are currently taking, or plan to take any hormonal substances or medications such as androgenic-anabolic steroids, growth hormone, or glucocorticoids (e.g., prednisone).

We will reserve the right to dismiss you at any time if we believe you do not follow the instructions provided for this study.

Initial Screening Session (~2 hours):

You will report to the Applied Physiology Laboratory (APL) (PEB 108).

1. You will be given an informed consent, brief medical history form, and an alcohol intake questionnaire.
2. We will then measure your height and weight. Body composition will be assessed using a very-low dose x-ray (DXA).
3. You will then perform a standardized warm-up, be familiarized with proper squat technique, and then perform a 1-repetition maximum.

Experimental Exercise Trials:

You will complete two experimental treatment conditions (Alcohol and Placebo, either once each or one condition twice). To account for hormonal variations during the menstrual cycle women will be "phased" so that they complete the experimental treatment sessions during their early follicular phase (days 2-7 after the start of menses). You will be asked to record your dietary intake for the two days before and the morning of the first treatment visit and to replicate that diet prior to the second treatment visit.

Treatment sessions

You will be asked to refrain from: 1) eating or drinking anything (except for water and non-caffeinated diet drinks) during the 2 hours leading up to each treatment visit, 2) any ingestion of ethanol for 84 hours (3.5 days) prior to each treatment visit, 3) consuming large amounts of caffeine on test day (no more than 1 cup of coffee allowed), 4) engaging in sexual activity for 24 hours prior to each treatment visit, and 5) performing any resistance exercise or intense aerobic exercise for 96 hours (4 days) prior to each visit. It will also be required that you have not donated blood within 8 weeks or plasma within 96 hours of the laboratory visits.

Sixty five minutes prior to the acute heavy resistance exercise (6x10 squats) you will consume a standardized meal replacement drink (Ensure Plus®) containing 8 kcal per kg body mass. After the meal replacement drink is ingested a catheter will be inserted in a superficial vein of the forearm.

Fifteen minutes before performing the acute heavy resistance exercise (50 min after the meal), you will complete a standardized warm-up. You will then complete 10 warm-up squats at 50% of 1-RM followed by the acute heavy resistance exercise. The acute heavy resistance exercise will

consist of 6 sets of 10 repetitions of Smith machine squats starting at 80% of 1-RM with 2 min of rest between sets. After the completion of the resistance exercise, you will rest in a chair for the remainder of the session (next 5 hours) and will not be allowed to sleep. To prevent anxiety and boredom you will be permitted to read, write, or watch TV/movies as long as the content will not cause you to experience any form of stress, anxiety, or other strong emotions, either positive or negative.

Alcohol ingestion

From 10-20 minutes post-exercise, you will consume either alcohol or no alcohol (Placebo condition) in an artificially sweetened and calorie free beverage. For the alcohol condition, a dose of 1.09 g of ethanol (Vodka, 40% v/v alcohol) per kg fat free body mass will be used. The alcohol will be diluted to a concentration of 15 % v/v absolute ethanol; for the Placebo condition the ethanol will be substituted with an equal volume of water.

Blood Draws

For each blood draw ~15 ml of blood will be collected (about 1 table spoon). For each of the 2 protocol visits a total of 300 ml (1.26 cups) of blood will be collected over approximately 5 hours. This is about half of the amount of blood typically taken for a Red Cross blood donation. Although this blood volume is replenished almost immediately we ask that as a safety precaution, you refrain from donating blood within the time period two weeks before the start of the study through two weeks after the end of the study.

Muscle biopsies

Muscle samples will be obtained before exercise and three and five hours post exercise by biopsies. Three separate sites 3 cm apart on the right thigh (vastus lateralis) will be used to obtain the three biopsies during the treatment visit. Each muscle sample will be obtained from the superficial portion of the vastus lateralis using microbiopsy procedures. Briefly, the skin over the muscle will be cleaned using betadine and a local anesthetic (1% lidocaine w/o epinephrine) will be injected under the skin and into the muscle. A pilot hole in the skin will be made with a regular 16 gauge syringe needle. Then, a Pro-Mag 14 gauge microbiopsy needle will be introduced barely past the fascia and the trigger mechanism will be engaged. This will advanced an inner cannula 25mm to cut the tissue. The needle will subsequently be removed. The needle puncture site will be covered with sterile gauze and compression applied to prevent bleeding. The site will then be covered with a small adhesive bandage (i.e., Band-Aid). For this method no scalpel incision or standard Bergstrom biopsy needle is used and thus no suture is needed or noticeable scar produced.

Foreseeable Risks: The potential risks involved in this study are as follows:

DXA Test

During the DXA testing you will be exposed to a very low dose of radiation. The dose exposure is equivalent to your exposure during a trans-Atlantic plane flight (i.e. Dallas to Europe) and poses no long-term health risks. It is important that if you are pregnant or think you may be pregnant that you do not complete this test and thus not participate in this study.

Resistance Exercise

The performance of muscular exercise and physical effort can entail the potential hazards of injury from over-exertion and/or accident. This study will be planned to avoid injury to the musculoskeletal system. Additional risks associated with resistance exercise and with the strength and power tests and training involve the possibility of muscle strains or pulls of the involved musculature, delayed muscle soreness 24 to 48 hours after exercise, muscle spasm, and in extremely rare instances, muscle tears. Such risks are very low.

The Principal Investigator (Dr. Jakob Vingren) is a National Strength and Conditioning Association (NSCA) Certified Strength and Conditioning Specialist recertified with Distinction (CSCS*D).

Venous Blood Draws

We will take blood samples from a small, flexible tube (catheter) which will be inserted in a vein in your arm. There will be discomfort when the catheter initially punctures your skin. There is a small chance that you might develop a bruise at the site of the puncture and/or become dizzy/faint. Additionally, it is possible that an infection might occur. Insertion of the catheter will be done by a skilled technician using sterile techniques.

Fingerstick Blood Collection
After your venous blood collections, we will puncture your fingertip skin using a needle. Sterile, single-use supplies will be used for your fingertip sampling. Blood will be collected into a series of capillary tubes and/or filter paper for additional blood analyses.

Muscle Biopsy

There is a small chance that you might develop a bruise at the site of the puncture and/or become dizzy/faint. Additionally, it is possible that an infection might occur. All biopsy procedures will be performed by Dr. Vingren who is trained in the microbiopsy procedure. You will be provided with an informational take-home sheet after the biopsies that addresses care of the needle puncture site and you will be provided with additional Band-Aids and topical antibiotic.

Benefits to the Participants or Others: There is limited direct benefit to you for participating in this study; however, all your data will be explained to you and interpreted for you so that a maximum amount of educational understanding and use of the data will be achieved.

Participation in this study will also afford you instruction in correct weight-lifting techniques and you will learn your endocrine and physical response to resistance exercise and alcohol ingestion.

The results could have substantial indirect benefits to society. Given that 60 % of athletes believe that their alcohol intake does not affect their performance, we propose that providing an evidence-based report generated from data using a rigorous experimental model may have a strong impact on reducing alcohol consumption. Importantly, identifying hormonal biomarkers associated with tissue regeneration has translational implications in clinical testing, treatment and intervention.

Compensation for Participants: You will receive $300 for completion of all aspects of this study.

Procedures for Maintaining Confidentiality of Research Records: All data will be kept in coded participant files in the primary investigator's locked files. Participant codes will be used when statistical analyses are performed or when experimental feedback sheets are provided to you. All investigators, professional staff, and technicians are aware of the confidentiality involved with this study and have completed the confidentiality training required by the University. Your data will not be available or divulged to anyone outside of the experimental research team. The data files will be kept for 3 years after the study is terminated. The confidentiality of your individual information will be maintained in any publications or presentations regarding this study.

Questions about the Study: If you have any questions about the study, you may contact *Dr. Jakob Vingren* at telephone number (940) 565 3899.

Review for the Protection of Participants: This research study has been reviewed and approved by the UNT Institutional Review Board (IRB). The UNT IRB can be contacted at (940) 565-3940 with any questions regarding the rights of research participants.

Circumstances for Dismissal from Project

Your participation in this study can be terminated:

- If you do not keep study appointments
- If you do not follow the instructions you are given
- If Dr. Vingren determines that staying in the study is harmful to your health or is not in your best interest
- If Dr. Vingren decides to stop or cancel the study

APPENDIX B

FINAL CURRICULUM VITAE OF GRADUATE WORK

Curriculum Vita

1. Personal Information

Anthony Adam Duplanty, M.S., ACSM-HFS
Teaching Fellow / Research Assistant
Department of Kinesiology, Health Promotion, and Recreation
Mailing address: 1155 Union Circle #310769 Denton, TX 76203-5017
Phone: (940) 595-4696 Fax: (940) 565-4904
Email: Anthony.Duplanty@unt.edu

2. Education

Ph.D. in Biology specializing in Exercise Physiology (ABD)
University of North Texas, Denton, TX
Dissertation title: "Effect of Acute Alcohol Ingestion on Resistance Exercise Induced mTOR Signaling in Human Muscle"
Mentor: Jakob L. Vingren, Ph.D.
Anticipated completion date: June 2015

M.S. in Kinesiology
University of North Texas, Denton, TX
Mentor: Jakob L. Vingren, Ph.D.
Project: "*Effect of Resistance Training on the Male Athlete Triad in Runners"*
Completed: August 2012

B.S. in Kinesiology
University of North Texas, Denton, TX
Minor: Health Promotion
Completed: May 2010

3. Professional Experience

Begin-End Year	Place of Employment	Job Title
Fall 2010 – present	Department of Kinesiology, Health Promotion and Recreation University of North Texas, Denton, TX	Teaching Fellow
Fall 2010 – present	Applied Physiology Laboratory University of North Texas, Denton, TX	Research Assistant

4. Scholarly Publication Record

Data–based or theoretical refereed papers (7 total)

Donahue, R. B., Vingren, J. L., Duplanty, A. A., & Kraemer, W. J. (in press) Effect of whole-body vibration warm-up on footspeed quickness, *Journal of Strength and Conditioning Research*

Budnar, R. G., Duplanty, A. A., Hill, D. W., McFarlin, B. K., & Vingren, J. L. (2014) The acute hormonal response to the kettlebell swing exercise, *Journal of Strength and Conditioning Research,* 28(10):2793-2800

Duplanty, A. A. Vingren, J. L &. Keller, J. (2014) Special populations: Physical Activity and Intellectual Disability, *Strength and Conditioning Journal,* 36(2):26-28

Duplanty, A. A. Vingren, J. L &. Keller, J. (2014) One-on-One: Exercise Training Recommendations: Working with Individuals with Intellectual Disabilities, *Strength and Conditioning Journal,* 36(2):29-31

Shaner, A. A., Vingren, J. L., Hatfield., D. L., Budnar, R. G., Duplanty, A. A., & Hill, D. W. (2014) Hormonal Response to Free Weight and Machine Weight Resistance Exercise. *Journal of Strength and Conditioning Research* 28(4):1032-1040.

Vingren, J. L., Hill, D. W., Buddhadev, H. H., & Duplanty, A. A. (2013). Post-resistance exercise ethanol ingestion and acute testosterone bioavailability. *Medicine and Science in Sport and Exercise,* 45(9):1825-32

Buddhadev, H. H., Vingren, J. L., Duplanty, A. A., & Hill, D. W. (2012). Mechanisms underlying the reduced performance measures from using equipment with a counterbalance weight system. *Journal of Strength and Conditioning Research,* 26(3):641-647

Manuscripts in review or final preparation (7 total)

Vingren, J.L., Budnar, R. G., Luk, H. Y., McKenzie, A. L., Duplanty, A. A., Layman, T., Levitt, D., & Armstrong, L. E., (in review-minor revisions) The acute testosterone, growth hormone, cortisol, and interleukin-6 response to 164-km road cycling in a hot environment. *Journal of Sport Science*

Luk, H. Y., Vingren, J.L, McKenzie, A. L., Duplanty, A. A., Budnar, R. G., Levitt, D., Fernandez, A., Lee, E. C., & Armstrong, L. E. (in review-major revisions) Leukocyte subset changes in response to a 164-km road cycle ride in a hot environment. *International Journal of Exercise Science*

Duplanty, A.A, Vingren, J.L., Hill, D.W., McFarlin, B.K., Padilla, P.A. (in final preparation) Effect of Acute Alcohol Ingestion on Resistance Exercise Induced mTOR Signaling in Human Muscle.

Duplanty, A.A., Vingren, J.L., DiMarco, N.M., Hill, D.W. (in final preparation) Factors that contribute to bone mineral density in male runners.

Vingren , J. L., Curtis, J. H., Lee, E. C., Duplanty, A. A., McFarlin, B. & Hill, D. W. (in final preparation) Effect of 6-weeks of resistance training on cytokines in HIV+ men with chemical dependence.

Koziris, L. P., Vingren, J. L., Chatterton, R. T. & Duplanty, A. A. (in final preparation) Blood biomarkers and performance during competitive swim training, *British Journal of Sports Medicine*

Luk, H. Y., McKenzie, A. L., Lee, E. C., Armstrong, L. E., Duplanty, A. A., Budnar, R. G., Levitt, D., McFarlin, B. K.,& Vingren, J.L. (in final preparation) Cytokine response to a 164-km bicycle ride in a hot ambient temperature. *European Journal of Applied physiology*

Vingren, J.L, Levitt, D., Idemudia, N., Cregar, C., Duplanty, A. A., Budnar, R. G., Luk, H. Y., & Hill, D. W. (in final preparation) Effect of binge drinking following heavy eccentric resistance exercise on muscle power recovery in men.

5. <u>Peer Reviewed Abstracts Presented</u>

<u>International (1 total):</u>

Luk, H. Y., Duplanty, A. A., Levitt, D. E., Budnar, R. G., McFarlin, B. K., Hill, D. W., & Vingren, J. L. (submitted for June 2015) Increased Satellite Cell Proliferation when Cultured with Post-Exercise Serum: Role of Testosterone? Annual Conference of the European College of Sport Science, Malmo, Sweden.

<u>National</u> (27 total):

Vingren J. L., Budnar R. G., Duplanty A. A., Levitt D. E., Luk H., Fernandez A., & Hill, D. W. (submitted for July 2015). Effect of ethanol ingestion on the acute hormonal response to heavy resistance exercise in women. The National Strength and Conditioning Association's National Conference, Orlando, Florida.

Levitt, DE, Duplanty, AA, Budnar, RG, Luk, HY, Fernandez, A, Layman, TJ, Fancher, DL, McFarlin, BK, Hill, DW, and Vingren, JL. (submitted for July 2015). The effect of alcohol consumption after heavy resistance exercise on lipopolysaccharide-stimulated IL-1β, TNF-α, and IL-10. The National Strength and Conditioning Association's 38th Annual Conference, Orlando, FL.

Budnar R. G., Levitt D. E., Luk H., Fernandez A., Duplanty A. A., Layman, T. J., Blumenthal, H., Trost, Z., & Vingren J. L. (Accepted for May 2015) Alcohol consumption 24h following eccentric low back muscle damage on muscular performance recovery in untrained individuals. Annual meeting of the American College of Sports Medicine, San Diego, California.

Vingren J. L., Duplanty A. A., Budnar R. G., Luk H., Levitt D. E., Fernandez A., Hill, D. W., & DiMarco, N. M., (Accepted for May 2015) Greater total and regional bone mineral density in adult male long-distance runners who resistance train. Annual meeting of the American College of Sports Medicine, San Diego, California.

Duplanty, A.A., Budnar, R.G., Luk, H.Y., Fernandez, A., Levitt, D.E., Venable, A.S., Hill, D.W., DiMarco, N.M., McFarlin, B.K., Vingren, J.L. (July 2014) Engaging in resistance training is associated with greater femoral and spinal bone mineral density in male long distance runners. The National Strength and Conditioning Association's National Conference, Las Vegas, Nevada.

Luk, H. Y., Levitt, L. E., Duplanty, A. A., Budnar, R. G., Fernandez, A., Layman, T. J., McKenzie, A., Lee, E. C., Armstrong, L. E., Hill, D. W., McFarlin, B. K., Williamson, K. & Vingren, J. L. (July 2014) the effect of ultra-endurance cycling in hot environment on th2 cytokine. The National Strength and Conditioning Association's National Conference, Las Vegas, Nevada.

Budnar, R.G., Vingren, J.L., Duplanty, A.A., Fernandez, A., McKenzie, A.L., Luk, H.Y., Levitt, D.E., Layman, T.J., Hill, D.W., McFarlin, B.K., Williamson, K., Armstrong, L.E. (July 2014) Acute hormonal response to 164km road cycling in a hot environment. The National Strength and Conditioning Association's National Conference, Las Vegas, Nevada.

Fernandez, A., Duplanty, A.A., Budnar, R.G., Luk, H.Y., Levitt, D.E., Layman, T.J., Hill, D.W., Vingren, J.L. (July 2014) Exercise intervention on performance measures related to cardiovascular and neuromuscular health in HIV+ women recovering from substance abuse. The National Strength and Conditioning Association's National Conference, Las Vegas, Nevada.

Layman, T.J., Duplanty, A.A., Budnar, R.G., Luk, H.Y., Cregar, C.M., Idemudia, N.O., Fernandez, A., Levitt, D.E., Hill, D.W., Vingren, J.L. (July 2014) Binge drinking following heavy eccentric resistance exercise: Effect on muscle power recovery in women. The National Strength and Conditioning Association's National Conference, Las Vegas, Nevada.

Levitt, D.E., Luk, H.Y., Duplanty, A.A., Budnar, R.G., Fernandez, A., Layman, T.J., McKenzie, A.L., Lee, E.C., Hill, D.W., Armstrong, L.E., McFarlin, B.K., Vingren, J.L. (July 2014) The pro-inflammatory cytokine response to ultra-endurance cycling in an extreme environment. The National Strength and Conditioning Association's National Conference, Las Vegas, Nevada.

Vingren, J. L., Duplanty, A. A., Budnar, R. G., Luk, H. Y., Fernandez, A., Miller, J & Hill, D. W. (May 2014) Effect of combined aerobic and resistance training on HPA axis reactivity in HIV+ women undergoing treatment for substance abuse. Annual meeting of the American College of Sports Medicine, Orlando, Florida

Budnar, R. G., Luk, H. Y., Duplanty, A. A., Fernandez, A., Armstrong, L. E., McKenzie A. L., Johnson, E. C., Kupchak, B. R., Lee, E. C., Williamson, K., & Vingren, J. L. (May 2014) Effects of

Long Distance Cycling in a Hot Ambient Temperature on White Blood Cell Count. Annual meeting of the American College of Sports Medicine, Orlando, Florida

Duplanty, A.A., Vingren, J.L., Hill., D.W., Fernandez, A., Budnar, R.G., Leschak, N. M., Wright, H.H., DiMarco, N.M., & Meyer N.L. (May 2014) Influence of Race on the Female Athlete Triad. Annual meeting of the American College of Sports Medicine, Orlando, Florida

McKenzie A. L., Vingren, J. L., Kunces, L. J., Saenz, C., McDermott, B. P., Ganio, M. S., Luk, H. Y., Duplanty, A. A, Budnar, R. G., Kupchak, B. R., Lee, E. C., & Armstrong, L. E. (May 2014) Endogenous antioxidant enzyme response to a 164km cycling event in the heat. Annual meeting of the American College of Sports Medicine, Orlando, Florida.

Vingren, J. L., Adinoff, B., Duplanty, A. A., Budnar, R. G., Luk, H. Y., Xiao, H., & Hill, D. W. (March 2014) Muscle glucocorticoid receptors and long-term alcohol abuse: Preliminary findings. 13th Biennial Advances in Skeletal Muscle Biology in Health and Disease Conference, Gainesville, Florida

Vingren, J. L., Idemudia, N. O., Cregar, C. M., Duplanty, A. A. Budnar, R.G. and Hill, D. W., (July 2013) Binge drinking following heavy eccentric resistance exercise: effect on muscle power. The National Strength and Conditioning Association's National Conference, Las Vegas, Nevada.

Budnar, R.G. Vingren, J. L., Hill, D. W., and Duplanty, A. A. (July 2013) The acute hormonal response to the kettlebell swing exercise. The National Strength and Conditioning Association's National Conference, Las Vegas, Nevada.

Budnar, R.G. Birdwell, R., Moody, C., Hill, D. W., Duplanty, A. A., Jackson, A. W., & Vingren, J.L. (May 2013) Functional movement screen^{tm} scores in relation to injury risk and performance in collegiate track and field athletes. Annual meeting of the American College of Sports Medicine, Indianapolis, Indiana.

Vingren, J. L., Curtis, J. H., Duplanty, A. A., Lee, E. C., Budnar, R. G., McFarlin, B. K., & Hill, D. W. (April 2013) Effect of resistance training on circulating Th1 cytokines in HIV+ men recovering from substance abuse. Experimental Biology, Boston, Massachusetts.

Duplanty, A. A., Vingren, J. L., Curtis, J. H., Lee, E. C., Budnar, R. G., McFarlin, B. K., & Hill, D. W. (April 2013) Effect of resistance training on circulating Th2 cytokines in HIV+ men recovering from substance abuse. Experimental Biology, Boston, Massachusetts.

Vingren, J.L., Curtis, J. H., Duplanty, A. A., Cregar, C. M., & Hill, D. W. (July 2012) Effect of resistance training on circulating tumor necrosis factor-α in HIV+ men recovering from substance abuse. The National Strength and Conditioning Association's National Conference, Providence, Rhode Island.

Curtis, J.H., Vingren, J.L, Duplanty, A.A., Cregar, C.M, Cantu, J.F, Hill, D.W. (2012) Effect of Resistance Training on Vcam-1 and Cortisol in HIV+ Men Recovering from Substance Abuse.

Annual meeting of the American College of Sports Medicine, San Francisco, CA. *Medicine and Science in Sports and Exercises.*

Idemudia, N. O., Vingren, J. L., Buddhadev, H. H., Duplanty, A.A., Williams, K. R., Chua, J., & Hill, D. W. (May 2012). Binge drinking following resistance exercise: Effect on muscle power recovery. Annual meeting of the American College of Sports Medicine, San Francisco, California.

Cregar, C. M., Vingren, J. L., Buddhadev, H. H., Duplanty, A. A., & Hill, D. W. (July 2011) Effect of alcohol ingestion on the acute cortisol and estradiol response to resistance exercise. The National Strength and Conditioning Association's National Conference, Las Vegas, Nevada. *Journal of Strength and Conditioning Research*. 26(1), S87.

Vingren, J. L., Buddhadev, H. H., Duplanty, A. A., & Hill, D. W. (2011). Effect of alcohol ingestion on the acute testosterone response to resistance exercise. The National Strength and Conditioning Association's National Conference, Las Vegas, Nevada. *Journal of Strength and Conditioning Research*. 26(1), S5.

Duplanty, A. A., Buddhadev, H. H., Hill, D. W., Driver, S., Goggin, N. L., & Vingren, J. L. (May 2011) Effect of Using a Counter-balanced Smith Machine on Performance Measurements for Concentric-Only Bench Press Throws. Annual meeting of the American College of Sports Medicine, Denver, Colorado. *Medicine and Science in Sports and Exercises*, 43(5), S.

Buddhadev, H. H., Duplanty, A. A., Hill, D. W., Driver, S., Goggin, N. L., & Vingren, J. L. (May 2011) Use of Counter-balanced Smith Machine Affects Performance Measurements for Rebound Bench Press Throws. Annual meeting of the American College of Sports Medicine, Denver, Colorado. *Medicine and Science in Sports and Exercises*, 43(5), S.

<u>Regional</u> (8 total):
Duplanty, A. A., Vingren, J. L., Hill, D. W., Fernandez, A., Budnar, R. G., Leschak, N. M., Wright, H. H., Meyer, N. L., and DiMarco, N. M. (Feb 2014) Influence of Race on the Female Athlete Triad. Annual meeting of the Texas chapter of the American College of Sports Medicine, Fort Worth, Texas. *International Journal of Exercise Science: Conference Proceedings*: Vol. 2: Iss. 6, Article 19.

Luk, H. Y., Vingren, J. L., Duplanty, A. A., Fernandez, A., Miller, J., and Hill, D. W. (Feb 2014) Effect Of Combined Aerobic And Resistance Training On HPA Axis Reactivity In HIV+ Women Undergoing Treatment For Substance Abuse. Annual meeting of the Texas chapter of the American College of Sports Medicine, Fort Worth, Texas. *International Journal of Exercise Science: Conference Proceedings*: Vol. 2: Iss. 6, Article 16.

Budnar, R. G. Jr, Luk, H. Y., Duplanty, A., Fernandez, A., Armstrong, L., McKenzie, A., Johnson, E., Kupchak, B., Lee, E., Williamson, K., and Vingren, J. (Feb 2014) Effects of Long Distance Cycling in a Hot Ambient Temperature on White Blood Cell Count. Annual meeting of the Texas chapter

of the American College of Sports Medicine, Fort Worth, Texas. *International Journal of Exercise Science: Conference Proceedings*: Vol. 2: Iss. 6, Article 36.

Budnar, R.G. Birdwell, R., Moody, C., Hill, D. W., Duplanty, A. A., Jackson, A. W., & Vingren, J.L. (2013) Functional movement screen^{tm} scores in relation to injury risk and performance in collegiate track and field athletes. Annual meeting of the Texas chapter of the American College of Sports Medicine, Austin, Texas. *International Journal of Exercise Science: Conference Abstract Submissions.*

Curtis, J. H., Vingren, J. L., Duplanty, A. A., Cregar, C. M., Cantu, J., & Hill, D. W. (2012). Effect of Resistance Training on VCAM-1 and Cortisol in HIV+ Men with Chemical Dependence. Annual meeting of the Texas chapter of the American College of Sports Medicine, Austin, Texas. *International Journal of Exercise Science: Conference Abstract Submissions.*

Idemudia, N. O., Vingren, J. L., Buddhadev, H. H., Duplanty, A.A., Williams, K. R., Chua, J., & Hill, D. W. (2012). Binge Drinking Following Resistance Exercise: Effect on Muscle Power Recovery. Annual meeting of the Texas chapter of the American College of Sports Medicine, Austin, Texas. *International Journal of Exercise Science: Conference Abstract Submissions: Vol. 2: Iss. 4, Article 46.*

Duplanty, A. A., Buddhadev, H. H., Hill, D. W., Driver, S., Goggin, N. L., & Vingren, J. L. (2011) Effect of Using a Counter-balanced Smith Machine on Performance Measurements for Concentric-Only Bench Press Throws. Annual meeting of the Texas chapter of the American College of Sports Medicine, Austin, Texas. International Journal of Exercise Science: Conference Abstract Submissions 2(3), Article 47

Buddhadev, H. H., Duplanty, A. A., Hill, D. W., Driver, S., Goggin, N. L., & Vingren, J. L. (2011) Use of Counter-balanced Smith Machine Affects Performance Measurements for Rebound Bench Press Throws. Annual meeting of the Texas chapter of the American College of Sports Medicine, Austin, Texas. *International Journal of Exercise Science: Conference Abstract Submissions 2(3): Article 48.*

6. <u>Grants</u>

Duplanty, A. A., & Vingren J. L. (2014) *Effect of alcohol ingestion on resistance exercise induced mTOR signaling*. ($1,000) Dissertation Support Grant, College of Education, University of North Texas.

Duplanty, A. A., & Vingren J. L. (2013) *Effect of alcohol ingestion on resistance exercise induced mTOR signaling*. ($9,960) Graduate Research Grant (Doctoral), National Strength and Conditioning Association.

Duplanty, A.A., Vingren, J.L., DiMarco, N.M., Hill, D.W. (2013) *Factors that contribute to bone mineral density in male runners.* ($1,000) Doctoral Student Research Development Award, Texas Chapter of American College of Sports Medicine.

Duplanty, A.A., Vingren, J.L., DiMarco, N.M., Hill, D.W. (2012) *Factors that contribute to bone mineral density in male runners.* ($1,000) Thesis Support Grant, College of Education, University of North Texas.

7. Journal Activity

Reviewer

2013-present	Guest reviewer, *Journal of Strength and Conditioning Research*
2014-present	Guest reviewer, *International Journal of Exercise Science*

8. Membership and Service in Professional Organizations

Professional Memberships

2007-present	American College of Sports Medicine
2011-present	National Strength and Conditioning Association
2012-present	American Physiology Society

Offices in Organizations

2011-2012	President, Strength and Conditioning Professional Development Club, University of North Texas

Service to Professional Organizations

2015	Grant reviewer for the National Strength and Conditioning Association Foundation's Doctoral Research Grants
2014	Research session moderator at the National Conference of the National Strength and Conditioning Association in Las Vegas, Nevada
2013	Research session moderator at the National Conference of the National Strength and Conditioning Association in Las Vegas, Nevada

9. Awards

2014	Annual Chair's Award for Outstanding Doctoral Student in the Department of Kinesiology, Health Promotion and Recreation, University of North Texas. ($1,000) Dissertation Development Award
2008	*Outstanding Student Employee Award*, University of North Texas

10. Instructional Activities

Areas of expertise

- Exercise physiology

- Biomechanics
- Exercise testing and prescription

Courses taught

Course	# of semesters taught
KINE 3050 Biomechanics	2
PHED 1210 Beginning weight training	2
PHED 1211 Intermediate weight lifting	7
PHED 1220 Running	1
PHED 1221 Walking	3

Teaching assistant duties

Course	# of semesters assisting
KINE 3050 Biomechanics	6
KINE 3080 Exercise physiology	2
KINE 4320 Exercise testing and prescription	2
KINE 2050 Sociology of sport	2
KINE 2030 Introduction to Kinesiology	2

Guest lectures

Course
KINE 3050 Biomechanics
KINE 5301 Physiology of exercise (graduate course)

11. Credentials

2007-present	Certified Health and Fitness Specialist – American College of Sports Medicine
2007-present	Adult CPR, AED, and First Aid- American Red Cross
2010-present	Certified to use Dual-energy X-ray Absorptiometry (DXA) – State of Texas
2010-present	Human Participant Protections Education for Research Teams - National Institute of Health (NIH)
2010-present	Bloodborne Safety Training-UNT
2010-present	Biosafety Level 2 Training- UNT

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