2015

Intramuscular Anabolic Signaling and Endocrine Response Following Different Resistance Exercise Protocols In Trained Men

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INTRAMUSCULAR ANABOLIC SIGNALING AND ENDOCRINE RESPONSE FOLLOWING DIFFERENT RESISTANCE EXERCISE PROTOCOLS IN TRAINED MEN

by

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BS The College of New Jersey, 2009
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A dissertation submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
in the College of Education and Human Performance
at the University of Central Florida
Orlando, Florida

Spring Term

2015

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ABSTRACT

The mammalian/mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway appears to be the primary regulator of protein synthesis and growth. While resistance exercise paradigms are often divided into hypertrophy (HYP) and strength (STR) protocols, it is unknown whether these protocols differentially stimulate mTORC1 signaling. The purpose of this study was to examine mTORC1 signaling in conjunction with circulating hormone concentrations following a typical lower-body HYP and STR resistance exercise protocol. Ten resistance-trained men (24.7±3.4y; 90.1±11.3kg; 176.0±4.9cm) performed each resistance exercise protocol in a random, counterbalanced order. Blood samples were obtained at baseline (BL), immediately (IP), 30 minutes (30P), 1 hour (1H), 2 hours (2H), and 5 hours (5H) post-exercise. Fine needle muscle biopsies were completed at BL, 1H, and 5H. Electromyography of the vastus lateralis was also recorded during each protocol. HYP and STR produced a similar magnitude of muscle activation across sets. Myoglobin and lactate dehydrogenase concentrations were significantly greater following STR compared to HYP (p=0.01-0.02), whereas the lactate response was significantly higher following HYP compared to STR (p=0.003). The GH, cortisol, and insulin responses were significantly greater following HYP compared to STR (p=0.0001-0.04). No significant differences between protocols were observed for the IGF-1 or testosterone response. Intramuscular anabolic signaling analysis revealed a significantly greater (p=0.03) phosphorylation of IGF-1 receptor at 1H following HYP compared to STR. Phosphorylation status of all other signaling proteins including mTOR (mammalian target of rapamycin), p70S6k (ribosomal S6 kinase 1), and RPS6 (ribosomal protein S6) were not significantly different between trials. Despite significant differences in markers of muscle

damage and the endocrine response following STR and HYP, both protocols appeared to elicit similar mTORC1 activation in resistance-trained men.

ACKNOWLEDGMENTS

I would like to thank the participants for their time, effort, and dedication to complete the resistance exercise protocols in this study. I would also like to thank my colleagues and friends at the Institute of Exercise Physiology and Wellness for their assistance with data collection and analysis.

To my committee, thank you for your guidance, expertise, and mentoring during this project. It has been a pleasure working with each of you throughout my higher education. I would like to thank my advisor, Dr. Jay Hoffman, for being an inspiration to pursue my doctorate in exercise physiology. I would not be the teacher, researcher, and scientist I am today without his support and encouragement.

I would also like to thank my parents, Sergio and Grace, my brother, Devin, my aunt, Carol, and my girlfriend, Mailyn, for their much appreciated love and support.

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CHAPTER I

Introduction

Resistance exercise paradigms are often divided into distinct hypertrophy or strength protocols. Hypertrophy protocols typically involve high volumes (3-6 sets; 8-12 repetitions), moderate intensities [< 85% 1 repetition maximum (1-RM)], and short rest intervals (30 – 90 seconds), while strength protocols typically involve high intensities (≥ 85% 1-RM), low volumes (2-6 sets; ≤ 6 repetitions), and longer rest intervals (3-5 minutes) (ACSM, 2009). Although changes in the acute program variables surrounding a resistance exercise prescription have been suggested to promote differing anabolic responses and hypertrophic adaptation in skeletal muscle (Kraemer and Ratamess, 2005), the optimal parameters of a resistance training program for regulating muscle growth remain unclear (Adams and Bamman, 2012). The stimulus from muscle contraction during resistance exercise of differing intensities results in varying biochemical responses regulating the rate of protein synthesis, known as mechanotransduction (Hornberger, 2011). At the cellular level, skeletal muscle adaptation is the result of the cumulative effects from transient changes in gene expression following acute bouts of exercise (Coffey and Hawley, 2007). Thus, maximizing the resistance exercise-induced anabolic response stimulates the greatest potential for hypertrophic adaptation with training.

Acute program variables, including exercise intensity, volume, and rest interval, influence the endocrine response following resistance exercise (Kraemer and Ratamess, 2005). Specifically, hypertrophy-style resistance exercise has been suggested to produce significantly greater elevations in both anabolic and catabolic hormones compared to strength-style resistance exercise (Crewther et al., 2008, Hakkinen and Pakarinen, 1993, Kraemer et al., 1990, Linnamo et al., 2005, McCaulley et al., 2009, Smilios et al., 2003, Uchida et al., 2009). Systemic elevations

of circulating hormones increase the likelihood of interaction with receptors located within muscle tissue, and has been suggested to contribute to muscle growth consequent to resistance training (Kraemer and Ratamess, 2005). However, the mechanisms of exercise-mediated muscle hypertrophy have been suggested to be solely an intrinsic process which is not influenced by transient changes in circulating hormones (Mitchell et al., 2013, West et al., 2010a, West et al., 2010b, West et al., 2009). Although a high volume, moderate intensity training protocol (i.e., designed to elicit muscle hypertrophy) is thought to stimulate a greater endocrine response, physiological fluctuations in ostensibly anabolic hormones have not been shown to enhance muscle protein synthesis (West et al., 2009), intramuscular anabolic signaling (Spiering et al., 2008, West et al., 2009), or resistance training-induced muscle hypertrophy (Mitchell et al., 2013).

The mammalian/mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway appears to be the primary regulator of muscle protein synthesis and growth (Drummond et al., 2009, Goodman, 2014, Hornberger et al., 2006). Phosphorylation of signaling proteins within the mTORC1 pathway regulates translation initiation, the rate-limiting step in protein synthesis (Brian et al., 2012, Welle et al., 1999). Insulin and growth factors lead to the phosphorylation of protein kinase B (Akt) which activates mTORC1 signaling by inactivating TSC2 (a primary inhibitor of mTORC1) (Inoki et al., 2002). Resistance exercise also evokes a robust activation of mTORC1 signaling in human skeletal muscle (Camera et al., 2010, Coffey et al., 2006, Farnfield et al., 2011, Koopman et al., 2006). While the exact mechanism of mechanotransduction has yet to be fully elucidated, muscle contraction has been reported to inactivate TSC2 through an Akt-independent activation of mTORC1 (Hornberger et al., 2004, Jacobs et al., 2013). Muscle contraction also activates mTORC1 through phospholipase D-

induced (Hornberger et al., 2006, Wang et al., 2006) and diacylglycerol kinase ζ-induced phosphatidic acid production (You et al., 2014). mTORC1 activation subsequently phosphorylates mTOR and further downstream targets, p70S6k (ribosomal S6 kinase 1) and RPS6 (ribosomal protein S6) (Goodman, 2014). The magnitude of p70S6k phosphorylation has been shown to be a proxy marker of myofibrillar protein synthesis rates (Burd et al., 2010, Kumar et al., 2009), and also corresponds with resistance training-induced muscle hypertrophy (Baar and Esser, 1999, Mayhew et al., 2009, Mitchell et al., 2013, Terzis et al., 2008).

It remains unclear whether different resistance exercise protocols stimulate anabolic signaling in a similar or distinct manner. Multiple set resistance exercise elicits greater intramuscular anabolic signaling than single set exercise, indicating that exercise volume influences the muscle protein signaling response to exercise (Burd et al., 2010, Terzis et al., 2010). However, low- versus high-intensity unilateral leg extensions performed to volitional fatigue have yielded inconsistent findings (Burd et al., 2010, Mitchell et al., 2012). Greater mTORC1 activation has also been demonstrated following a high volume (5 x 10 RM) versus a very low volume (15 x 1 RM) bilateral leg press protocol (Hulmi et al., 2012). Evidence appears to indicate that additional factors including muscle fiber recruitment (Gehlert et al., 2014), time-under-tension (Burd et al., 2012), and metabolic stress (Popov et al., 2015) also influence intramuscular anabolic signaling. The optimal stimulus for maximizing the anabolic response from resistance exercise remains unclear, and it is also unknown whether a hypertrophy or strength resistance exercise protocol differentially stimulates intramuscular signaling. Thus, the purpose of this study was to examine intramuscular anabolic signaling in conjunction with circulating hormone concentrations following a typical hypertrophy (HYP) and strength (STR) lower-body resistance exercise protocol in well-trained men. Additionally, electromyography

(EMG) analysis of the vastus lateralis was performed to examine muscle activation patterns between each resistance exercise protocol.

Hypotheses

1. It was hypothesized that both HYP and STR would induce significant elevations in hormones including GH and cortisol, however HYP would induce a greater endocrine response compared to STR.
2. It was hypothesized that both HYP and STR would induce significant elevations in the phosphorylation states of downstream signaling proteins including p70S6k and RPS6, however there would be no difference between HYP and STR in phosphorylation states of signaling proteins within the mTORC1 pathway.
3. It was hypothesized that the endocrine response would not be associated with the phosphorylation states of signaling proteins within the mTORC1 pathway.

Assumptions (Theoretical)

1. Participants accurately answered the medical history and activity questionnaire.
2. All participants gave maximal effort when performing maximum strength testing and each resistance exercise protocol.
3. Participants duplicated the content, quantity, and timing of their diet during the 24-hours prior to each experimental trial.
4. Participants were well-rested and refrained from all forms of moderate to intense exercise during the 72-hours prior to each experimental trial.

Assumptions (Statistical)

1. The population from which the samples are drawn is normally distributed.

Limitations

1. The methods of studying intramuscular signaling *in vivo* in humans are accompanied with inherent limitations as it requires repeated biopsy sampling of a small population of muscle fibers at few, distinctive time points following exercise, and the analyzed tissue is assumed to be representative of the entire muscle.
2. The method of studying endocrine responses *in vivo* in humans are accompanied with inherent limitations as it requires repeated blood sampling at few, distinctive time points following exercise, and the analyzed sample is assumed to be representative of the circulating concentration.

CHAPTER II

Literature Review

Introduction

Skeletal muscle is critical for disease prevention (Braith and Stewart, 2006, Yanagita and Shiotsu, 2014), mobility and quality of life (Janssen et al., 2002, Peterson and Gordon, 2011), and whole body metabolism (Baskin et al., 2015). Skeletal muscle mass is also desired by many types of athletes to confer superior athletic ability, increase body size, and improve aesthetic appearance. Muscle mass accretion is dictated by the net difference in rates of protein synthesis and protein breakdown. If protein synthesis exceeds protein degradation, an increase in skeletal muscle mass can occur, which is known as hypertrophy (Goodman et al., 2011). The rate of protein synthesis appears to be more dynamic than that of protein breakdown, suggesting that growth of skeletal muscle is primarily dictated by regulation of muscle protein synthesis (Greenhaff et al., 2008). Hypertrophy is reflected by a greater muscle cross-sectional area (CSA), which may be attributable to increases in myofibrillar volume of individual muscle fibers (Lüthi et al., 1986, Paul and Rosenthal, 2002, Toigo and Boutellier, 2006). Among the wide variety of stimuli effecting net protein synthesis and muscle mass accretion including nutritional support, cytokines, and hormones, resistance exercise is known to be a major regulator for promoting hypertrophy. Resistance exercise can stimulate an increase in protein synthesis for up to 48 hours post-exercise (Chesley et al., 1992, MacDougall et al., 1995, Phillips et al., 1997, Yarasheski et al., 1993), and repeated bouts of resistance exercise (i.e., training) can significantly increase muscle CSA and muscle fiber hypertrophy (Aagaard et al., 2001, Bell et al., 2000, McCall et al., 1996, Seynnes et al., 2007).

Resistance exercise of various intensities, volumes, and rest intervals elicit muscular hypertrophy in human models [e.g., (Aagaard et al., 2001, Bell et al., 2000, McCall et al., 1996, Seynnes et al., 2007)]. However, the optimal parameters of a resistance training program for the regulation of muscle growth remain unclear (Adams and Bamman, 2012). The stimulus of muscle contraction that occurs during differing intensities of resistance exercise results in varying biochemical responses regulating the rate of protein synthesis, known as mechanotransduction (Hornberger, 2011). At the cellular level, skeletal muscle adaptation appears to be the result of the cumulative effects of transient changes in gene expression following acute bouts of exercise (Coffey and Hawley, 2007). Thus, maximizing the resistance exercise-induced anabolic response produces the greatest potential for hypertrophic adaptation with training. The purpose of this review is to examine the physiological response to resistance exercise with particular emphasis on the endocrine system and intramuscular anabolic signaling through the mammalian/mechanistic target of rapamycin complex 1 (mTORC1) pathway.

Magnitude of Hypertrophy Following Different Resistance Exercise Protocols

Controversy exists regarding an optimal training paradigm to maximize hypertrophic adaptation. Long-term studies evaluating the effects of different resistance exercise intensities on the magnitude of muscle hypertrophy have yielded inconclusive findings. Comparisons of high intensity versus low intensity resistance training programs for up to 12-weeks in previously untrained subjects showed no differences in muscle CSA as measured by magnetic resonance imaging (MRI) (Chestnut & Docherty, 1999, Hisaeda et al., 1996, Kraemer et al., 2004, Mitchell et al., 2012, Ogasawara et al., 2013, Popov et al., 2006, Tanimoto and Ishii, 2006), computed tomography (Lamon et al., 2009, Léger et al., 2006), dual-energy x-ray absorptiometry (DEXA)

(Alegre et al., 2015), and ultrasonography (Alegre et al., 2015, Tanimoto et al., 2008). However, Holm et al. (2008) found low-intensity loads (15.5% 1-RM) to be inferior to high-intensity loads (70% 1-RM) for evoking increases in quadriceps CSA assessed via MRI. Similarly, low-intensity loads were also shown to be inferior to high-intensity loads for increasing muscle fiber hypertrophy as assessed via muscle biopsy (Campos et al., 2002, Schuenke et al., 2012). Other investigations though have indicated that low-intensity loads (40-80% 1-RM) produce greater gains in muscle fiber CSA compared to high-intensity loads (90% 1-RM) (Choi et al., 1998, Masuda et al., 1999).

Distilling the data into an optimal intensity load recommendation for enhancing muscle hypertrophy is difficult due to the inconsistency of findings. Additionally, the contradictory nature of these findings may also be attributed to the different assessment methods (i.e., MRI, computed tomography, ultrasonography, vs. muscle biopsy), experimental designs (i.e., within- vs. between-subject designs), exercised musculature (i.e., single- vs. multi-joint movements), rest intervals utilized, and protocol parameters (i.e., equated vs. non-equated volume). A number of researchers equate volume to account for the potentially greater dose-response associated with hypertrophic adaptation (Krieger, 2010). Furthermore, these studies are collectively limited as observations of early-phase hypertrophic adaptations among untrained subjects. Greater training experience has shown to attenuate post-exercise anabolic responses including muscle protein synthesis rates (Kim et al., 2005, Phillips et al., 1999, Tang et al., 2008) and intracellular anabolic signaling (Coffey et al., 2006, Gonzalez et al., 2015, Nader et al., 2014, Tang et al., 2008). Therefore, these findings cannot be generalized to a well-trained population. Schoenfeld et al. (2014) recently assessed the magnitude of hypertrophy following eight weeks of a hypertrophy-style resistance training program versus a volume-equated strength-style program in

resistance-trained men and found no significant differences in muscle thickness of the biceps brachii assessed via ultrasonography. In conjunction with training intensity, factors including muscle fiber recruitment (Gehlert et al., 2014), time-under-tension (Burd et al., 2012), and metabolic stress (Popov et al., 2015) have all been suggested to influence intramuscular anabolic signaling. Furthermore, muscular adaptation following regimented resistance training is highly variable between individuals (Bamman et al., 2007, Hubal et al., 2005, Mitchell et al., 2013, Timmons, 2011). Several factors influence muscle remodeling and the magnitude of hypertrophy including nutritional support, muscle fiber type distribution, and genetic predisposition (Adams and Bamman, 2012, Koopman et al., 2006).

The intensity of training necessary to stimulate muscle growth has been suggested to be greater than 60% of an individual's 1-RM (McDonagh and Davies, 1984, Wernbom et al., 2007), while others have suggested that maximal growth occurs at training intensities between 80-95% of 1-RM (Fry, 2004). However, recent research has shown that intensity of training as low as 30% of 1-RM can be equally as effective at stimulating muscle protein synthesis and muscle hypertrophy when performed to volitional fatigue in previously untrained men (Burd et al., 2010, Mitchell et al., 2012, Ogasawara et al., 2013). Moreover, a majority of the scientific evidence supporting a greater anabolic response following a high volume, moderate intensity training protocol (i.e., designed to elicit muscle hypertrophy) has emerged from acute investigations indicating a superior endocrine response compared to other training paradigms (Crewther et al., 2008, Hakkinen and Pakarinen, 1993, Kraemer et al., 1990, Linnamo et al., 2005, McCaulley et al., 2009, Smilios et al., 2003, Uchida et al., 2009). However, the mechanisms of exercise-mediated muscle hypertrophy have been suggested to be solely an intrinsic process which is not influenced by transient changes in circulating hormones (Mitchell et al., 2013, West et al., 2010a,

West et al., 2010b, West et al., 2009). Thus, the acute activation of intrinsically located signaling proteins and the acute elevation of muscle protein synthesis may be more reflective of the potential to increase muscle mass with resistance training (West et al., 2010a). Whether a high volume, moderate intensity training protocol activates intramuscular anabolic signaling to a greater degree than other training paradigms remains to be determined.

Role of mTORC1 in Skeletal Muscle Adaptation to Resistance Exercise

One of the most widely recognized mechanisms for controlling muscle mass involves mechanical tension (Goldberg et al., 1974). Resistance exercise initiates a multifaceted series of events converting the stimulus of muscle contraction into biochemical responses regulating the rate of protein synthesis, known as mechanotransduction (Hornberger, 2011). The mechanisms involved in converting mechanical signals into the molecular events that control muscle growth are not completely understood, however phosphorylation of intramuscular signaling molecules appear to play an important role in skeletal muscle adaptation to resistance exercise (Hornberger, 2011). Protein phosphorylation is a reversible post-translational modification causing conformational changes in protein structure accompanied by an increase or decrease in enzymatic activity (Brian et al., 2012). Skeletal muscle protein synthesis appears to be regulated by the multi-protein phosphorylation cascade, mTORC1 (Drummond et al., 2009, Goodman, 2014, Hornberger et al., 2006). Upon activation, phosphorylation of upstream (i.e., IRS1, Akt, TSC2) and downstream (i.e., mTOR, p70S6k, RPS6) effectors of mTORC1 signal to promote anabolic and inhibit catabolic cellular functions providing a biochemical mechanism for controlling processes related to cell differentiation and muscle remodeling (Figure 1) (Goodman, 2014). The protein kinase mTOR (mammalian/mechanistic target of rapamycin) serves as a

critical protein which confers signaling to p70S6k and several other downstream signaling molecules that regulate protein synthesis and skeletal muscle mass (Goodman, 2014, Hornberger, 2011).

The mTORC1 complex plays an important regulatory role during the process of skeletal muscle hypertrophy (Bodine et al., 2001). mTORC1 is involved in many cell processes including the regulation of cell size, mRNA translation, biogenesis of mitochondria and ribosomes, and autophagy (Laplante and Sabatini, 2012). At the cellular level, mTORC1 functions as a critical regulator of translation initiation, the rate-limiting step in protein synthesis (Brian et al., 2012, Goodman, 2014). It appears that the phosphorylation of signaling molecules in response to resistance exercise is a prerequisite for increasing translation initiation and muscle protein synthesis. The inhibition of mTOR via rapamycin treatment has been consistently demonstrated to blunt increases in muscle protein synthesis (Anthony et al., 2000, Gundermann et al., 2014, Kubica et al., 2005) and prevent skeletal muscle hypertrophy, which normally occurs following prolonged resistance training (Bodine et al., 2001, Hornberger et al., 2003). In humans, rapamycin treatment has been shown to block the acute exercise-induced increase in muscle protein synthesis in addition to blunting several downstream components of the mTORC1 signaling pathway including p70S6k (ribosomal S6 kinase 1) (Drummond et al., 2009, Gundermann et al., 2014). Further, the magnitude of p70S6k phosphorylation has been shown to be a proxy marker of myofibrillar protein synthesis rates (Burd et al., 2010, Kumar et al., 2009) and also corresponds with resistance training-induced muscle hypertrophy (Baar and Esser, 1999, Mayhew et al., 2009, Mitchell et al., 2013, Terzis et al., 2008). Collectively, these observations suggest that mTOR acts as the primary regulator of intracellular anabolic signaling via phosphorylation of p70S6k and several other downstream signaling molecules that regulate

protein synthesis and skeletal muscle mass (Drummond et al., 2009, Goodman, 2014, Goodman et al., 2011, Hornberger et al., 2006). Although the exact mechanism underlying increased mTORC1 activation following resistance exercise remains relatively elusive, mechanical loading has been suggested to promote mTORC1 activation by increasing the activity of Rheb (Ras homolog enriched in brain) and increasing the abundance of phosphatidic acid (PA) (Marcotte et al., 2014).

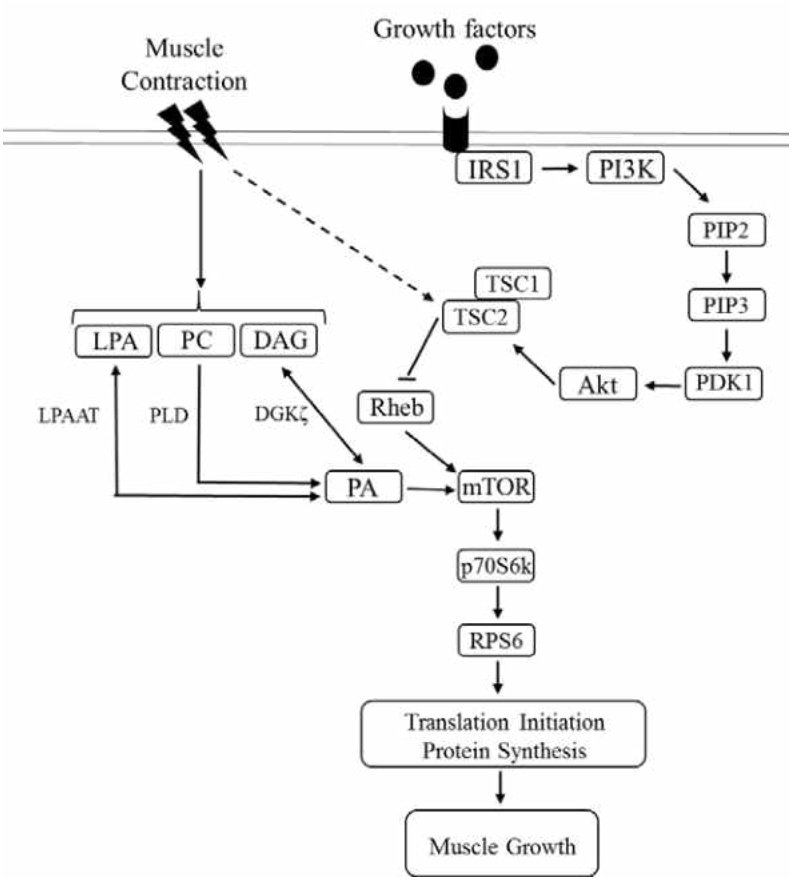

Figure 1. Simplistic overview of potential influences on mTORC1 signaling

mTORC1 activity is regulated by the modulation of tumor suppressor tuberous sclerosis complex 1/2 (TSC 1/2) activity (Laplante and Sabatini, 2012). TSC 1/2 negatively regulates mTORC1 activity by converting Rheb into its inactive GDP (guanosine diphosphate) bound state

(Sato et al., 2009). Tumor sclerosis complex 2 (TSC2) acts as the GTPase activating enzyme that keeps Rheb in the GDP-bound state (Tee et al., 2003). TSC2 phosphorylation inactivates the GTPase activating enzyme activity of TSC2 repressing the hydrolysis of Rheb-GTP (guanosine triphosphate) (Menon et al., 2014). When Rheb is in its active GTP-bound state, it translocates to the lysosome, allowing mTORC1 activity to continue (Menon et al., 2014, Sandri, 2008). Jacobs et al. (2013) showed that TSC2 localizes with Rheb at rest, however, following resistance exercise, TSC2 phosphorylation corresponds with the movement of TSC2 away from Rheb. In summary, resistance exercise-induced activation of mTORC1 requires the TSC2 complex (a negative regulator of Rheb) to be sequestered away from Rheb (Figure 2). However, it remains unclear what mediates TSC2 phosphorylation following resistance exercise (Marcotte et al., 2014). While insulin and growth factors phosphorylate TSC2 through Akt, resistance exercise-induced activation of mTORC1 appears to be Akt-independent (Hornberger et al., 2004). Several studies have shown that Akt phosphorylation either does not change (Burd et al., 2012, Coffey et al., 2006, Gonzalez et al., 2015), or decreases (Deldicque et al., 2008a, Deldicque et al., 2008b) following resistance exercise, despite downstream activation of mTORC1.

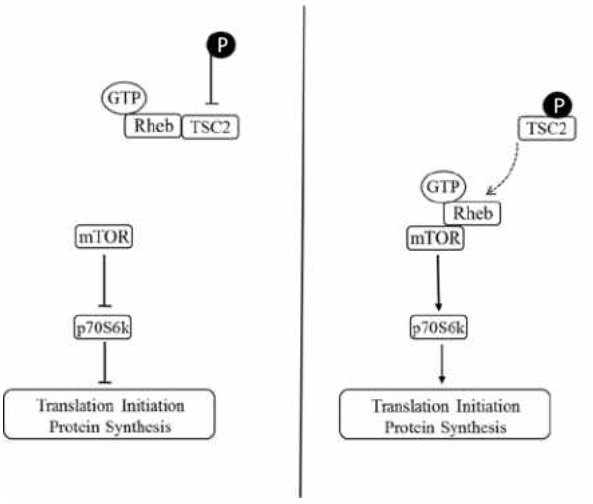

Figure 2. Simplistic overview mTORC1 activation via phosphorylation of TSC2

An additional mTORC1 activator associated with resistance exercise-induced muscle hypertrophy involves the lipid second messenger known as PA (Hornberger et al., 2006). Exogenous administration of PA or an over expression of enzymes that produce PA results in an increase in mTORC1 activation (Ávila-Flores et al., 2005, Tang et al., 2006, You et al., 2014). Similarly, limiting PA production attenuates mTORC1 activity (Hornberger et al., 2006). It has been suggested that PA mediates mTORC1 activation by competing with the FKBP12 (FK506 binding protein 12)-rapamycin complex for binding to the FKBP12-rapamycin-binding (FRB) domain of mTOR (Chen and Fang, 2002, Fang et al., 2001). PA may also promote mTORC1 activation as a primary effector of Rheb (Sun et al., 2008). GTP-bound Rheb has been shown to activate phospholipase D (PLD), an enzyme that generates PA from phosphatidylcholine (Sun et al., 2008). Additionally, PA may promote mTORC1 activation through the ERK (extracellular signal-regulated kinases) signaling pathway (Winter et al., 2010). PA can be synthesized by various classes of enzymes such as PLD, diacylglycerol kinase ζ (DGKζ), and lysophosphatidic acid acyltransferases (LPAAT) (Foster et al., 2014, Hornberger et al., 2006, Wang et al., 2006, You et al., 2014). Joy et al. (2014) found that stimulating myoblast cells with PA increased mTORC1 signaling, and trained subjects supplementing with PA significantly improved skeletal muscle hypertrophy following 8 weeks of resistance training (Joy et al., 2014). Thus, resistance exercise results in increased production of PA and evidence suggests that PA is a direct regulator of resistance exercise-induced mTORC1 signaling promoting muscle hypertrophy.

Growth Factor Activation of mTORC1

Within the mTORC1 signaling pathway, growth factors including insulin and IGF-1 bind to their respective receptors, which promote the inhibition of Rheb in an Akt-dependent pathway, resulting in increased mTORC1 activity (Menon et al., 2014). When insulin/IGF-1 bind to their receptors on the membrane, the receptor autophosphorylates creating a docking site for insulin receptor substrate 1 (IRS1) (Shepherd et al., 1998). IRS1 moves to the plasma membrane which subsequently recruits phosphatidylinositol-3 kinase (PI3K) (Shepherd et al., 1998). PI3K phosphorylates the membrane phospholipid phosphoinositol (4,5)-bisphosphate (PIP2), resulting in phosphoinositol (3,4,5)-trisphosphate (PIP3) (Alessi and Cohen, 1998). PIP3 causes the co-localization of Akt and 3-phosphoinositide-dependent protein kinase-1 (PDK-1) to the membrane resulting in Akt phosphorylation (Alessi et al., 1997). Subsequently, TSC2 is phosphorylated by Akt resulting in relocalization away from Rheb (Inoki et al., 2002, Menon et al., 2014). Akt also inhibits PRAS40 (proline-rich Akt substrate of 40 kDa), a negative regulator of mTORC1 signaling (Vander Haar et al., 2007). In summary, similar to resistance exercise-induced mTORC1 activation, insulin and growth factors activate mTORC1 via phosphorylation of TSC2 (Figure 2). However, insulin and growth factors activate mTORC1 through Akt, while resistance exercise induces an Akt-independent activation of mTORC1.

Association between Circulating Hormones, mTORC1 Signaling, and Muscle Growth

The endocrine system plays an integral role in the regulation of muscle mass. Hormonal factors influence muscle growth and development throughout life, and states of hormonal excess or deficiency alter the balance between skeletal muscle anabolism and catabolism (Solomon and Bouloux, 2006, Veldhuis et al., 2005). While the fundamental roles of hormones are imperative

for developmental growth and maintenance of skeletal muscle throughout a lifetime, the impact of physiological fluctuations (i.e., non-pharmacological-based changes) in anabolic hormones have been debated (Schroeder et al., 2013). Resting hormonal concentrations appear to be unaltered following resistance training programs of up to 24 weeks (Ahtiainen et al., 2003, McCall et al., 1999), therefore the post-exercise endocrine response has gained considerable speculation about its role in mediating increases in muscle size (Kraemer and Ratamess, 2005). Systemic elevations of circulating hormones presumably increase the likelihood of interaction with receptors located within the muscle tissue and have been speculated to contribute to muscle growth consequent to resistance training (Kraemer and Ratamess, 2005). However, in humans, elevations of the ostensibly anabolic hormones do not appear to be necessary for muscle hypertrophy (Wilkinson et al., 2006), intramuscular signaling (Spiering et al., 2008, West et al., 2009), or muscle protein synthesis (West et al., 2009), leading to the supposition that the mechanisms of muscle hypertrophy are intrinsically specific to activation of the exercised musculature (West et al., 2010a). Exogenous supra-physiological doses of testosterone have shown to significantly increase muscle protein synthesis and lean body mass (Ferrando et al., 1998, Griggs et al., 1989), especially when combined with resistance training (Bhasin et al., 1996, Bhasin et al., 2001). Additionally, administration of exogenous testosterone supplementation to restore normal physiological values in androgen deficient older men is associated with significant increases in muscle mass (Ferrando et al., 2002, Morley et al., 1993, Sih et al., 1997, Snyder et al., 1999, Tenover, 2000, Tenover, 1992). However, several studies suggest that physiological fluctuations of hormones are not required for resistance exercise-induced skeletal muscle hypertrophy (Marcotte et al., 2014). Hormones including testosterone,

growth hormone (GH), insulin, insulin-like growth factor-1 (IGF-1), and cortisol appear to affect developmental growth far more than exercise-induced muscle growth (Marcotte et al., 2014).

Overexpression of Rheb in skeletal muscle stimulates a PI3K/Akt-independent activation of mTORC1 providing evidence that a PI3K/Akt-independent activation of mTORC1 is sufficient to induce muscle hypertrophy (Goodman et al., 2010). Although it has been suggested that growth factor activation of the PI3K/Akt axis is also sufficient for skeletal muscle growth, these mechanisms do not appear to be necessary for maximizing mTORC1 activation or the hypertrophic response that occurs in response to resistance exercise (Hornberger, 2011, Marcotte et al., 2014). Resistance exercise and growth factors share the same final step in mTORC1 activation (via phosphorylation of TSC2) (Marcotte et al., 2014). Since the end result of both resistance exercise and growth factors is the movement of TSC2 away from Rheb via different upstream kinases, resistance exercise and growth factor exposure may not offer a synergistic effect.

Influence of Acute Endocrine and Intramuscular Signaling Response on Muscle Growth

Regardless of training status or age, substantial evidence indicates that resistance exercise protocols (i.e., high volumes [3-6 sets; 8-12 repetitions], moderate intensities [< 85% 1 repetition maximum (1-RM)], and short rest intervals [30 – 90 seconds]) which activate a large muscle mass appear to elicit the greatest acute elevations in testosterone and GH (Ahtiainen et al., 2005, Beaven et al., 2008, Boroujerdi and Rahimi, 2008, Crewther et al., 2008, Goto et al., 2003, Hakkinen and Pakarinen, 1993, Kraemer et al., 1995, Kraemer et al., 1999, Kraemer et al., 1990, Linnamo et al., 2005, McCaulley et al., 2009, Smilios et al., 2003, Uchida et al., 2009,

Villanueva et al., 2012). Studies investigating the acute hormonal response following different heavy resistance exercise protocols are presented in Table 1. Several studies have investigated the associations between acute exercise-induced hormone responses and changes in muscle size following a structured resistance training program (Table 2). McCall et al. (1999) found a significant correlation between acute exercise-induced GH elevations and the degree of both type I and type II muscle fiber hypertrophy following 15 weeks of resistance training in 11 recreationally-trained men. Ahtiainen et al. (2003) reported a significant correlation between changes in the acute testosterone response and the degree of muscle hypertrophy following 21 weeks of resistance training in 16 men (8 strength athletes and 8 non-athletes). However, both of these studies had a relatively small number of subjects, thereby limiting the ability to draw meaningful conclusions. In a more recent study examining a larger cohort of 56 untrained men, West and Phillips (2012) reported that the acute systemic hormonal response of GH and cortisol were weakly correlated with resistance training-induced changes in muscle fiber CSA ($r=0.28$-0.36; $p's<0.05$) explaining 8% and 12% of the variance, respectively. Although cortisol, a catabolic hormone, weakly correlated with changes in lean body mass ($r=0.29$; $p<0.05$), no significant correlations were observed between GH, testosterone, and IGF-1 and changes in lean body mass (West and Phillips, 2012). Additionally, the variability within the gains of muscle hypertrophy seen in 'high responders' and 'low responders' could not be explained by the acute hormone response (West and Phillips, 2012). Furthermore, Wilkinson et al. (2006) observed significant gains in hypertrophy in the absence of systemic changes in GH, testosterone, and IGF-1 (Wilkinson et al., 2006).

Table 1. Studies investigating the acute hormonal response following different heavy resistance exercise protocols

	Participants	Cross-over design?	Design	Protocols	Hormones Measured	Results
Beaven et al. 2008	15 trained men	Yes	Full-body	1. 4x10; 70% 1RM (2 min rest) 2. 3x5; 85% 1RM (3 min rest) 3. 5x15; 55% 1RM (1 min rest) 4. 3x5; 40% 1RM (3 min rest)	Testosterone Cortisol (salivary)	Protocols 1, 2, & 4 elicited significant decreases in cortisol following exercise. No significant differences in testosterone between protocols.
Crewther et al. 2008	11 recreationally trained men	Yes	Lower-body	1. 8x6; 45% 1RM (3 min rest) 2. 10x10; 75% 1RM (2 min rest) 3. 6x4; 88% (4 min rest)	Testosterone Cortisol (salivary)	Only protocol 2 elicited significant increases in testosterone and cortisol concentration following exercise.
Hakkinen & Pakarinen, 1993	10 trained men	Yes	Lower-body	1. 10x10; 70% 1RM (3 min rest) 2. 20x1; 100% 1RM (3 min rest)	Testosterone Cortisol GH	Protocol 1 elicited significant increases in testosterone, cortisol and growth hormone following exercise. Protocol 2 elicited significant increase in growth hormone following exercise.
Kraemer et al. 1990	9 recreationally trained men	Yes	Full-body	1. 3x10; 10RM (1 min rest) 2. 5x5; 5RM (3 min rest)	Testosterone Cortisol GH	Protocol 1 elicited significantly greater growth hormone following exercise. Both protocols significantly increased testosterone however not at the same magnitude and duration (no difference in AUC). Both protocols showed only random acute increases in cortisol.
Linnamo et al. 2005	8 recreationally active men	Yes	Full-body	1. 5x10; 10RM (2 min rest) 2. 5x10; 70% 10RM (2 min rest)	Testosterone GH	Only protocol 1 elicited significant increases in growth hormone and testosterone following exercise.
McCaulley et al. 2009	10 trained men	Yes	Lower-body	1. 4x10; 75% 1RM (1.5 min rest) 2. 11x3; 90% 1RM (5 min rest)	Testosterone Cortisol	Only protocol 1 elicited a significant increase in testosterone and cortisol following exercise.
Raastad et al. 2000	7 trained men	Yes	Lower-body	1. 3x3; 3RM (6 min rest) (Squat & Front Squat) & 3x6; 6RM (4 min rest) (Leg Extension) 2. 3x3; 70% 3RM (6 min rest) (Squat & Front Squat) & 3x6; 76% 6RM (4 min rest) (Leg Extension)	Testosterone Cortisol GH IGF-1 Insulin	Protocol 1 elicited significantly greater testosterone AUC as compared to Protocol 2. Protocol 1 elicited significantly greater cortisol AUC as compared to Protocol 2. No significant difference in growth hormone, IGF-1, or insulin between protocols.
Smilios et al. 2002	11 trained men	Yes	Full-body	1. *x5; 88% 1RM (3 min rest) 2. *x10; 75% 1RM (2 min rest) 3. *x15; 60% 1RM (1 min rest) (*Each was performed using 2, 4, & 6 sets)	Testosterone Cortisol GH	Protocol 2 and 3 elicited significantly greater growth hormone and cortisol following exercise. No significant differences were observed for testosterone for any protocol.
Uchida et al. 2009	27 trained men	No	Upper-body	1. 4x~20; 50% 1RM (2 min rest) 2. 5x~11; 75% 1RM (2 min rest) 3. 10x~4; 90% 1RM (2 min rest) 4. *8x~4;110% 1RM (2 min rest) (*Eccentric only)	Testosterone Cortisol	Protocol 2 elicited significantly greater cortisol following exercise. No differences in testosterone following each protocol.

Mitchell et al. (2013) examined post-exercise changes in anabolic hormone concentrations (testosterone, GH, and IGF-1) and intramuscular signaling to examine the association with muscle fiber hypertrophy following 16 weeks of training. Post-exercise increases in these circulating hormones did not appear to be related to training-induced hypertrophy, whereas acute increases in p70S6k phosphorylation and androgen receptor protein content were highly associated ($r=0.54-0.60$, $p's<0.05$) with resistance training-induced hypertrophy (Mitchell et al., 2013). The magnitude of p70S6k phosphorylation has shown to be associated with myofibrillar protein synthesis rates ($r=0.31-0.34$; $p's<0.05$) (Burd et al., 2010, Kumar et al., 2009), and its acute phosphorylation following resistance exercise has been reported to correlate with muscle hypertrophy following training in both rodents ($r=0.998$; $p<0.05$) (Baar and Esser, 1999) and untrained men ($r=0.53-0.89$; $p's<0.05$) (Mayhew et al., 2009, Terzis et al., 2008). However, not all studies have found such a relationship (Mitchell et al., 2012). Nevertheless, correlations between transient changes in muscular and systemic markers following acute bouts of exercise and training-induced muscle hypertrophy are not evidence of a causative role for cellular adaptations in the trained muscle (Mitchell et al., 2014).

Table 2. Indirect research investigating the hormonal influence on muscle hypertrophy

	Participants	Study length	Results
McCall et al. 1999	11 recreationally trained men	12 wks	Significant correlation between acute GH elevation and the degree of type I (r=0.70) and type II (r=0.71) muscle fiber hypertrophy.
Ahtiainen et al. 2003	8 physically active men; 8 strength athletes	21 wks	Significant correlation between acute testosterone elevation and change in muscle CSA (r=0.76).
West & Phillips 2012	56 recreationally active men	12 wks	Significant correlation between acute GH elevation and the degree of type I fiber hypertrophy (r=0.36). Significant correlation between acute cortisol elevation and the degree of type II fiber hypertrophy (r=0.35) and changes in lean body mass (r=0.29).
Mitchell et al. 2013	23 recreationally active men	16 wks	No correlation between acute testosterone, GH, or IGF-1 elevation and muscle hypertrophy.

Studies have also been conducted to directly examine whether exercise-induced elevations in anabolic hormones are necessary for, or could enhance intramuscular anabolic signaling and growth. To investigate the influence of varying concentrations of circulating hormones consequent to resistance exercise on intramuscular anabolic signaling, Spiering et al. (2008) compared untrained men performing a lower-body resistance exercise protocol designed to maintain basal hormone concentrations preceded by rest or a bout of high-volume upper-body resistance exercise designed to elicit a large increase in circulating hormones. The trial eliciting a high hormonal response did not enhance markers of mTORC1 signaling in the vastus lateralis compared to the trial that did not elicit an increase in hormonal concentrations. West et al. (2009) examined untrained men performing an elbow flexor resistance exercise protocol designed to maintain basal hormone concentrations followed by rest or a bout of high-volume lower-body resistance exercise designed to elicit a large increase in circulating hormones. The

trial eliciting a transient increase in endogenous hormones did not enhance anabolic signaling or muscle protein synthesis in the biceps brachii, despite a threefold increase from resting concentrations in testosterone following resistance exercise (West et al., 2009). In a subsequent study by the same research team, untrained men were compared performing a 15-week resistance training program with the elbow flexors followed by rest or a bout of high-volume lower-body resistance exercise designed to elicit a large increase in circulating hormones. Results showed no difference between conditions in training-induced muscle hypertrophy of the biceps brachii (West et al., 2010b). Other investigators though provide conflicting evidence. Rønnestad and colleagues (2011) examined untrained men performing bouts of high-volume lower-body resistance exercise prior to elbow flexor exercises for 11 weeks and demonstrated that the increased concentrations of serum testosterone and growth hormone occurring prior to performing the elbow flexor exercise yielded greater increase in CSA of the arm flexors compared to elbow flexor exercises performed in isolation. The authors hypothesized that their findings may be related to the order of the exercises. This contrasts with others who suggest that changes in the post-exercise circulating concentrations of testosterone, growth hormone, and IGF-1, and the subsequent interaction within skeletal muscle is not influenced by the order of the resistance exercises (West et al., 2012). Evidence to date appears to suggest that exposing exercising muscle to an acute elevation in endogenous hormones does not enhance intramuscular signaling, and limited research has been able to support the potential benefits of transient increases in endogenous hormones and their role in enhancing muscle growth.

Table 3. Direct research investigating the hormonal influence on muscle hypertrophy

	Participants	Study length	Results
Acute			
Spiering et al. 2008	7 physically active men	2 trials	No additive effect from elevated circulating hormones on intramuscular anabolic signaling.
West et al. 2009	8 recreationally active men	2 trials	No additive effect from elevated circulating hormones on intramuscular anabolic signaling or muscle protein synthesis.
Prolonged			
West et al. 2010	12 untrained men	15 wks	No additive effect from elevated circulating hormones on whole muscle, type I, or type II CSA.
Ronnestad et al. 2011	11 untrained men	11 wks	Significant increase in muscle CSA as a result of elevated circulating hormones.

Effect of Resistance Exercise Variables on Activation of mTORC1

Resistance exercise evokes a robust activation of mTORC1 signaling in untrained and recreationally active men in both fed (Apró and Blomstrand, 2010, Deldicque et al., 2010, Farnfield et al., 2009, Hulmi et al., 2009, Karlsson et al., 2004) and fasted states (Dreyer et al., 2006, Dreyer et al., 2010, Drummond et al., 2009, Roschel et al., 2011, Terzis et al., 2008). Resistance exercise-induced mTORC1 activation has also been observed in experienced, resistance-trained men (Areta et al., 2013, Glover et al., 2008, Gonzalez et al., 2015), yet the optimal resistance exercise parameters for maximizing the anabolic response remain unclear.

Multiple set resistance exercise elicits greater intramuscular anabolic signaling than single set exercise, indicating that exercise volume influences the muscle protein signaling response to exercise (Burd et al., 2010, Terzis et al., 2010). However, low- versus high-intensity unilateral leg extensions performed to volitional fatigue have yielded inconclusive results (Burd et al., 2010, Mitchell et al., 2012). Burd et al. (2010b) found low intensity loads (30% RM) to be

more effective than high intensity loads (90% RM) in recreationally active men for inducing mTORC1 signaling at four hours post-exercise (Burd et al., 2010), while Mitchell et al. (2012) found high intensity loads (80% RM) to be more effective than lower intensity loads (30% RM) for inducing mTORC1 signaling in untrained men at one hour post-exercise. Nevertheless, no difference in the magnitude of hypertrophy was observed following 10 weeks of training at the different intensities to volitional fatigue (Mitchell et al., 2012). mTORC1 signaling has also shown to be greater following a high volume (5 x 10 RM) compared to lower volume, but higher intensity (15 x 1 RM) bilateral leg press exercise (Hulmi et al., 2012). The lack of any clear benefit from training program design is likely related to additional evidence that suggests that the intramuscular anabolic signaling response is influenced by a host of other factors including muscle fiber recruitment (Gehlert et al., 2014), time-under-tension (Burd et al., 2012), and metabolic stress (Popov et al., 2015).

Exercise-induced metabolic stress may also play a role in acute activation of mTORC1 signaling. Metabolic stress results from exercise that primarily relies on anaerobic glycolysis as it major energy provider. Lactate directly affects muscle cells *in vitro* by increasing satellite cell activity as well as mTOR and p70S6k phosphorylation (Oishi et al., 2015). Elevations in blood lactate have also been demonstrated to be weakly associated ($r=0.38$; $p<0.05$) with intramuscular anabolic signaling following resistance exercise in trained men (Popov et al., 2015). Lactate production may contribute to increased mTORC1 signaling (Gundermann et al., 2012), however the mechanisms by which metabolic stress influences anabolic signaling are not fully elucidated and warrant further investigation.

Acute activation of mTORC1 signaling may also be influenced by mode of contraction. Eccentric only resistance exercise has been suggested to provide a stronger anabolic stimulus than concentric only resistance exercise (Cuthbertson et al., 2006, Eliasson et al., 2006, Moore et al., 2005, Rahbek et al., 2014). Eccentric contractions have been demonstrated to produce a more rapid rise in myofibrillar muscle protein synthesis than concentric only contractions (Cuthbertson et al., 2006, Moore et al., 2005). Maximal eccentric contractions have also shown to significantly activate p70S6k and RPS6 up to 2 hours into recovery, while maximal concentric and submaximal eccentric contractions failed to induce changes in Akt, mTOR, p70S6k, or RPS6 phosphorylation status (Eliasson et al., 2006). Similarly, Rahbek (2014) demonstrated that maximal eccentric contractions triggered a greater acute anabolic signaling response compared to concentric contractions. However, no differences were noted in myofibrillar protein synthesis rates or exercise-induced hypertrophy following 12 weeks of high-volume resistance training (Rahbek et al., 2014). Increases in muscle size following 9 weeks of unilateral resistance training has also shown to be unrelated to muscle contraction type when matched for both exercise intensity and total external work (Moore et al., 2012). Thus, eccentric contractions, which emphasize greater tension and stretching of the muscle, may yield a greater acute anabolic response, yet whether it translates into greater hypertrophy with training remains questionable.

It is important to note that the anabolic response following resistance exercise appears to be highly variable between individuals (Bamman et al., 2007, Coffey et al., 2006, Davidsen et al., 2011, Hubal et al., 2005). A number of factors influence the muscle remodeling process following resistance exercise including nutritional intake and genetic predisposition (Marcotte et al., 2014, Phillips, 2014). Nevertheless, several studies have suggested that training status can also impact resistance exercise-induced intramuscular anabolic signaling. Coffey et al. (2006)

reported that prior training history blunts the anabolic signaling responses involved in the adaptation to resistance exercise. Chronic resistance training in rats also attenuates p70S6k phosphorylation following an acute exercise bout (Ogasawara et al., 2013). Similarly, in humans, the duration of protein synthesis following a bout of resistance exercise was reduced following eight weeks of resistance training (Tang et al., 2008). Additionally, our lab recently demonstrated that highly trained, stronger individuals have an attenuated acute anabolic response following a high-volume resistance exercise protocol (Gonzalez et al., 2015). Thus, a potential lower adaptive ability among highly trained individuals may, in part, account for the diminished hypertrophic adaptation among athletes with increased training experience (Häkkinen et al., 1987, Hoffman et al., 1991).

Conclusion

Skeletal muscle adaptation appears to be the result of the cumulative effects of transient changes in gene expression following acute bouts of exercise (Coffey and Hawley, 2007). The mechanisms involved in converting mechanical signals into the molecular events that control muscle growth are not completely understood, however skeletal muscle protein synthesis appears to be regulated by the multi-protein phosphorylation cascade, mTORC1. Thus, maximizing resistance exercise-induced mTORC1 signaling should yield the greatest potential for hypertrophic adaptation with training (Baar and Esser, 1999, Mayhew et al., 2009, Mitchell et al., 2013, Terzis et al., 2008). While a majority of the research to date shows that mTORC1 signaling is not influence by post-exercise transient changes in circulating hormones (Mitchell et al., 2013, West et al., 2010a, West et al., 2010b, West et al., 2009), resistance exercise-induced mTORC1 activation appears to be a very multifaceted process which is influenced by a number

of factors. It appears that resistance exercise protocols that maximize muscle fiber recruitment, time-under-tension, and metabolic stress will contribute to maximizing intramuscular anabolic signaling, however the optimal resistance exercise parameters for maximizing the anabolic response remain unclear, and it is unknown whether different resistance exercise paradigms differentially stimulate intramuscular anabolic signaling.

CHAPTER III

Methods

Participants

Ten resistance-trained men (24.7 ± 3.4 y; 90.1 ± 11.3 kg; 176.0 ± 4.9 cm; 14.1 ± 6.1% body fat) were recruited to participate in this randomized, cross-over design research study. Strict recruitment criteria were implemented to increase homogeneity of the sample. Inclusion criteria required participants to be between the ages of 18 and 35 years, a minimum of one year of resistance training experience, and the ability to squat a weight equivalent to their body mass. Participants had 6.7 ± 4.6 years of resistance training experience with an average maximum barbell back squat of 172.7 ± 25.2 kg. All participants were free of any physical limitations that may affect performance. Additionally, all participants were free of any medications and performance enhancing drugs, as determined by a health and activity questionnaire. Following an explanation of all procedures, risks, and benefits, each participant provided his informed consent prior to participation in this study. The research protocol was approved by the New England Institutional Review Board prior to participant enrollment.

Maximal Strength Testing

Prior to experimental trials, participants reported to the Human Performance Laboratory (HPL) to establish maximal strength (1-RM) on all lifts involved in the exercise protocol. Prior to maximal strength testing, participants performed a standardized warm-up consisting of five minutes on a cycle ergometer against a light resistance, 10 body weight squats, 10 body weight walking lunges, 10 dynamic walking hamstring stretches, and 10 dynamic walking quadriceps stretches. The 1-RM test for the barbell back squat and leg press were performed using methods

previously described by Hoffman (2006). Briefly, each participant performed two warm-up sets using a resistance of approximately 40-60% and 60-80% of his perceived maximum, respectively. For each exercise, 3-4 subsequent trials were performed to determine the 1-RM. A 3-5 minute rest period was provided between each trial. For all other exercises, the 1-RM was assessed using a prediction formula based on the number of repetitions performed to fatigue using a given weight (Brzycki, 1993). Trials not meeting the range of motion criteria for each exercise or where proper technique was not used were discarded.

Experimental Trials

On the morning of each trial, participants reported to the HPL after a 10-hour overnight fast and having refrained from all forms of moderate to vigorous exercise for the previous 72 hours. Experimental trials were performed in a balanced, randomized order, and each experimental trial was separated by a minimum of one week to ensure adequate recovery. Each participant performed experimental trials at the same time of day to avoid diurnal variations. Participants provided a urine sample upon arrival to the HPL for analysis of urine specific gravity (USG) by refractometry to ensure an adequate hydration status (USG $\leq$ 1.020 defined as euhydration).

During each experimental trial, participants performed the standardized warm-up routine described above, followed by a lower-body resistance exercise protocol. Table 4 depicts the hypertrophy (HYP) and strength (STR) resistance exercise protocols. The HYP protocol utilized a load of 70% 1-RM for sets of 10-12 repetitions with a 1-minute rest period length between sets and exercises. The STR protocol utilized a load of 90% 1-RM for sets of 3-5 repetitions with a 3-minute rest period length between sets and exercises. Both protocols included six sets of

barbell back squats and four sets of bilateral leg press, bilateral hamstring curls, bilateral leg extensions, and seated calf raises. During each protocol, participants were verbally encouraged to complete each set. If the participant was unable to complete the desired number of repetitions, spotters provided assistance until the participant completed the remaining repetitions. Subsequently, the load for the next set was adjusted so that participants were able to perform the specific number of repetitions for each set.

Table 4. Resistance exercise protocols

	Hypetrophy Protocol (HYP)			Strength Protocol (STR)		
Exercise Order	**Sets x Repetitions**	**Intensity**	**Rest Time**	**Sets x Repetitions**	**Intensity**	**Rest Time**
1. Barbell Back Squats	6 x 10-12			6 x 3-5		
2. Bilateral Leg Press	4 x 10-12			4 x 3-5		
3. Bilateral Hamstring Curls	4 x 10-12	70% 1-RM	1 minute	4 x 3-5	90% 1-RM	3 minutes
4. Bilateral Leg Extensions	4 x 10-12			4 x 3-5		
5. Seated Calf Raises	4 x 10-12			4 x 3-5		

Following each resistance exercise protocol, participants remained in the laboratory for all post-exercise assessments. Blood samples were obtained at six time points over the course of the study: baseline (BL), immediately post-exercise (IP), 30 minutes post-exercise (30P), 1 hour post-exercise (1H), 2 hours post-exercise (2H), and 5 hours post-exercise (5H). Fine needle muscle biopsies were completed at BL, 1H, and 5H.

To control for diet, participants were provided a standardized low protein, low carbohydrate breakfast (7 grams protein; 3 grams carbohydrate; 13 grams fat) following BL assessments. Immediately following IP blood sampling, participants were also provided a flavored drink (355 mL, 0 grams protein, 2.5 grams carbohydrates, 0 gram fat). Participants were permitted to drink water ad libitum during experimental trials, and volume of water consumption was recorded.

Muscle Activation

To investigate muscle activation, EMG analysis of the vastus lateralis of the non-dominant leg was assessed during every repetition for the multi-joint exercises (barbell back squat and bilateral leg press) during each resistance exercise protocol. A bipolar surface electrode arrangement (Quinton, Milwaukee, WI, USA) was placed at two-thirds of the line between the anterior superior iliac spine and superior lateral aspect of the patella, with the reference electrode placed over the tibial tuberosity. The skin beneath the electrodes was shaved, abraded, and cleaned with alcohol to keep inter-electrode impedance below 5,000 ohms. EMG signals were obtained with a differential amplifier (MP150 BIOPAC Systems, Inc., Santa Barbara, CA, USA) sampled at 1,000 Hz. Data was sent in real time to a computer via Bluetooth and recorded for later analysis. To eliminate variance, all EMG preparation and electrode attachment was conducted by a single technician, and the foot placement and anatomical positioning of participants were recorded and kept consistent during each experimental trial. EMG signals were band-pass filtered from 10 Hz to 500 Hz and expressed as root mean square amplitude values by software (AcqKnowledge v4.2, BIOPAC Systems, Inc., Santa Barbara, CA). The average root mean square (RMS; microvolts) was calculated for each repetition by the software. For the purpose of normalizing EMG analysis, maximal voluntary isometric contraction (MVIC) of the bilateral leg extension was obtained during the maximal strength testing visit (Burden, 2010). All RMS values were normalized as a percent of MVIC. Test-retest reliability for the RMS of MVIC of the bilateral leg extension in our laboratory has been established (ICC=0.88). MVIC was conducted in a bilateral leg extension machine with the knees flexed at 105.6 ± 4.2° and hands grasping the handlebars for stability. Participants were asked to extend the knee exerting maximal force against an immoveable resistance for 5 seconds.

The highest MVIC EMG value was used as the reference with which to normalize EMG signals. EMG data were reported as percentage of MVIC.

Blood Measurements

During each experimental trial, blood samples were obtained using a Teflon cannula placed in a superficial forearm vein using a three-way stopcock with a male luer lock adapter and plastic syringe. The cannula was maintained patent using an isotonic saline solution (Becton Dickinson, Franklin Lakes, NJ, USA). BL blood samples were obtained following a 15-minute equilibration period. IP blood samples were taken within one minute of exercise cessation. Participants were instructed to lie in a supine position for 15 minutes prior to 30P, 1H, 2H, and 5H blood draws.

All blood samples were collected into three 6 ml Vacutainer® tubes. Blood samples were drawn into either plain, sodium heparin, or K_2EDTA treated tubes. A small aliquot of whole blood was removed and used for determination of hematocrit and hemoglobin concentrations. The blood in the plain tube was allowed to clot at room temperature for 30 minutes and subsequently centrifuged at 3,000×g for 15 minutes along with the remaining whole blood from the other tubes. The resulting serum and plasma was placed into separate micro-centrifuge tubes and frozen at −80°C for later analysis.

Biochemical Analysis

Blood lactate concentrations were analyzed from plasma using an automated analyzer (Analox GM7 enzymatic metabolite analyzer, Analox instruments USA, Lunenburg, MA, USA). Hematocrit concentrations were analyzed from whole blood via microcentrifugation (CritSpin,

Westwood, MA, USA) and microcapillary technique. Hemoglobin concentrations were analyzed from whole blood using an automated analyzer (HemoCue, Cypress, CA, USA). Plasma volume shifts were calculated using the formula established by Dill & Costill (1974). To eliminate inter-assay variance, all samples were analyzed in duplicate by a single technician. Coefficient of variation for each assay was 1.4% for blood lactate; 0.4% for hematocrit; and 0.6% for hemoglobin.

Circulating concentrations of IGF-1, insulin, testosterone, GH, and cortisol were assessed via enzyme-linked immunosorbent assays (ELISA) and a spectrophotometer (BioTek Eon, Winooski, VT, USA) using commercially available kits. Myoglobin concentrations were determined via ELISA (Calbiotech, Spring Valley, CA, USA) and a spectrophotometer. Lactate dehydrogenase (LDH) concentrations were determined via ELISA (Sigma-Aldrich, St. Louis, MO, USA) and a spectrophotometer. To eliminate inter-assay variance, all samples for each assay were thawed once and analyzed in duplicate in the same assay run by a single technician. Coefficient of variation for each assay was 6.5% for IGF-1; 8.1% for insulin; 4.8% for testosterone; 4.9% for GH; 5.3% for cortisol; 4.1% for myoglobin; and 4.8% for LDH.

Fine Needle Muscle Biopsy Procedure

Fine needle muscle biopsies were performed on the vastus lateralis muscle of the participant's dominant leg using a spring-loaded, reusable instrument with 14-gauge disposable needles and a coaxial introducer (Argon Medical Devices Inc., Plano, TX, USA). Following local anesthesia with 2 mL of 1% lidocaine applied into the subcutaneous tissue, a small incision to the skin was made and an insertion cannula was placed perpendicular to the muscle until the fascia was pierced. The biopsy needle was inserted through the cannula and a muscle sample

was obtained by the activation of a trigger button, which unloaded the spring and activated the needle to collect a muscle sample. Multiple biopsy passes at each time point were made with the cannula in place, thus avoiding repeated skin punctures. Each muscle sample was removed from the biopsy needle using a sterile scalpel and was subsequently placed in a cryotube, rapidly frozen in liquid nitrogen, and stored at -80˚C. All muscle biopsies were performed by a licensed physician.

Intramuscular Anabolic Signaling Analysis

Tissue samples were thawed and kept on ice for preparation and homogenization. A lysis buffer with protease inhibitor (EMD Millipore, Billerica, MA, USA) was added to each sample at a rate of 500 µl per 10 mg of tissue. Samples were homogenized using a Teflon pestle and sonication (Branson, Danbury, CT, USA). Tissue samples were then placed on a plate shaker (Thermo Fisher Scientific Inc., Waltham, MA, USA) for 10 minutes at 4˚C and subsequently centrifuged at 10,000×g for 5 minutes. The supernatant was aspirated and used for analysis.

Multiplex enzyme-linked immunosorbent assay (ELISA) was used to quantify the phosphorylation status of proteins specific to the mTORC1 signaling pathway using MAGPIX® (Luminex, Austin, TX, USA) and a multiplex signaling assay kit (EMD Millipore, Billerica, MA, USA) according to manufacturer's guidelines. Multiplex ELISA has been validated (Hwang, 2011) and previously used to determine the phosphorylation status of proteins in the mTORC1 signaling pathway (Gonzalez et al., 2015, Sharma et al., 2012, Sharma et al., 2012). Samples were analyzed for phosphorylation of insulin-like growth factor-1 receptor (IGF1R) at Tyr 1135/1136, insulin receptor (IR) at Tyr 1162/1163, insulin receptor substrate 1 (IRS1) at Ser 636, tumor sclerosis complex 2 (TSC2) at Ser 939, protein kinase B (Akt) at Ser 473, mTOR at

Ser 2448, p70S6k at Thr 412, and ribosomal protein S6 (RPS6) at Ser 235/236. Total protein quantification was conducted using a detergent compatible (DC) protein assay kit (Bio-Rad, Hercules, CA, USA). Homogenized samples were diluted prior to being loaded and results are reported as fluorescence intensity expressed relative to total protein content. To eliminate inter-assay variance, all tissue samples were thawed once and analyzed in duplicate in the same assay run by a single technician. The average coefficient of variation for phospho-protein analysis was 8.4%.

Dietary Logs

Participants were instructed to maintain their normal dietary intake leading up to experiment trials. Participants were then instructed to record as accurately as possible everything they consumed during the 24 hours prior to the first experimental trial. For the following experimental trial, participants were required to duplicate the content, quantity, and timing of their daily diet during the 24 hours prior. Participants were instructed not to eat or drink (except water) within 10 hours of reporting to the HPL for experimental trials.

Statistical Analysis

Prior to statistical procedures, all data was assessed for normal distribution, homogeneity of variance, and sphericity. If the assumption of sphericity was violated, a Greenhouse-Geisser correction was applied. Biochemical changes were analyzed using a two factor (trial x time) analysis of variance (ANOVA) with repeated measures on time. In the event of a significant F ratio, LSD post-hoc tests were used for pairwise comparisons. Area under the curve (AUC) was also calculated for biochemical measures using a standard trapezoidal technique. AUC analysis

was analyzed via paired samples t-tests. Percent changes from baseline measures were calculated for anabolic signaling, and Pearson's product-moment correlation was used to examine the association between circulating hormones and intramuscular anabolic signaling. Mean muscle activation of each set of squat and leg press were analyzed using a two factor (trial x set) ANOVA. For effect size, partial eta squared statistics were calculated, and according to Green et al. (2000), 0.01, 0.06, and 0.14 were interpreted as small, medium, and large effect sizes, respectively. Significance was accepted at an alpha level of $p \leq 0.05$ and all data are reported as mean ± SD.

CHAPTER IV

Results

Resistance Exercise Protocol

All participants were adequately hydrated (USG ≤ 1.020) prior to each trial, and no significant differences were noted between trials for baseline USG (p=0.98). No significant differences were noted for water consumption during each protocol (p=0.34). As anticipated, significant differences between trials were noted for workout volume (p=0.01). Workout volume (sets x load x reps) was significantly greater for HYP (45300.0 ± 13919.8 kg) compared to STR (33633.5 ± 5661.9 kg).

Muscle Activation

Analysis of muscle activation during the squat exercise revealed no significant effect across the six sets (F=3.0; p=0.07; η^2=0.16), and no significant interactions were noted (F=1.1; p=0.36; η^2=0.07) (Figure 3A). In addition, no significant differences were noted in muscle activation during each of the four sets of leg press (F=2.3; p=0.09; η^2=0.12), and no significant interactions were noted (F=1.3; p=0.27; η^2=0.07) (Figure 3B).

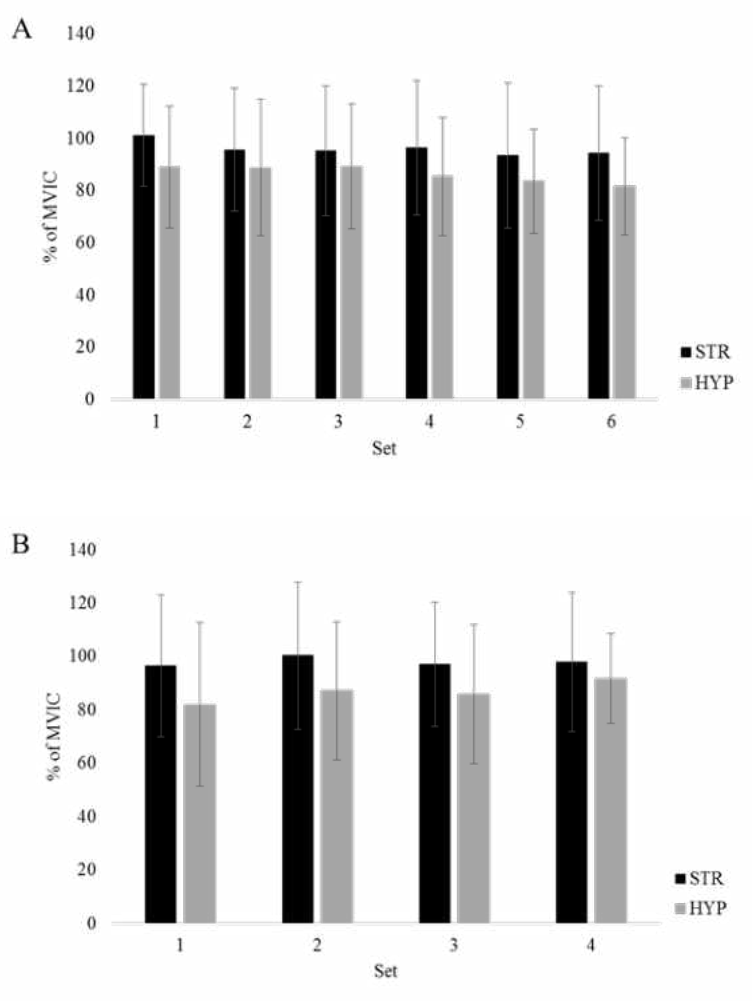

Figure 3. A. Muscle activation during the squat exercise. B. Muscle activation during the leg press exercise. STR=Strength protocol; HYP=Hypertrophy protocol; MVIC=Maximal voluntary isometric contraction. All data are reported as means ± SD.

Biochemical Analysis

Myoglobin

Significant time effects were noted for myoglobin ($F=46.7$; $p=0.0001$; $\eta^2=0.72$). Myoglobin concentrations were significantly elevated from BL at IP, 30P, and 1H ($p<0.001$). Significant interactions were also noted for myoglobin ($F=5.8$; $p=0.02$; $\eta^2=0.25$). Myoglobin concentrations were significantly greater during STR compared to HYP at both IP ($p=0.02$) and

30P (p=0.01) (Table 5). AUC (BL-1H) analysis indicated myoglobin concentrations during STR were significantly greater than HYP (p=0.02).

Table 5. Myoglobin concentration following resistance exercise. Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post. All data are reported as means ± SD. * = Significant difference between STR and HYP ($p \leq 0.05$). # = Significant difference from BL ($p \leq 0.05$).

	BL	**IP**	**30P**	**1H**
		* #	* #	#
STR	29.3 ± 8.6	164.3 ± 93.5	201.6 ± 106.8	199.9 ± 104.3
HYP	35.0 ± 13.4	91.9 ± 26.1	104.9 ± 34.5	141.9 ± 51.0

Lactate Dehydrogenase

Significant time effects were noted for LDH ($F=13.1$; $p=0.0001$; $\eta^2=0.42$). LDH concentrations were significantly elevated from BL at all time-points ($p<0.001$). No significant interactions were noted for LDH ($F=0.8$; $p=0.53$; $\eta^2=0.04$). AUC analysis indicated LDH concentrations during STR were significantly greater than HYP (p=0.01) (Figure 4).

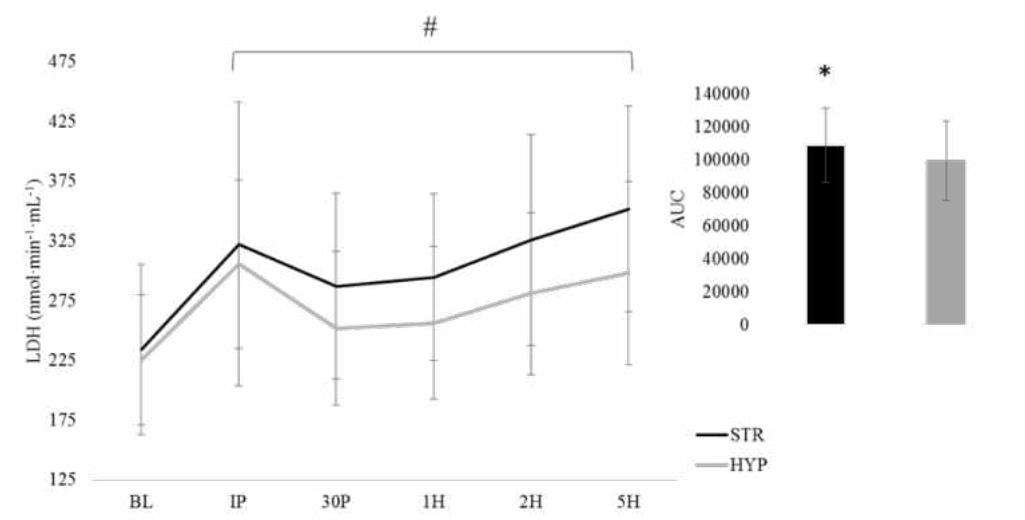

Figure 4. Lactate dehydrogenase (LDH) concentration following resistance exercise. Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Inset: area under the curve (AUC). All data are reported as means ± SD. * = Significant difference between STR and HYP ($p \leq 0.05$). # = Significant difference from BL ($p \leq 0.05$).

Lactate

Significant time effects were noted for lactate ($F=154.3$; $p=0.0001$; $\eta^2=0.90$). Lactate concentrations were significantly elevated from BL at all time-points ($p<0.001$). Significant interactions were also noted for lactate ($F=27.5$; $p=0.0001$; $\eta^2=0.60$). Lactate concentrations were significantly greater during HYP compared to STR at IP ($p=0.0001$), 30P ($p=0.0001$), and 1H ($p=0.001$). AUC analysis indicated that lactate concentrations during HYP were significantly greater than STR ($p=0.003$) (Figure 5).

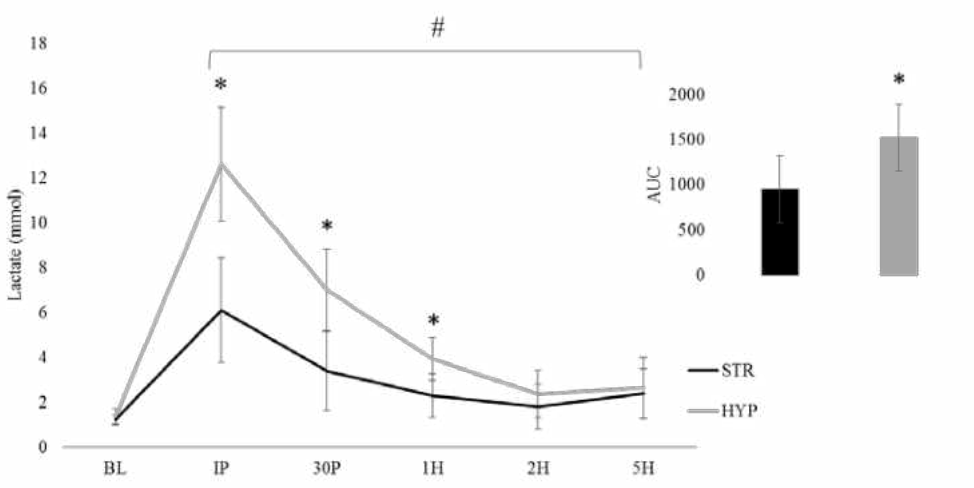

Figure 5. Lactate concentration following resistance exercise. Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Inset: area under the curve (AUC). All data are reported as means ± SD. * = Significant difference between STR and HYP ($p \leq 0.05$). # = Significant difference from BL ($p \leq 0.05$).

Hormonal Analysis

Insulin-like Growth Factor-1

Significant time effects were observed for IGF-1 ($F=5.2$; $p=0.0001$; $\eta^2=0.23$). IGF-1 concentrations were significantly elevated from BL at IP ($p=0.0001$), 1H ($p=0.02$), and 5H ($p=0.02$). No significant interactions were noted for IGF-1 ($F=2.3$; $p=0.06$; $\eta^2=0.11$). AUC analysis revealed no significant differences between trials ($p=0.39$) (Figure 6).

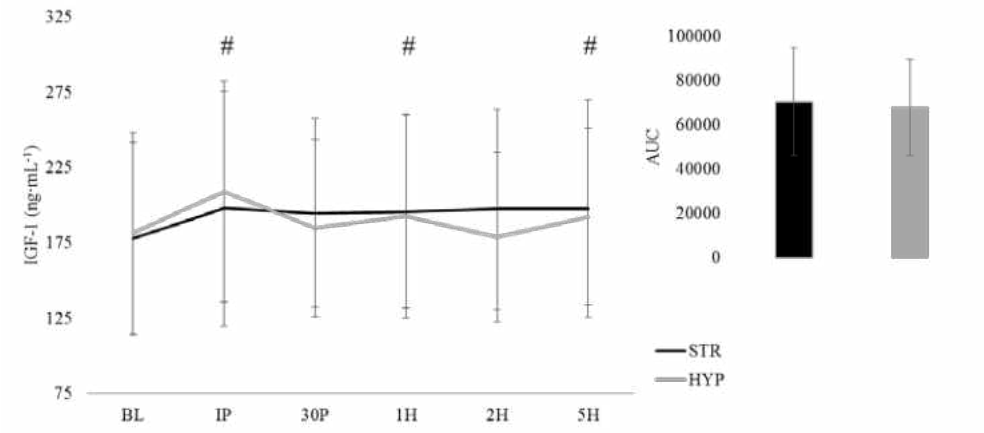

Figure 6. Insulin-like growth factor-1 (IGF-1) concentration following resistance exercise. Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Inset: area under the curve (AUC). All data are reported as means ± SD. # = Significant difference from BL ($p \leq 0.05$).

Insulin

Significant time effects were observed for insulin ($F=5.6$; $p=0.03$; $\eta^2=0.24$). Insulin concentrations were significantly elevated from BL at IP ($p=0.004$) and 30P ($p=0.02$). No significant interactions were noted for insulin ($F=1.1$; $p=0.31$; $\eta^2=0.06$), however AUC analysis indicated that the insulin response during HYP was significantly greater than STR ($p=0.04$) (Figure 7).

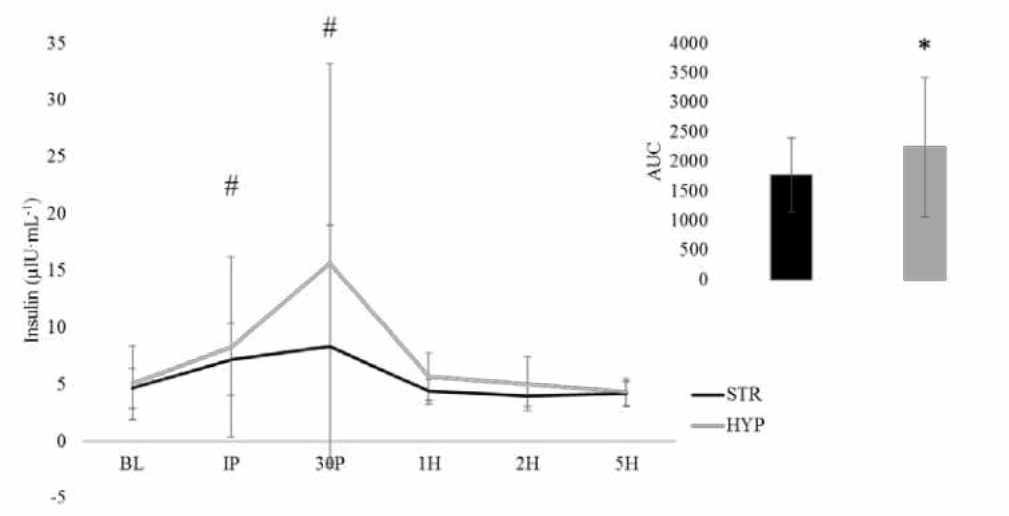

Figure 7. Insulin concentration following resistance exercise. Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Inset: area under the curve (AUC). All data are reported as means ± SD. * = Significant difference between STR and HYP ($p \leq 0.05$). # = Significant difference from BL ($p \leq 0.05$).

Testosterone

Significant time effects were observed for testosterone ($F=5.1$; $p=0.02$; $\eta^2=0.22$). Testosterone concentrations were significantly decreased from BL at 1H ($p=0.04$) and 2H ($p=0.03$). No significant interactions were noted for testosterone ($F=1.6$; $p=0.22$; $\eta^2=0.08$). AUC analysis revealed no significant differences between trials ($p=0.44$) (Figure 8).

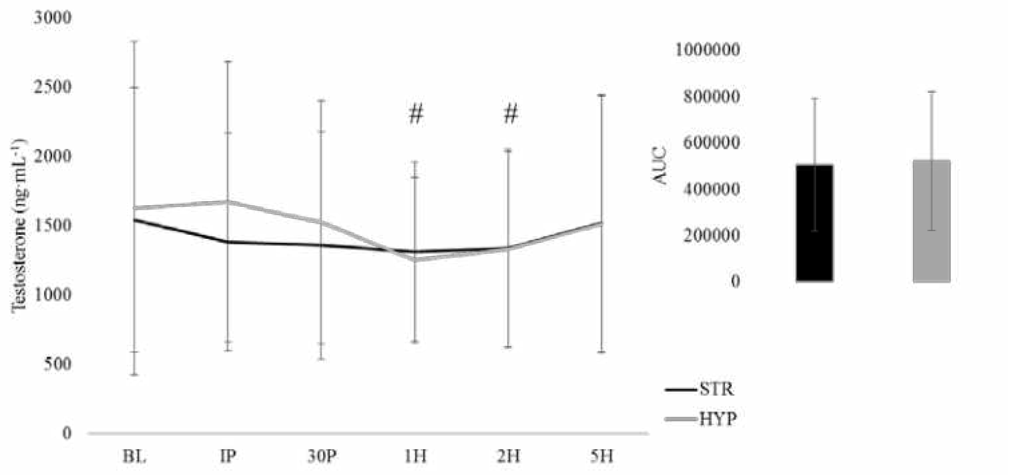

Figure 8. Testosterone concentration following resistance exercise. Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Inset: area under the curve (AUC). All data are reported as means ± SD. # = Significant difference from BL ($p \leq 0.05$).

Growth Hormone

Significant time effects were observed for GH ($F=44.5$; $p=0.0001$; $\eta^2=0.71$). GH concentrations were significantly elevated from BL at IP ($p=0.0001$), 30P ($p=0.0001$), and 1H ($p=0.01$). Significant interactions were also noted for GH ($F=22.4$; $p=0.0001$; $\eta^2=0.56$). GH concentrations were significantly greater during HYP compared to STR at IP ($p=0.0001$), 30P ($p=0.0001$), and 1H ($p=0.02$). AUC analysis indicated that the GH response during HYP was significantly greater than STR ($p=0.0001$) (Figure 9).

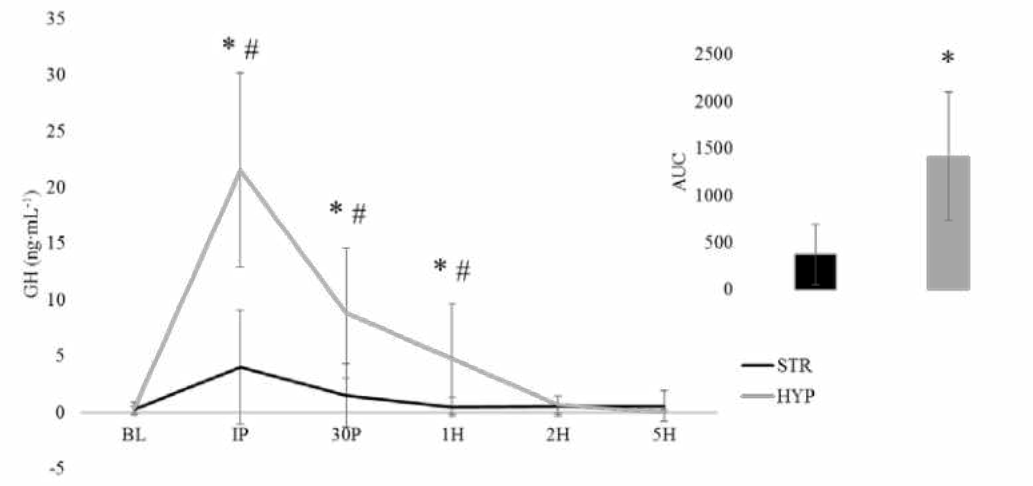

Figure 9. Growth hormone (GH) concentration following resistance exercise. Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Inset: area under the curve (AUC). All data are reported as means ± SD. * = Significant difference between STR and HYP ($p \leq 0.05$). # = Significant difference from BL ($p \leq 0.05$).

Cortisol

Significant time effects were observed for cortisol ($F=29.2$; $p=0.0001$; $\eta^2=0.62$). Cortisol concentrations were significantly elevated from BL at IP ($p=0.001$), 30P ($p=0.001$), and 1H ($p=0.01$), and significantly decreased from BL at 5H ($p=0.0001$). Significant interactions were also noted for cortisol ($F=8.4$; $p=0.0001$; $\eta^2=0.32$). Cortisol concentrations were significantly greater during HYP compared to STR at IP ($p=0.01$), 30P ($p=0.001$), 1H ($p=0.003$) and 2H ($p=0.02$). AUC analysis indicated that the cortisol response during HYP was significantly greater than STR ($p=0.003$) (Figure 10).

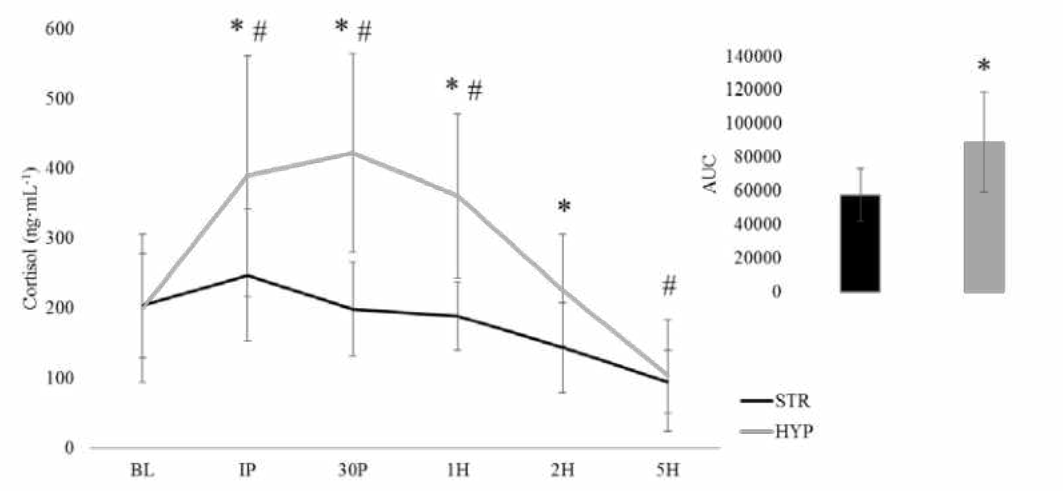

Figure 10. Cortisol concentration following resistance exercise. Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Inset: area under the curve (AUC). All data are reported as means ± SD. * = Significant difference between STR and HYP ($p \leq 0.05$). # = Significant difference from BL ($p \leq 0.05$).

Plasma Volume Shifts

Relative to BL, plasma volume shifts were significantly different between trials at IP ($p=0.02$). The difference between trials was not significant for any other time-point. During HYP, plasma volume decreased at IP, -8.0 ± 7.7; increased at 30P, 2.1 ± 9.4; increased at 1H, 7.2 ± 14.0; increased at 2H, 3.7 ± 5.0; and decreased at 5H, -1.6 ± 5.5. During STR, plasma volume decreased at IP, -1.6 ± 3.1; increased at 30P, 3.3 ± 3.6; increased at 1H, 4.0 ± 3.0; increased at 2H, 7.2 ± 7.3; and decreased at 5H, -2.6 ± 4.0. Blood variables were not corrected for plasma volume shifts due to the importance of molar exposure at the tissue receptor level.

Intramuscular Anabolic Signaling

Insulin-like Growth Factor-1 Receptor

No significant differences were noted for phosphorylation of IGF1R over time (F=1.1; p=0.35; η^2=0.06), however significant interactions were noted for phosphorylation of IGF1R (F=4.1; p=0.02; η^2=0.19). Phosphorylation of IGF1R was significantly greater at 1H (p=0.03) for HYP compared to STR. No other significant interactions were noted (Figure 11).

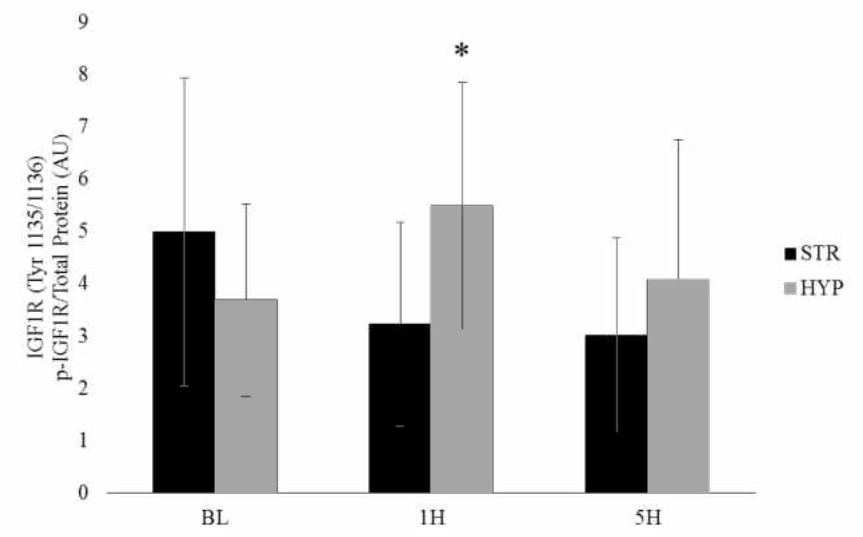

Figure 11. Phosphorylation of IGF1R (Tyr 1135/1136). Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Phosphorylation status of signaling proteins were determined relative to total protein concentration and are therefore reported as arbitrary units (AU). All data are reported as means ± SD. * = Significant difference between STR and HYP ($p \leq 0.05$).

Insulin Receptor

No significant differences were noted for phosphorylation of IR over time (F=0.1; p=0.95; η2=0.003), and no significant interactions were noted for phosphorylation of IR (F=1.4; p=0.26; η^2=0.07) (Figure 12).

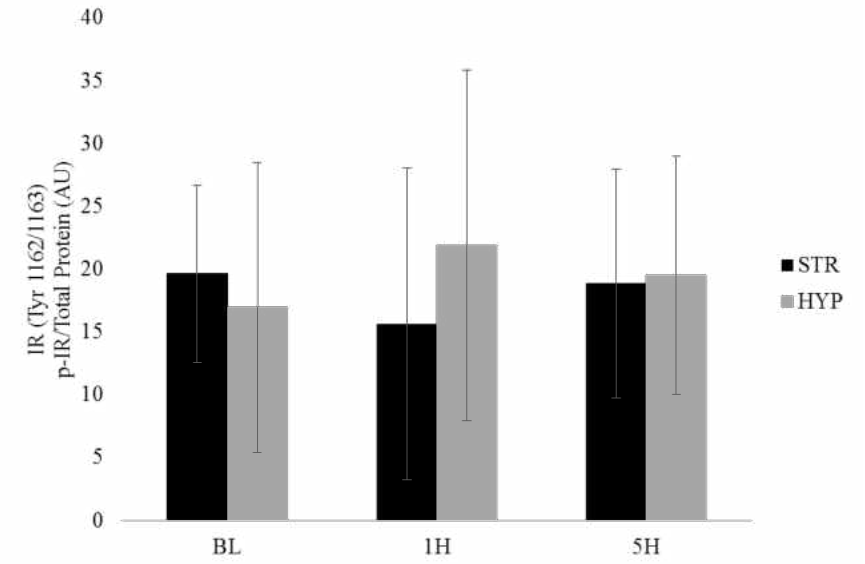

Figure 12. Phosphorylation of IR (Tyr 1162/1163). Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Phosphorylation status of signaling proteins were determined relative to total protein concentration and are therefore reported as arbitrary units (AU). All data are reported as means ± SD.

Insulin Receptor Substrate 1

No significant differences were noted for phosphorylation of IRS1 over time ($F=1.3$; $p=0.29$; $\eta^2=0.07$), and no significant interactions were noted for phosphorylation of IRS1 ($F=0.1$; $p=0.88$; $\eta^2=0.01$) (Figure 13).

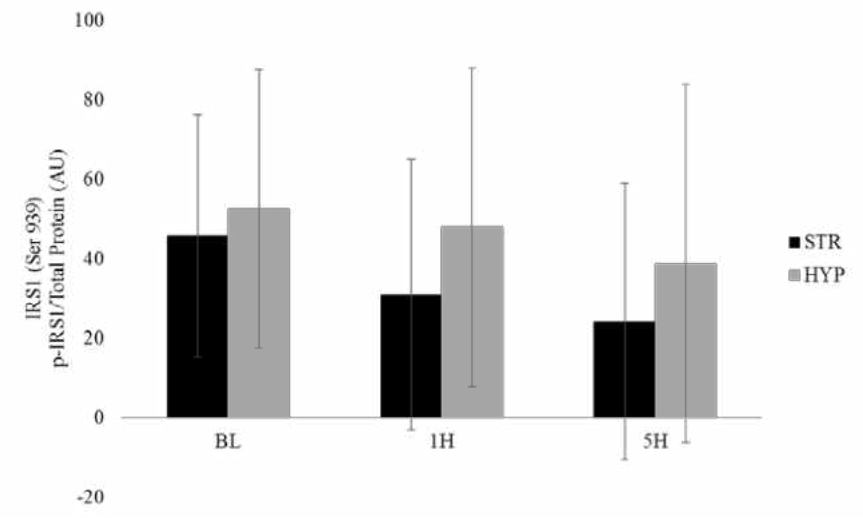

Figure 13. Phosphorylation of IRS1 (Ser 939). Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Phosphorylation status of signaling proteins were determined relative to total protein concentration and are therefore reported as arbitrary units (AU). All data are reported as means ± SD.

Tumor Sclerosis Complex 2

Significant time effects were observed for phosphorylation of TSC2 ($F=5.4$; $p=0.01$; $\eta^2=0.23$). Phosphorylation of TSC2 was significantly decreased from BL at 1H ($p=0.04$) and 5H ($p=0.01$). No significant interactions were noted for phosphorylation of TSC2 ($F=0.3$; $p=0.78$; $\eta^2=0.01$) (Figure 14).

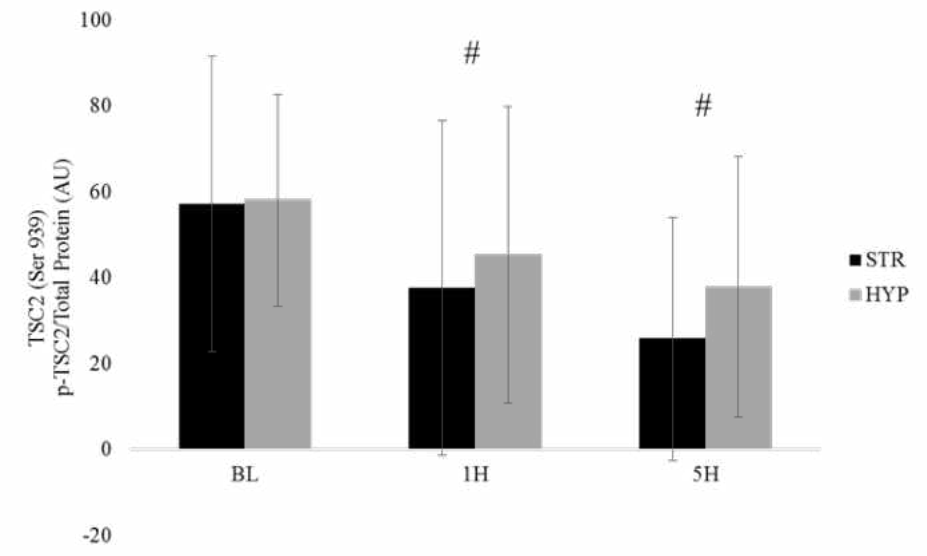

Figure 14. Phosphorylation of TSC2 (Ser 939). Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Phosphorylation status of signaling proteins were determined relative to total protein concentration and are therefore reported as arbitrary units (AU). All data are reported as means ± SD. # = Significant difference from BL ($p \leq 0.05$).

Protein Kinase B

Significant time effects were observed for phosphorylation of Akt ($F=4.4$; $p=0.04$; $\eta 2=0.20$). Phosphorylation of Akt was significantly decreased from BL at 5H ($p=0.02$). No significant interactions were noted for phosphorylation of Akt ($F=0.8$; $p=0.40$; $\eta^2=0.04$) (Figure 15).

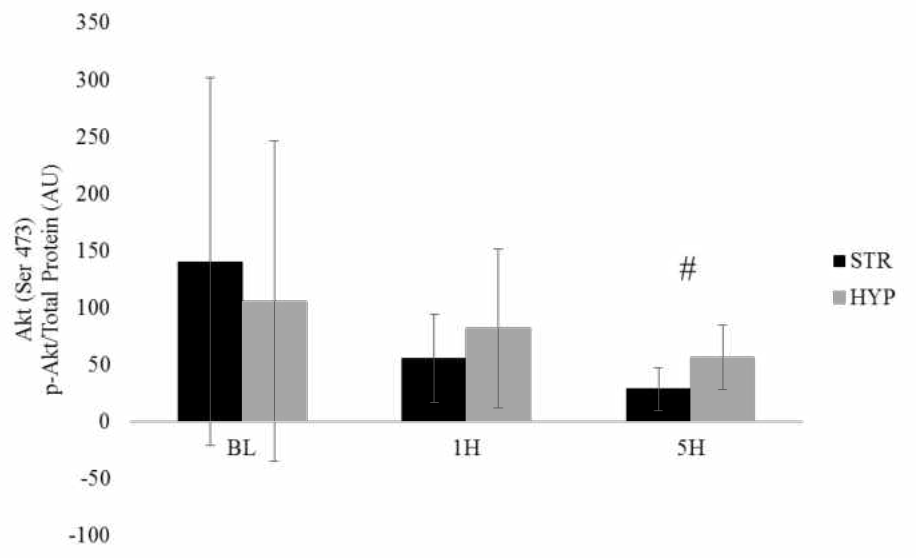

Figure 15. Phosphorylation of Akt (Ser 473). Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Phosphorylation status of signaling proteins were determined relative to total protein concentration and are therefore reported as arbitrary units (AU). All data are reported as means ± SD. # = Significant difference from BL ($p \leq 0.05$).

Mammalian/mechanistic Target of Rapamycin

Significant time effects were observed for phosphorylation of mTOR ($F=4.5$ $p=0.02$; $\eta^2=0.21$). Phosphorylation of mTOR was significantly decreased from BL at 5H ($p=0.01$). No significant interactions were noted for phosphorylation of mTOR ($F=0.002$; $p=0.99$; $\eta^2=0.0001$) (Figure 16).

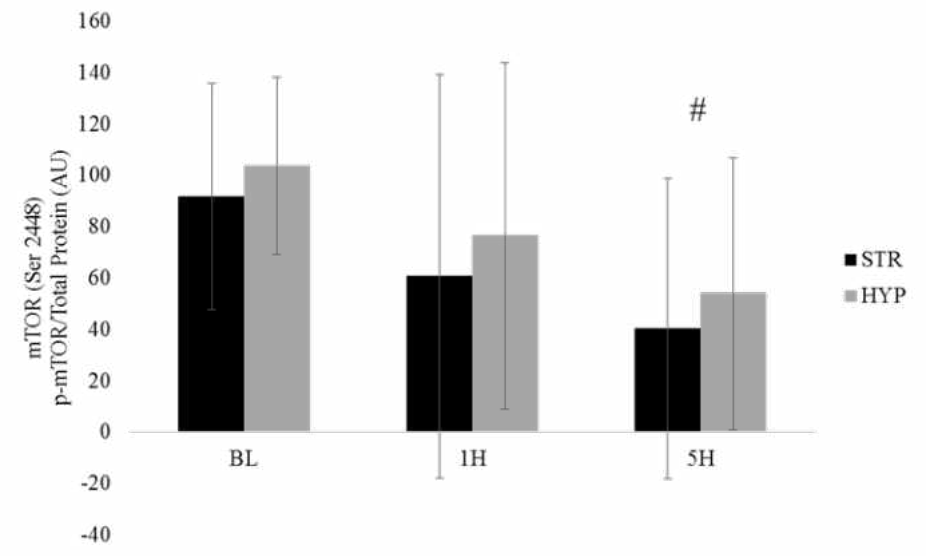

Figure 16. Phosphorylation of mTOR (Ser 2448). Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Phosphorylation status of signaling proteins were determined relative to total protein concentration and are therefore reported as arbitrary units (AU). All data are reported as means ± SD. # = Significant difference from BL ($p \leq 0.05$).

Ribosomal S6 Kinase 1

No significant differences were noted for phosphorylation of p70S6k over time ($F=2.4$; $p=0.11$; $\eta^2=0.12$), and no significant interactions were noted for phosphorylation of p70S6k ($F=0.2$; $p=0.82$; $\eta^2=0.01$) (Figure 17).

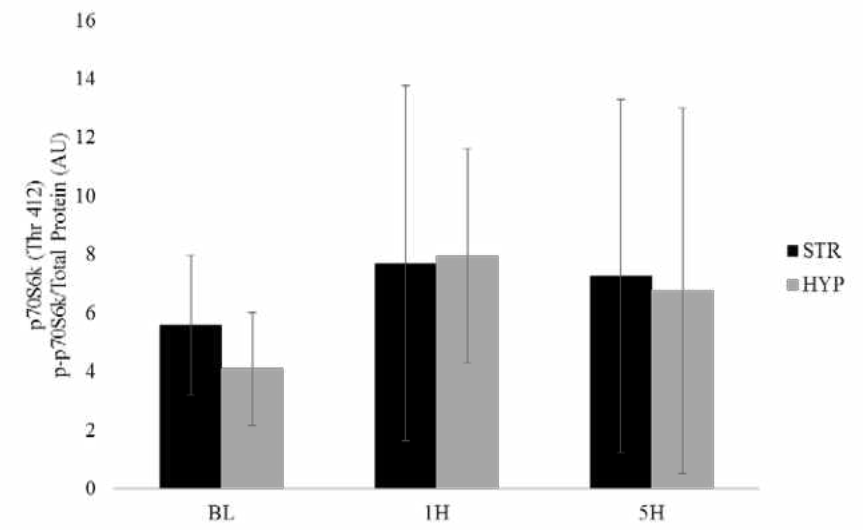

Figure 17. Phosphorylation of p70S6k (Thr 412). Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Phosphorylation status of signaling proteins were determined relative to total protein concentration and are therefore reported as arbitrary units (AU). All data are reported as means ± SD.

Ribosomal Protein S6

Significant time effects were observed for phosphorylation of RPS6 ($F=12.3$; $p=0.0001$; $\eta^2=0.41$). Phosphorylation of RPS6 was significantly elevated from BL at 1H ($p=0.001$) and 5H ($p=0.0001$). No significant interactions were noted for phosphorylation of RPS6 ($F=0.3$; $p=0.77$; $\eta^2=0.02$) (Figure 18).

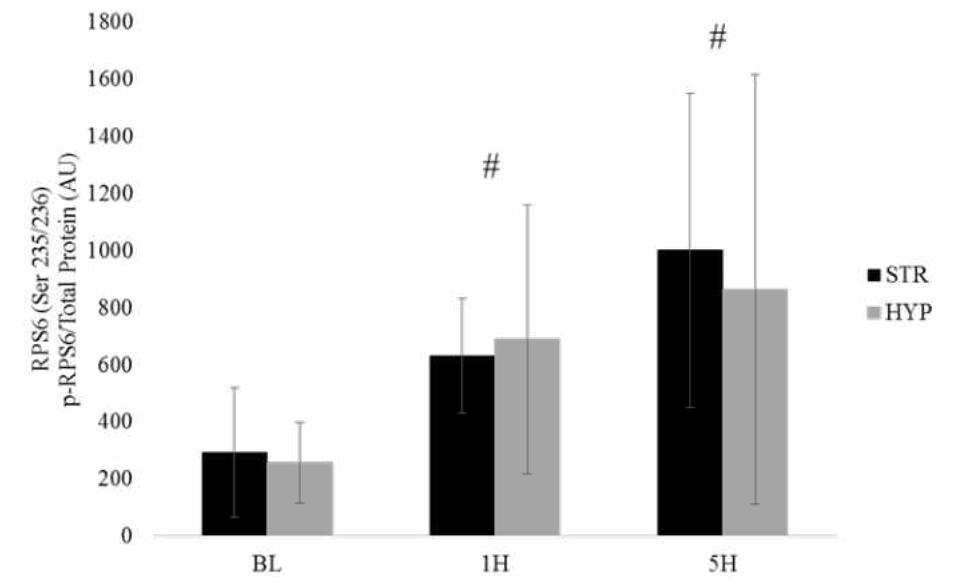

Figure 18. Phosphorylation of RPS6 (Ser 235/236). Groups: STR=Strength protocol; HYP=Hypertrophy protocol. Time points: BL=Baseline; IP=Immediately-post; 30P=30-min post; 1H=one hour post; 2H=two hours post; 5H=five hours post. Phosphorylation status of signaling proteins were determined relative to total protein concentration and are therefore reported as arbitrary units (AU). All data are reported as means ± SD. # = Significant difference from BL ($p \leq 0.05$).

Association between Intramuscular Anabolic Signaling and Circulating Hormones

Phosphorylation of IGF1R at 1H was significantly correlated with insulin concentration at IP (r=0.59; p=0.01), 30P (r=0.46; p=0.04), and 1H (r=0.75; p=0.0001). Phosphorylation of IGF1R at 1H was also significantly correlated with GH concentration at IP (r=0.45; p=0.04). Phosphorylation of Akt at 1H was significantly correlated with GH concentration at IP (r=0.52; p=0.02), 30P (r=0.74; p=0.0001), and 1H (r=0.61; p=0.004), and significantly correlated with IGF-1 concentration at 30P (r=0.52; p=0.02) and 1H (r=0.47; p=0.004). Phosphorylation of RPS6 at 1H was inversely correlated with IGF-1 concentration at 30P (r = - 0.53; p=0.02) and 1H (r= - 0.51; p=0.02). No additional relationships were noted between the phosphorylation status of all other signaling proteins at 1H and the circulating concentrations of IGF-1, insulin, testosterone, GH, or cortisol at IP, 30P, or 1H.

Phosphorylation of Akt at 5H was significantly correlated with GH concentration at IP (r=0.47; p=0.04). Phosphorylation of IRS1 at 5H was inversely correlated with testosterone concentration at 1H (r= - 0.51; p=0.03) and 2H (r= - 0.50; p=0.03). Phosphorylation of TSC2 at 5H was inversely correlated with testosterone concentration at 1H (r= - 0.46; p=0.04). The phosphorylation of p70S6k and RPS6 at 5H was inversely correlated with GH concentration at 2H (r= - 0.46; p=0.04, and r= - 0.45; p=0.04, respectively). Phosphorylation of RPS6 at 5H was also significantly correlated with testosterone concentration at 5H (r=0.47; p=0.04). No additional relationships were noted between the phosphorylation status of all other signaling proteins at 5H and the circulating concentration of IGF-1, insulin, testosterone, GH, or cortisol at IP, 30P, 1H, 2H, or 5H.

Correlational analysis between AUC (BL-1H and BL-5H) of the endocrine measures examined and changes in signaling proteins at 1H and 5H indicated that AUC for the cortisol response was significantly correlated with the percent change in phosphorylation of IR (r=0.58; p=0.01 and r=0.44; p=0.05, respectively) and the percent change in phosphorylation of IGF1R (r=0.57; p=0.01 and r=0.45; p=0.04, respectively), while AUC (BL-1H) for the IGF-1 response to exercise was significantly correlated with the percent change in phosphorylation of IGF1R (r=0.48; p=0.03) at 1H. However, AUC (BL-1H) for the IGF-1 response was inversely correlated with the percent change in phosphorylation of RPS6 (r= - 0.45; p=0.04) at 1H. The percent change in all other signaling proteins were not correlated with the AUC for IGF-1, insulin, testosterone, GH, and cortisol concentration.

CHAPTER V

Discussion

Resistance exercise initiates a multifaceted biochemical response regulating muscle protein synthesis and growth. In the current study, signaling proteins within the mTORC1 pathway were examined in conjunction with circulating hormone concentrations following two different resistance exercise protocols in resistance-trained men. The HYP and STR protocols employed were designed to be typical lower-body resistance exercise protocols used primarily for hypertrophy and strength development, respectively (ACSM, 2009). Although workout volume was designed to be different a priori, both protocols required participants to use an intensity load that required maximal effort to achieve the required repetition range (i.e., reach momentary muscular failure). This effort appeared to cause greater changes in markers of muscle damage (i.e., myoglobin and LDH concentrations) during STR, but greater changes in a metabolic marker of stress (i.e., lactate concentration) during HYP. Significant differences in the endocrine response were also observed between protocols. GH, cortisol, and insulin responses were significantly greater during HYP than STR, however no differences between protocols were observed for either the IGF-1 or testosterone response. Intramuscular anabolic signaling analysis revealed that only the phosphorylation of IGF1R at 1H was significantly greater during HYP than STR, while no other differences were noted in the phosphorylation of all other signaling proteins between HYP and STR.

The intensity used during each resistance exercise protocol produced similar muscle activation across sets in both the squat and leg press exercises. Muscle activation is influenced by the firing rate and number of motor units activated (Fuglsang-Frederiksen and Rønager, 1988), and motor units appear to be recruited in accordance with the size principle during

voluntary muscle contraction (Henneman et al., 1965). However, it has been suggested that lighter loads (20-30% 1-RM) lifted to momentary muscular failure will result in a similar amount of muscle fiber recruitment compared with heavier loads (50-80% 1-RM), thus promoting similar muscular adaptations (Barcelos et al., 2015, Burd et al., 2012, Mitchell et al., 2012). Additionally, the relationship between intensity and muscle protein synthesis may reach a plateau between intensities of ~60-90% of 1-RM (Kumar et al., 2009). The results of this study indicated that STR and HYP elicited similar muscle activation, however it is important to note that during HYP, considering the greater volume of training, the muscle activation was provided for a longer period of time.

Resistance exercise can induce significant microtrauma to muscle fibers (Nosaka et al., 2003). Myoglobin and LDH concentrations have been used extensively as markers of muscle damage and may indicate the integrity of the muscle cell membrane (Gonzalez et al., 2014, Jamurtas et al., 2005, Nosaka et al., 2003, Rodrigues et al., 2010). Although both STR and HYP protocols elicited significant elevations in these markers, greater changes in myoglobin and LDH concentrations were observed following STR. Although microtrauma to skeletal muscle fibers is accompanied with an inflammatory response, indirect markers of muscle damage have not shown to be a consistent indicator of exercise-mediated adaptation (Brentano and Martins, 2011). Further, muscle hypertrophy has been observed in the relative absence of muscle damage (Brentano and Martins, 2011, Flann et al., 2011). Although both protocols elicited significant increases in circulating myoglobin and LDH concentrations, the role of exercise-induced elevations of markers of muscle damage in promoting gene expression influencing skeletal muscle adaptation remains unclear. Despite differences in markers of muscle damage between trials, intramuscular anabolic signaling did not appear to differ between the protocols.

Exercise-induced metabolic stress may also play a role in acute activation of mTORC1 signaling. Metabolic stress results from exercise that primarily relies on anaerobic glycolysis as its major energy provider. Lactate directly affects muscle cells *in vitro* by increasing mTOR and p70S6k phosphorylation (Oishi et al., 2015), and elevations in blood lactate have previously been demonstrated to be weakly associated (r=0.38) with intramuscular anabolic signaling following resistance exercise in trained men (Popov et al., 2015). Resistance exercise protocols that utilize moderate to high intensities (60-85% 1-RM) and volumes (3-6 sets), with relatively short rest intervals (<90 seconds) appear to elicit the greatest increase in blood lactate concentrations (Gotshalk et al., 1997, Kraemer et al., 1991, Kraemer et al., 1990, Linnamo et al., 2005, McCaulley et al., 2009, Rahimi et al., 2010, Smilios et al., 2003). Furthermore, the lactate response in training programs that are focused on stimulating muscle hypertrophy has previously been shown to be significantly greater than resistance training programs that are focused on maximal strength development (McCaulley et al., 2009, Smilios et al., 2003). In the current study, elevated blood lactate concentrations were observed following STR and HYP, however the lactate response was greater following HYP. Despite large differences in blood lactate concentrations between protocols, intramuscular anabolic signaling did not appear to be different. Lactate production may contribute to mTORC1 activation, however the mechanisms by which metabolic stress influences anabolic signaling are not fully elucidated and warrant further investigation.

Acute program variables, including exercise intensity, volume, and rest, have been shown to influence the endocrine response following resistance exercise (Kraemer and Ratamess, 2005). Regardless of training status or age, heavy resistance exercise appears to be a potent stimulus for acute increases in circulating anabolic hormones (Ahtiainen et al., 2005, Beaven et al., 2008,

Boroujerdi and Rahimi, 2008, Crewther et al., 2008, Goto et al., 2003, Hakkinen and Pakarinen, 1993, Kraemer et al., 1995, Kraemer et al., 1999, Kraemer et al., 1990, Linnamo et al., 2005, McCaulley et al., 2009, Smilios et al., 2003, Uchida et al., 2009, Villanueva et al., 2012). Furthermore, high volume, short rest resistance exercise protocols are associated with greater elevations of growth hormone (Kraemer et al., 1990, Smilios et al., 2003), testosterone (Crewther et al., 2008, McCaulley et al., 2009), and cortisol (Crewther et al., 2008, McCaulley et al., 2009, Smilios et al., 2003, Uchida et al., 2009) when compared to high intensity, long rest resistance exercise programs. The results of this present study appear to be consistent with some, but not all of the previous investigations. The GH, cortisol, and insulin response to exercise was significantly greater following HYP compared to STR, while no significant differences between the protocols were observed for IGF-1 or testosterone. Nevertheless, the role of transient hormonal increases in the adaptive response to resistance exercise is not well understood (Schroeder et al., 2013). It has been suggested that elevations in circulating concentrations of these hormones increase the likelihood of hormone-receptor interaction and thus enhance the probability of a physiological effect (Ahtiainen et al., 2003, Kraemer et al., 1990, Kraemer and Ratamess, 2005). However, the mechanisms of exercise-mediated muscle hypertrophy have been suggested to be solely an intrinsic process which may not be influenced by transient changes in circulating hormones (Mitchell et al., 2013, West et al., 2010a, West et al., 2010b, West et al., 2009).

To the best of our knowledge, this appears to be the first study to compare intramuscular anabolic signaling responses following high volume, short rest and high intensity, long rest resistance exercise paradigms that are typically used by bodybuilders and strength/power athletes, respectively. mTORC1 signaling analysis revealed a greater phosphorylation of IGF1R

at 1H following HYP compared to STR, while the phosphorylation status of all other signaling proteins did not appear to be different between the two different training protocols. However, the IGF-1 receptor may not be necessary for resistance exercise-induced mTORC1 signaling and muscle growth (Spangenburg et al., 2008). Using a transgenic mouse model, Spangenburg and colleagues (2008) reported that both Akt and p70S6k activation can be induced independent of a functioning IGF-1 receptor. Downstream signaling proteins, including mTOR, p70S6k, and RPS6, appeared to have similar activation patterns following both the HYP and STR protocols. Both resistance exercise protocols resulted in significant elevations in RPS6 phosphorylation, while not stimulating any change in p70S6k phosphorylation. The lack of any significant change in p70S6k phosphorylation following both resistance exercise protocols may be related to the greater training experience and muscle strength of the participants (Gonzalez et al., 2015). Several studies have suggested that a greater training status can attenuate resistance exercise-induced intramuscular anabolic signaling (Coffey et al., 2006, Ogasawara et al., 2013, Tang et al., 2008). The protein kinase mTOR serves as a critical protein which confers signaling to p70S6k and several other downstream signaling molecules that regulate protein synthesis and skeletal muscle mass (Goodman, 2014, Hornberger, 2011). The phosphorylation of p70S6k regulates several factors involved in translation initiation and protein synthesis (Goodman, 2014), and the phosphorylated state of p70S6k has been shown to be a proxy marker of myofibrillar protein synthesis rates (Kumar et al., 2009, West et al., 2010a) and exercise-induced hypertrophy (Baar and Esser, 1999, Mayhew et al., 2009, Mitchell et al., 2013, Terzis et al., 2008). Although the exact role of RPS6 in the regulation of protein synthesis remains unclear, RPS6 is a downstream target of p70S6k with the potential to regulate protein synthesis and is commonly used as an indirect marker of mTORC1 activation (Goodman, 2014). Based upon the

results of the present study, it appears that HYP and STR resistance exercise protocols elicit similar acute mTORC1 activation in resistance-trained men.

Despite significant differences in the endocrine response following HYP and STR, both protocols stimulated similar mTORC1 activation following resistance exercise. Though it is well appreciated that hormones play an important role in regulating muscle mass, there is much discrepancy in the literature on the capacity of transient hormonal elevations to increase muscle protein synthesis in humans (Schroeder et al., 2013). In the current study, correlational analysis between systemic hormone concentrations and intramuscular signaling proteins was conducted to examine this relationship. Following resistance exercise, insulin, IGF-1, GH, and cortisol showed significant correlations ($r=0.45$-0.75; $p<0.05$) with the phosphorylation of upstream signaling proteins (i.e., IGF1R, IR, Akt). Downstream signaling proteins (i.e., mTOR, p70S6k, RPS6) were not associated with systemic hormone concentrations, with the exception of testosterone concentration at 5H being moderately correlated ($r=0.47$; $p=0.04$) with the phosphorylation of RPS6 at 5H. Although the exact mechanism underlying increased mTORC1 activation following resistance exercise remains relatively elusive, mTORC1 has been suggested to be activated by increasing the activity of Rheb (Ras homolog enriched in brain) (Marcotte et al., 2014). mTORC1 activation requires phosphorylation of TSC2 (a negative regulator of Rheb), which subsequently causes TSC2 to be sequestered away from Rheb allowing mTORC1 to be turned on (Marcotte et al., 2014). Resistance exercise and growth factors including insulin and IGF-1 lead to the phosphorylation of TSC2 (Inoki et al., 2002, Jacobs et al., 2013, Menon et al., 2014). When insulin/IGF-1 bind to their membrane receptors, TSC2 is subsequently phosphorylated via Akt (Inoki et al., 2002, Menon et al., 2014), whereas resistance exercise-induced activation of mTORC1 appears to be Akt-independent (Hornberger et al., 2004). It

remains unclear what mediates TSC2 phosphorylation following resistance exercise (Marcotte et al., 2014). Nevertheless, resistance exercise and growth factors share the same final step in mTORC1 activation (via phosphorylation of TSC2) (Marcotte et al., 2014). Since the end result of both resistance exercise and growth factors is the movement of TSC2 away from Rheb via different upstream kinases, resistance exercise and hormonal exposure may not offer a synergistic effect. This appears to be consistent with the results of the present study, in which insulin, IGF-1, GH, and cortisol were predominately associated with only upstream signaling proteins. Furthermore, previous research has demonstrated that physiological fluctuations in anabolic hormones do not enhance muscle protein synthesis (West et al., 2009), intramuscular anabolic signaling (Spiering et al., 2008, West et al., 2009), or resistance training-induced muscle hypertrophy (Mitchell et al., 2013). The prominent role of acute increases in hormones such as GH and cortisol may be to meet a greater metabolic demand caused by the resistance exercise protocol, rather than promoting muscle protein synthesis.

The current study investigated the acute anabolic response following two typical lower-body resistance exercise paradigms in experienced, resistance-trained men. The results of this study may reflect the lower adaptive ability among highly trained individuals, accounting for the attenuated signaling responses in comparison to untrained individuals (Coffey et al., 2006, Gonzalez et al., 2015, Nader et al., 2014, Tang et al., 2008). Although the stimulation of muscle protein synthesis appears to requires mTORC1 activation (Anthony et al., 2000, Gundermann et al., 2014, Kubica et al., 2005), a dissociation between anabolic signaling and muscle protein synthesis may exist (Greenhaff et al., 2008, Mitchell et al., 2015). We also recognize that the methods of studying intramuscular signaling in vivo in humans are accompanied with inherent limitations as it requires repeated biopsy sampling of a small population of muscle fibers at a

few, distinctive time points following exercise and the analyzed tissue is assumed to be representative of the entire muscle.

In conclusion, STR appeared to cause greater changes in markers of muscle damage (e.g., myoglobin and LDH concentrations), but greater changes in lactate concentration were observed following HYP. The GH, cortisol, and insulin response to exercise was significantly greater following HYP than STR. However, the phosphorylation status of signaling proteins within mTORC1 were not significantly different between HYP and STR, with the exception of IGF1R. Phosphorylation of IGF1R was significantly greater following HYP at 1H compared to STR. Despite significant differences in lactate, myoglobin, LDH, and hormone concentrations following STR and HYP, the regulation of signaling proteins within mTORC1 appeared to be similar following both protocols in resistance-trained men.

APPENDIX A: UCF IRB APPROVAL LETTER

University of Central Florida Institutional Review Board
Office of Research & Commercialization
12201 Research Parkway, Suite 501
Orlando, Florida 32826-3246
Telephone: 407-823-2901, 407-882-2901 or 407-882-2276
www.research.ucf.edu/compliance/irb.html

Notice that UCF will Rely Upon Other IRB for Review and Approval

From : **UCF Institutional Review Board**
FWA00000351, IRB00001138

To : **Adam Michael Gonzalez**

Date : **August 21, 2014**

IRB Number: **SBE-14-10394**

Study Title: **The effect of different heavy resistance exercise protocols and a proprietary blend protein supplement on protein signaling, hormonal, and pro-inflammatory immune response following resistance exercise**

Dear Researcher:

The research protocol noted above was reviewed by the University of Central Florida designated Reviewer on August 21, 2014. The UCF IRB accepts the New England's Institutional Review Board review and approval of this study for the protection of human subjects in research. **The expiration date will be the date assigned by the New England's Institutional Review Board and the consent process will be the process approved by that IRB.**

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

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

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

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

Signature applied by Patria Davis on 08/21/2014 10:10:13 AM EDT

IRB Coordinator

APPENDIX B: NEIRB APPROVAL LETTER

NEW ENGLAND INSTITUTIONAL REVIEW BOARD

August 20, 2014

Adam M. Gonzalez, MEd
University of Central Florida
12494 University Boulevard
Orlando, FL 32816

Re: (IRB# 14-272): "The Effect of Different Heavy Resistance Exercise Protocols and a Proprietary Blend Protein Supplement on Protein Signaling, Hormonal, and Pro-Inflammatory Immune Response Following Resistance Exercise"

This is to inform you that New England Institutional Review Board (NEIRB)'s Tuesday Board has approved the above-referenced research protocol and the participation of the above-referenced investigative site in the research. The approval period is **8/20/2014 to 7/7/2015. Your study number is 14-272. Please be sure to reference either this number or the name of the principal investigator in any correspondence with NEIRB.**

Continued approval is conditional upon your compliance with the following requirements:

- A copy of the **Informed Consent Document, NEIRB version 1.0**, approved as of 8/20/2014 is enclosed. Only NEIRB-approved informed consent documents should be used. It must be signed by each subject prior to initiation of any protocol procedures. In addition, each subject must be given a copy of the signed consent form.
- The following must be promptly reported to NEIRB: changes to the study site, and all unanticipated problems that may involve risks or affect the safety or welfare of subjects or others, or that may affect the integrity of the research.
- Approval is valid for enrollment of the number of subjects indicated on your submission form. If you anticipate enrolling more than this number of subjects, NEIRB approval must be obtained prior to exceeding the approved enrollment number.
- All protocol amendments and changes to approved research must be submitted to the IRB and not be implemented until approved by the IRB except where necessary to eliminate apparent immediate hazards to the study subjects.
- Compliance with all federal and state laws pertaining to this research, and with NEIRB's SOPs.
- The enclosed recruitment/subject materials have been approved (Recruitment Script, Dietary Logs and Instructions, Confidential Medical and Activity History Questionnaire, Physical Activity Readiness Questionnaire (PAR-Q), Soreness Recovery Scale, and Visual Analog Scale). Advertisements, letters, internet postings and any other media for subject recruitment must be submitted to NEIRB and approved prior to use. Please refer to *NEIRB Guidelines for Recruitment and Advertising*, available at www.neirb.com.
- All deaths, life-threatening problems or serious or unexpected adverse events, *whether related to the study article or not*, must be reported to the IRB. The Serious Adverse Event Form is available at www.neirb.com.
- Any and all necessary FDA approvals must be received prior to your initiation of the trial. If this study is being conducted under an IDE, a copy of the FDA IDE approval letter must be submitted to NEIRB.
- The study cannot continue after 7/7/2015 until re-approved by NEIRB. A Study Renewal Report must be completed and returned to NEIRB prior to the expiration of the approval period.

Full AAHRPP

NEW ENGLAND INSTITUTIONAL REVIEW BOARD

- When the study is completed, terminated, or if it is not being renewed - complete and submit a Study Completion Report to NEIRB. The Study Completion Report can be accessed via the NEIRB website at www.neirb.com.

Sharon Wang, CIP
Lead Administrator

Copy: NEIRB Chair
University of Central Florida IRB
Enclosures

Full AAHRPP Accreditation

85 WELLS AVENUE, SUITE 107 • NEWTON, MASSACHUSETTS 02459 • PHONE (617) 243-3924 • FAX (617) 969-1310 • www.neirb.com

APPENDIX C: INFORMED CONSENT

The effect of different heavy resistance exercise protocols and a proprietary blend protein supplement on protein signaling, hormonal, and pro-inflammatory immune response following resistance exercise

Informed Consent

Principal Investigator:	Adam M. Gonzalez, M.Ed.
Sub-Investigator(s):	Jay R. Hoffman, Ph.D. Jeffrey R. Stout, Ph.D. Maren S. Fragala, Ph.D. David H. Fukuda, Ph.D. Leonardo P. Oliveira, M.D.
Faculty Supervisor:	Jay R. Hoffman, Ph.D.
Sponsor:	Funding is tentative from MusclePharm Corp.
Investigational Site:	Institute of Exercise Physiology and Wellness Sport and Exercise Science College of Education and Human Performance University of Central Florida
Phone Number(s):	407-823-2367 201-376-2592 (including after hours)

Introduction:

Researchers at the University of Central Florida (UCF) study many topics. To do this we need the help of people who agree to take part in a research study. You are being asked to take part in a research study which will include about twelve people at UCF. You have been asked to take part in this research study because you are an active young adult male with at least one year of resistance training experience. You must be between the age of 18 and 35 years of age to be included in the research study.

Approved by NEIRB on 8/20/2014
NEIRB Version 1.0

The person doing this research is Adam M. Gonzalez of UCF College of Education and Human Performance. Because the researcher is a doctoral student, he is being guided by Dr. Jay R. Hoffman, a UCF faculty supervisor in the Sport and Exercise Science Department.

What you should know about a research study:

- Someone will explain this research study to you.
- A research study is something you volunteer for.
- Whether or not you take part is up to you.
- You should take part in this study only because you want to.
- You can choose not to take part in the research study.
- You can agree to take part now and later change your mind.
- Whatever you decide it will not be held against you.
- Feel free to ask all the questions you want before you decide.

Purpose of the research study:

The purpose of this study is to examine how the body responds (intramuscular protein signaling, hormonal, and pro-inflammatory immune response) following two different resistance exercise protocols. This study will also examine the effect of a protein/coconut blend ingestion on this response following a resistance exercise protocol. It is unknown whether a hypertrophic protocol (low intensity/high volume) and a strength protocol (high intensity/low volume) differentially stimulate the processes that aid in muscle recovery and remodeling following resistance exercise. Additionally, protein consumption may stimulate these processes and potentially aid in muscle recovery and remodeling. In summary, the investigators of this study are interested in determining how the body responds to two different resistance exercise protocols and if the body responds differently with the addition of a protein/coconut blend ingestion.

What you will be asked to do in the study:

Screening Visit:

You will be asked to read and sign this consent form before any study-related procedures are performed. During the screening visit, the following will be done:

- Physical activity readiness questionnaire (PAR-Q)
- Your age, race and gender will be collected
- Self-reported confidential medical and activity history questionnaire

Upon being admitted to the study, you will be assigned a subject number. You will complete each of the following experimental trials in a random order on three separate occasions:

1. Acute low volume, high intensity resistance exercise protocol + Placebo
2. Acute high volume, low intensity resistance exercise protocol + Placebo
3. Acute high volume, low intensity resistance exercise protocol + Protein/coconut supplement

Approved by NEIRB on 8/20/2014
NEIRB Version 1.0

Study Protocol

All procedures are being done solely for research purposes. You will report to the Human Performance Laboratory (HPL) on four separate occasions. On the first visit, anthropometric assessments including height, body mass, and body fat percentage will be measured. Body fat percentage will be assessed via skinfold analysis using a 4-site skinfold test. You will then be tested for maximal strength (1 repetition maximum: 1-RM) on all lifts involved in the exercise protocol. Prior to maximal strength testing, you will perform a standardized warm-up consisting of 5 minutes on a cycle ergometer against a light resistance, 10 body weight squats, 10 body weight walking lunges, 10 dynamic walking hamstring stretches, 10 body-weight push-ups, and 10 dynamic walking quadriceps stretches.

You will then report to the HPL for three experimental trials on three separate occasions. Prior to each trial, you will be instructed to refrain from all forms of moderate to vigorous exercise for a minimum of 72 hours and to report to the HPL following a 10 hour overnight fast. Experimental trials will be performed in a randomized order. Upon arrival to the HPL for each experimental trial, you will provide a urine sample to be analyzed for hydration status.

During each experimental trial, you will perform the same standardized warm-up followed by a lower-body resistance training protocol designed to incorporating large amounts of muscle mass. Table 1 depicts the training routine for the high intensity (HI) and the high volume (HV) protocols. The HI protocol will utilize a load of 90% 1-RM for sets of 3-5 repetitions with a 3-min rest period length between sets and exercises. The HV protocol will utilize a load of 70% 1-RM for sets of 10-12 repetitions with a 1-min rest period length between sets and exercises. Both protocols will include 6 sets of barbell back squats and 4 sets of bilateral leg press, bilateral hamstring curls, bilateral leg extensions, and seated calf raises. During each trial, if you are unable to complete the desired number of repetitions, spotters will provide assistance until you complete the remaining repetitions. Then, the loads will be adjusted so that you can perform the specific number of repetitions for each set.

Table 1. Resistance Exercise Protocols

	High Intensity Protocol (HI)			High Volume Protocol (HV)		
Exercise Order	**Sets x Repetitions**	**Intensity**	**Rest Time**	**Sets x Repetitions**	**Intensity**	**Rest Time**
1. Barbell Back Squats	6 x 3-5			6 x 10-12		
2. Bilateral Leg Press	4 x 3-5			4 x 10-12		
3. Bilateral Hamstring Curls	4 x 3-5	90% 1-RM	3 minutes	4 x 10-12	70% 1-RM	1 minute
4. Bilateral Leg Extensions	4 x 3-5			4 x 10-12		
5. Seated Calf Raises	4 x 3-5			4 x 10-12		

Following each exercise protocols, you will remain in the HPL for all post-exercise assessments. Each experimental trial will be separated by a minimum of seven days to ensure proper recovery.

Blood samples will be obtained at six time points over the course of the study: baseline (BL), immediately post-exercise (IP), 30 minutes post-exercise (30min), 1 hour post-exercise (1H), 2 hours post-exercise (2H), and 5 hours post-exercise (5H). Fine needle muscle biopsies will be completed at BL, 1H, and 5H. All blood samples and biopsies will be taken at the same time of day to avoid diurnal variations.

Approved by NEIRB on 8/20/2014
NEIRB Version 1.0

You will be provided water and permitted to drink water during experimental trials. You will also be provided a standardized low protein, low carbohydrate breakfast following BL assessments. Post-workout drinks will be provided following IP assessments. Following each protocol, you will be provided either a placebo or a protein/coconut supplement. The supplement is an over-the-counter fluid beverage containing protein and coconut water. The supplement contains 120 calories (6 grams carbohydrates; 20 grams protein; 1 gram fat). The placebo will be identical in taste and appearance, but will contain only coconut flavored water. The supplement and placebo will be made by MusclePharm Corp. The experimental trials will be randomized and the researchers will be blinded regarding which drink is provided. The placebo and supplement drinks will be kept in the refrigerator prior to administration. The use of the supplement in this study is not intended to make or support any health claims.

Electromyography

Electromyography (EMG) is a technique for recording the electrical activity produced by the muscles. EMG activity of the vastus lateralis of the dominant leg will be assessed during maximal strength testing and experimental trials for barbell back squats, bilateral leg press, and bilateral leg extensions. A surface electrode arrangement will be placed on the lower thigh of your dominant leg. The skin beneath the electrodes will be shaved and cleaned with alcohol to maintain a proper signal. EMG assessment does not cause any discomfort and will not interfere with your ability to perform the exercise routine.

Subjective Measures of Soreness, Pain, and Recovery

You will be instructed to assess your subjective feelings of leg soreness and leg pain, as well as your perceived recovery status, using scale questionnaires at BL, IP, 30min, 1H, 2H, and 5H.

Blood Measurements

During each experimental trial, all blood samples will be obtained using a Teflon cannula by personnel trained in phlebotomy with extensive experience in both research and clinical settings. A cannula is a hollow tube, which can be inserted into the opening of a vein and serve as a channel for the transport of fluid. The cannula prevents the need for multiple needle pricks from being performed. The risks associated with the placement of the cannula are not any different than that experienced by a normal blood draw using a needle and syringe. The cannula will be kept open following each blood draw with an infusion of a saline solution. This solution contains salt that is similar to the osmolarity of the blood and acts to minimize potential blood clotting within the cannula that may occur with prolonged use. The cannula placement will not interfere with your ability to perform the exercise routine. BL blood samples will be obtained following a 15-min equilibration period. IP blood samples will be taken within 1 minute of exercise cessation. Following the resistance exercise protocol, you will remain in the HPL for subsequent blood draws. You will be instructed to lie in a supine position for the 15 minutes prior to 30min, 1H, 2H, and 5H blood draws. The total volume of blood that will be obtained during each trial will not exceed 156 ml, and 468 ml for the study as a whole. This is approximately 10.5 tablespoons per trial and 31.6 tablespoons for the entire study. To put the total volume of blood being drawn in proper perspective, one pint (475 ml; 32.1 tablespoons) of blood is typically drawn when donating blood. All blood draws will be conducted under sterile conditions. As an additional

safeguard in preventing contamination, new disposable gloves will be used for all blood draws. All blood samples collected will be frozen until analysis. The discomforts associated with the blood drawing procedures are minimal, but sometimes bruising and infection may occur, and the arm may become sore. This soreness usually resolves in a few days. If it persists, contact your doctor.

Biopsy Procedures

All muscle biopsies will be performed by a trained licensed physician. The fine needle muscle biopsy involves the removal of a small piece of muscle tissue from the vastus lateralis of the dominant leg using a sterile hollow needle. Fine needle biopsies are a technique which allows a researcher to obtain multiple muscle samples without large incisions that accompany other methods. Additionally, the pain level of a fine needle biopsy has been reported to be minimal with individuals comparing it to a "pushing sensation" with most participants engaging in normal physical activity the same day. The area over the outside of the lower thigh muscle (vastus lateralis muscle) will be carefully cleaned. A small amount of lidocaine (anesthetic) will be injected into and under the skin. You will likely experience a small pinching sensation while the numbing agent is injected. After the area is completely numbed, a small incision to the skin will be made and a hollow needle will be placed perpendicular to the muscle until the muscle is pierced. Following placement of the biopsy needle into the biopsy device, the unit will be inserted through the cannula. A muscle sample is obtained by the activation of a trigger button, which unloads the spring and activates the needle to collect a small muscle sample. The biopsy needle is then slid out of the insertion cannula while the cannula is maintained in place, thus avoiding repeated skin punctures. During the time that the sample is being taken (about 5 seconds), you may feel the sensation of pressure in the thigh and on some occasions this is moderately painful. However, the discomfort very quickly passes and you will be quite capable of performing exercise and daily activities. The whole biopsy procedure will be repeated up to 6 times in order to obtain sufficient muscle tissue. There may be some minimal bleeding when the needle is removed which may require application of pressure for a few minutes. Following the biopsy, the incision will be treated with a sterile dressing and wrapped in a bandage.

Dietary Logs

You will be instructed to maintain your normal dietary intake leading up to experiment trials. Then, you will be instructed to record as accurately as possible everything you consume during the 24 hours prior to the first experimental trial. You will be instructed not to eat or drink (except water) within 10 hours of reporting to the HPL for experimental trials. You will be required to duplicate the content, quantity, and timing of your daily diet during the 24 hours prior to the remaining experimental trials.

Location:

All testing will be conducted in the Institute of Exercise Physiology and Wellness at the University of Central Florida.

Time required:

We expect that you will be in this research study for approximately 4 weeks. This will consist of a preliminary trial and three experimental trials separated by at least 7 days. The preliminary trial will last approximately an hour and a half and the three experimental trials may last up to 9 hours.

Funding for this study:

This research study is tentatively being paid for by MusclePharm Corporation.

Risks:

The workout consists of exercises that are common to the training program of individuals with experience in weight lifting. It is expected that you will experience the normal soreness that often accompanies these workouts.

The risks associated with the blood draw may include some momentary pain at the time the needle is inserted into the vein. It is also possible for a bruise to develop at the site that the needle entered the skin or for individuals to report dizziness and possibly faint after the blood is drawn. It is also rare, but possible, to develop minor infections and pain after the blood draw. To minimize the risks, the skin area where the needle or cannula is inserted will be cleaned and prepared with a disinfectant wipe before the needle or cannula is inserted. Needles and cannulas are sterile, and gloves are worn by the person trained in obtaining blood. During experimental trials, a cannula will be used. This is to minimize the number of needle sticks. Upon the removal of the cannula, the puncture site will be covered with a bandage. The total amount of blood drawn during each testing point will not exceed 1.76 tablespoons. The total amount of blood that will be obtained during the study will not exceed 31.6 tablespoons. To put the volume of blood being drawn in proper perspective, one pint (32.1 tablespoons) of blood is typically drawn when donating blood. To reduce the risk of dizziness and fainting from the blood draws, the cannula will be inserted and all blood draws will occur while you are lying flat on your back.

The risks associated with muscle biopsies include momentary pain at the site of sampling during the time the needle is inserted, the possible appearance of a scar and potential bruising and/or soreness, and involuntary muscle contraction at the site of sampling. To limit these risks, a trained physician and technicians will obtain muscle biopsies using sterile techniques. Additionally, the sampling site will be sterilized prior to the procedure. There will be a total of 3-6 samples obtained during each time point for a total of approximately 90 mg (0.09g) of muscle tissue per sample. This will total approximately 270 mg per trial, and approximately 540mg (0.54g) over the course of the study. To put this in perspective, 1 oz is equal to approximately 28g. To reduce the risk of dizziness and fainting, all procedures will occur while you are lying flat on your back. Lidocaine (anesthetic) will be used to minimize the level of discomfort. The risks associated with lidocaine include irritation of the skin, bruising, and, although rare, allergic reaction. Symptoms of an allergic reaction may include a rash, itching, and swelling. Once the local anesthetic wears off, your leg may feel tight and often there is the sensation of a deep bruise or "Charlie Horse". However, the tightness in the muscle usually disappears within 2 days and participants routinely begin exercising at normal capacity within 2 days.

The risks associated with a 10-hour fast are feelings of hunger and tiredness.

The potential risk associated with ingestion of the protein/coconut supplement includes an allergic reaction (itching, rash, cough, diarrhea, etc.). The product does contain coconut, milk, and soy.

The loss of confidentiality is a potential risk of being in the study, but we will do everything to make sure that your information is protected.

Benefits:

There are no direct benefits to participants.

Compensation or payment:

Upon completion of the study, you will receive a $200 payment for your participation. However, if you only complete certain parts of the study, you may be able to be compensated for what you completed. You will receive $25 for completing the first experimental trial, $50 for completing the second experimental trial, and an additional $125 for completing the third experimental trial. No compensation will be provided if you do not complete any experimental trials.

Medical care and compensation for injury

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

Alternatives:

There are no other alternatives available outside of this study.

Cost:

There is no cost to you to be in the study.

Confidentiality:

Records of your participation in this study will be held confidential so far as permitted by law. The study investigator or it's designee, and, under certain circumstances, New England Institutional Review Board (NEIRB), will be able to inspect and have access to confidential data that identifies you by name. Any publication or presentation of the data will not identify you. By signing this consent form, you authorize the study investigator to release your medical records to the sponsor and the IRB.

The results of this study will be published as a group as part of a scientific publication. No individual results will be published or shared with any person or party. All information attained from the medical and activity questionnaire or performance tests will be held in strict confidence. Individual results will remain confidential and only be relayed to you upon request. All medical and activity questionnaires, as well as data collection sheets will be kept in a locked cabinet during and following the study. All information will be destroyed 5 years from the end of the study and not used for other research purposes. Participant folders and blood storage tubes will be marked with an I.D. number to protect

against a breach of confidentiality and the ID number will be removed upon disposal. Participant names and I.D. numbers will be stored apart from the blood samples. The identifiers will be removed from the samples and destroyed when the samples are disposed of.

Study contact for questions about the study or to report a problem:
If you have questions, concerns, or complaints, or think the research has hurt you, talk to Adam M. Gonzalez, Doctoral Student, Exercise Physiology, College of Education and Human Performance at (201) 376-2592 or by email at adam.gonzalez@ucf.edu. You may also contact Dr. Jay R. Hoffman, Institute of Exercise Physiology and Wellness, Sport and Exercise Science at (407) 823-2367 or by email at jay.hoffman@ucf.edu.

IRB contact about your rights in the study or to report a complaint:
If you have questions about your rights as a research subject, or other concerns about the research, you can contact the New England Institutional Review Board (NEIRB) at 1-800-232-9570.

Withdrawing from the study:
You have the right to discontinue participation without penalty, regardless of the status of the study. Your participation in the study may also be terminated at any time by the researchers in charge of the project or by New England IRB. This could be based upon your refusal to follow study instructions or follow study protocol.

For Students and Employees of University of Central Florida:
Your participation in this study is voluntary. You are free to withdraw your consent and discontinue participation in this study at any time without prejudice or penalty. Your decision to participate or not participate in this study will in no way affect your continued enrollment, grades, employment, or your relationship with individuals who may have an interest in this study. _____initials

(Please note you will be participating in this study on your own time; not during regular working hours or class time.)

VOLUNTEER'S STATEMENT:

I have been given a chance to ask questions about this research study. These questions have been answered to my satisfaction. I may contact Adam Gonzalez if I have any more questions about taking part in this study. Adam Gonzalez or the company he is employed by is being paid by the sponsor for my participation in this study.

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

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

New England Institutional Review Board
Telephone: 1-800-232-9570

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

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

_____ _____
Study Participant (signature) Date

_____
Print Participant's Name

_____ _____
Person who explained this study (signature) Date

Approved by NEIRB on 8/20/2014
NEIRB Version 1.0

APPENDIX D: MEDICAL HISTORY QUESTIONNAIRE AND PAR-Q

Human Performance Laboratory
University of Central Florida

Confidential Medical and Activity History Questionnaire

Participant #_____

When was your last physical examination? _____

1. **List any medications, herbals or supplements you currently take or have taken the last month**:

Medication	Reason for medication
_____	_____
_____	_____
_____	_____
_____	_____

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

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

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

Year of hospitalization	Reason
_____	_____
_____	_____
_____	_____

5. **Illnesses and other Health Issues**

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

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

Sickle cell anemia	yes	no
Cystic fibrosis	yes	no
Water retention problems	yes	no
Heart pacemaker	yes	no
Epilepsy	yes	no
Convulsions	yes	no
Dizziness/fainting/unconsciousness	yes	no
Asthma	yes	no
Shortness of breath	yes	no
Chronic respiratory disorder	yes	no
Chronic headaches	yes	no
Chronic cough	yes	no
Chronic sinus problem	yes	no
High blood pressure	yes	no
Heart murmur	yes	no
Heart attack	yes	no
High cholesterol	yes	no
Diabetes mellitus or insipidus	yes	no
Rheumatic fever	yes	no
Emphysema	yes	no
Bronchitis	yes	no
Hepatitis	yes	no
Kidney disease	yes	no
Bladder problems	yes	no
Tuberculosis (positive skin test)	yes	no
Yellow jaundice	yes	no
Auto immune deficiency	yes	no
Anemia	yes	no
Endotoxemia	yes	no
Thyroid problems	yes	no
Hyperprolactinemia	yes	no
Anorexia nervosa	yes	no
Bulimia	yes	no
Stomach/intestinal problems	yes	no
Arthritis	yes	no
Back pain	yes	no
Gout	yes	no
Hepatic encephalopathy	yes	no
Mania	yes	no
Hypermania	yes	no
Monosodium glutamate hypersensitivity	yes	no
Seizure disorders	yes	no

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Any others (specify):_____
_____
_____

Do you smoke cigarettes or use any other tobacco products?	yes	no
Do you have a history of drug or alcohol dependency?	yes	no
Do you ever have any pain in your chest?	yes	no
Are you ever bothered by racing of your heart?	yes	no
Do you ever notice abnormal or skipped heartbeats?	yes	no
Do you ever have any arm or jaw discomfort, nausea, Or vomiting associated with cardiac symptoms?	yes	no
Do you ever have difficulty breathing?	yes	no
Do you ever experience shortness of breath?	yes	no
Do you ever become dizzy during exercise?	yes	no
Are you pregnant?	yes	no
Is there a chance that you may be pregnant?	yes	no
Have you ever had any tingling or numbness in your arms or legs?	yes	no
Has a member of your family or close relative died of heart problems or sudden death before the age of 50?	yes	no
Has a health care practitioner ever denied or restricted your participation in sports for any problem	yes	no

If yes, please explain: _____
_____

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

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Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

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

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

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

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

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

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

→ **DELAY BECOMING MUCH MORE ACTIVE:**

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

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

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

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

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

CSEP | SCPE

Approved by NEIRB on 8/20/14
As Is X As Revised ____ Initials

REFERENCES

Aagaard, P., Andersen, J. L., Dyhre-Poulsen, P., Leffers, A.-M., Wagner, A., Magnusson, S. P., Halkjær-Kristensen, J.& Simonsen, E. B. (2001). A mechanism for increased contractile strength of human pennate muscle in response to strength training: Changes in muscle architecture. *The journal of physiology*, 534(2), 613-623.

ACSM (2009). American college of sports medicine position stand: Progression models in resistance training for healthy adults. *Medicine & science in sports & exercise*, 41(3), 687.

Adams, G. R.& Bamman, M. M. (2012). Characterization and regulation of mechanical loading-induced compensatory muscle hypertrophy. *Comprehensive Physiology*, 2(4), 2829-2870.

Ahtiainen, J. P., Pakarinen, A., Alen, M., Kraemer, W. J.& Hakkinen, K. (2005). Short vs. Long rest period between the sets in hypertrophic resistance training: Influence on muscle strength, size, and hormonal adaptations in trained men. *The Journal of Strength & Conditioning Research*, 19(3), 572-582.

Ahtiainen, J. P., Pakarinen, A., Alen, M., Kraemer, W. J.& Häkkinen, K. (2003). Muscle hypertrophy, hormonal adaptations and strength development during strength training in strength-trained and untrained men. *European journal of applied physiology*, 89(6), 555-563.

Alegre, L. M., Aguado, X., Rojas-Martín, D., Martín-García, M., Ara, I.& Csapo, R. (2015). Load-controlled moderate and high-intensity resistance training programs provoke similar strength gains in young women. *Muscle & nerve*, 51(1), 92-101.

Alessi, D. R.& Cohen, P. (1998). Mechanism of activation and function of protein kinase b. *Current opinion in genetics & development*, 8(1), 55-62.

Alessi, D. R., James, S. R., Downes, C. P., Holmes, A. B., Gaffney, P. R., Reese, C. B.& Cohen, P. (1997). Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase b. *Current Biology*, 7(4), 261-269.

Anthony, J. C., Yoshizawa, F., Anthony, T. G., Vary, T. C., Jefferson, L. S.& Kimball, S. R. (2000). Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. *The Journal of Nutrition*, 130(10), 2413-2419.

Apró, W.& Blomstrand, E. (2010). Influence of supplementation with branched-chain amino acids in combination with resistance exercise on p70s6 kinase phosphorylation in resting and exercising human skeletal muscle. *Acta physiologica*, 200(3), 237-248.

Areta, J. L., Burke, L. M., Ross, M. L., Camera, D. M., West, D. W., Broad, E. M., Jeacocke, N. A., Moore, D. R., Stellingwerff, T.& Phillips, S. M. (2013). Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *The Journal of physiology*, 591(9), 2319-2331.

Ávila-Flores, A., Santos, T., Rincón, E.& Mérida, I. (2005). Modulation of the mammalian target of rapamycin pathway by diacylglycerol kinase-produced phosphatidic acid. *Journal of Biological Chemistry*, 280(11), 10091-10099.

Baar, K.& Esser, K. (1999). Phosphorylation of p70s6k correlates with increased skeletal muscle mass following resistance exercise. *American Journal of Physiology-Cell Physiology*, 276(1), C120-C127.

Bamman, M. M., Petrella, J. K., Kim, J.-s., Mayhew, D. L.& Cross, J. M. (2007). Cluster analysis tests the importance of myogenic gene expression during myofiber hypertrophy in humans. *Journal of Applied Physiology*, 102(6), 2232-2239.

Bamman, M. M., Petrella, J. K., Kim, J., Mayhew, D. L.& Cross, J. M. (2007). Cluster analysis tests the importance of myogenic gene expression during myofiber hypertrophy in humans. *Journal of Applied Physiology*, 102(6), 2232-2239.

Barcelos, L. C., Nunes, P. R. P., de Souza, L. R. M. F., de Oliveira, A. A., Furlanetto, R., Marocolo, M.& Orsatti, F. L. (2015). Low-load resistance training promotes muscular adaptation regardless of vascular occlusion, load, or volume. *European journal of applied physiology*, 1-10.

Baskin, K. K., Winders, B. R.& Olson, E. N. (2015). Muscle as a "mediator" of systemic metabolism. *Cell Metabolism*, 21(2), 237-248.

Beaven, C. M., Gill, N. D.& Cook, C. J. (2008). Salivary testosterone and cortisol responses in professional rugby players after four resistance exercise protocols. *The Journal of Strength & Conditioning Research*, 22(2), 426-432.

Bell, G., Syrotuik, D., Martin, T., Burnham, R.& Quinney, H. (2000). Effect of concurrent strength and endurance training on skeletal muscle properties and hormone concentrations in humans. *European journal of applied physiology*, 81(5), 418-427.

Bhasin, S., Storer, T. W., Berman, N., Callegari, C., Clevenger, B., Phillips, J., Bunnell, T. J., Tricker, R., Shirazi, A.& Casaburi, R. (1996). The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. *New England Journal of Medicine*, 335(1), 1-7.

Bhasin, S., Woodhouse, L., Casaburi, R., Singh, A. B., Bhasin, D., Berman, N., Chen, X., Yarasheski, K. E., Magliano, L.& Dzekov, C. (2001). Testosterone dose-response relationships in healthy young men. *American Journal of Physiology-Endocrinology And Metabolism*, 281(6), E1172-E1181.

Bodine, S. C., Stitt, T. N., Gonzalez, M., Kline, W. O., Stover, G. L., Bauerlein, R., Zlotchenko, E., Scrimgeour, A., Lawrence, J. C.& Glass, D. J. (2001). Akt/mtor pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nature cell biology*, 3(11), 1014-1019.

Boroujerdi, S. S.& Rahimi, R. (2008). Acute gh and igf-i responses to short vs. Long rest period between sets during forced repetitions resistance training system. *South African Journal for Research in Sport, Physical Education and Recreation*, 30(2), 31-38.

Braith, R. W.& Stewart, K. J. (2006). Resistance exercise training its role in the prevention of cardiovascular disease. *Circulation*, 113(22), 2642-2650.

Brentano, M.& Martins, K. L. (2011). A review on strength exercise-induced muscle damage: Applications, adaptation mechanisms and limitations. *The Journal of sports medicine and physical fitness*, 51(1), 1-10.

Brian, M., Bilgen, E.& Diane, C. F. (2012). Regulation and function of ribosomal protein s6 kinase (s6k) within mtor signalling networks. *Biochemical Journal*, 441(1), 1-21.

Brzycki, M. (1993). Strength testing—predicting a one-rep max from reps-to-fatigue. *Journal of Physical Education, Recreation & Dance*, 64(1), 88-90.

Burd, N. A., Andrews, R. J., West, D. W., Little, J. P., Cochran, A. J., Hector, A. J., Cashaback, J. G., Gibala, M. J., Potvin, J. R.& Baker, S. K. (2012). Muscle time under tension during resistance exercise stimulates differential muscle protein sub-fractional synthetic responses in men. *The Journal of physiology*, 590(2), 351-362.

Burd, N. A., Holwerda, A. M., Selby, K. C., West, D. W., Staples, A. W., Cain, N. E., Cashaback, J. G., Potvin, J. R., Baker, S. K.& Phillips, S. M. (2010). Resistance exercise volume affects myofibrillar protein synthesis and anabolic signalling molecule phosphorylation in young men. *The Journal of physiology*, 588(16), 3119-3130.

Burd, N. A., Mitchell, C. J., Churchward-Venne, T. A.& Phillips, S. M. (2012). Bigger weights may not beget bigger muscles: Evidence from acute muscle protein synthetic responses after resistance exercise. *Applied Physiology, Nutrition, and Metabolism*, 37(3), 551-554.

Burd, N. A., West, D. W., Staples, A. W., Atherton, P. J., Baker, J. M., Moore, D. R., Holwerda, A. M., Parise, G., Rennie, M. J.& Baker, S. K. (2010). Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men. *PLoS ONE*, 5(8), e12033.

Burden, A. (2010). How should we normalize electromyograms obtained from healthy participants? What we have learned from over 25years of research. *Journal of Electromyography and Kinesiology*, 20(6), 1023-1035.

Camera, D. M., Edge, J., Short, M. J., Hawley, J. A.& Coffey, V. G. (2010). Early time course of akt phosphorylation after endurance and resistance exercise. *Medicine & science in sports & exercise*, 42(10), 1843-1852.

Campos, G. E., Luecke, T. J., Wendeln, H. K., Toma, K., Hagerman, F. C., Murray, T. F., Ragg, K. E., Ratamess, N. A., Kraemer, W. J.& Staron, R. S. (2002). Muscular adaptations in response to three different resistance-training regimens: Specificity of repetition maximum training zones. *European journal of applied physiology*, 88(1-2), 50-60.

Chen, J.& Fang, Y. (2002). A novel pathway regulating the mammalian target of rapamycin (mtor) signaling. *Biochemical pharmacology*, 64(7), 1071-1077.

Chesley, A., MacDougall, J., Tarnopolsky, M., Atkinson, S.& Smith, K. (1992). Changes in human muscle protein synthesis after resistance exercise. *Journal of Applied Physiology*, 73(1383-1383.

Chestnut, J. L.& Docherty, D. (1999). The effects of 4 and 10 repetition maximum weight-training protocols on neuromuscular adaptations in untrained men. *The Journal of Strength & Conditioning Research*, 13(4), 353-359.

Choi, J., Takahashi, H., Itai, Y.& Takamatsu, K. (1998). The difference between effects of" power-up type" and" bulk-up type" strength training exercises-with special reference to muscle cross-sectional area, muscular strength, anaerobic power and anaerobic endurance. *Japanese Journal of Physical Fitness and Sports Medicine*, 47(1), 119-129.

Coffey, V., Zhong, Z., Shield, A., Canny, B., Chibalin, A., Zierath, J.& Hawley, J. (2006). Early signaling responses to divergent exercise stimuli in skeletal muscle from well-trained humans. *The FASEB Journal*, 20(1), 190-192.

Coffey, V. G.& Hawley, J. A. (2007). The molecular bases of training adaptation. *Sports medicine*, 37(9), 737-763.

Coffey, V. G., Zhong, Z., Shield, A., Canny, B. J., Chibalin, A. V., Zierath, J. R.& Hawley, J. A. (2006). Early signaling responses to divergent exercise stimuli in skeletal muscle from well-trained humans. *The FASEB journal*, 20(1), 190-192.

Crewther, B., Cronin, J., Keogh, J.& Cook, C. (2008). The salivary testosterone and cortisol response to three loading schemes. *The Journal of Strength & Conditioning Research*, 22(1), 250-255.

Cuthbertson, D. J., Babraj, J., Smith, K., Wilkes, E., Fedele, M. J., Esser, K.& Rennie, M. (2006). Anabolic signaling and protein synthesis in human skeletal muscle after dynamic shortening or lengthening exercise. *American Journal of Physiology-Endocrinology and Metabolism*, 290(4), E731-E738.

Davidsen, P. K., Gallagher, I. J., Hartman, J. W., Tarnopolsky, M. A., Dela, F., Helge, J. W., Timmons, J. A.& Phillips, S. M. (2011). High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microrna expression. *Journal of applied physiology*, 110(2), 309-317.

Deldicque, L., Atherton, P., Patel, R., Theisen, D., Nielens, H., Rennie, M. J.& Francaux, M. (2008a). Decrease in akt/pkb signalling in human skeletal muscle by resistance exercise. *European journal of applied physiology*, 104(1), 57-65.

Deldicque, L., Atherton, P., Patel, R., Theisen, D., Nielens, H., Rennie, M. J.& Francaux, M. (2008b). Effects of resistance exercise with and without creatine supplementation on gene expression and cell signaling in human skeletal muscle. *Journal of Applied Physiology*, 104(2), 371-378.

Deldicque, L., De Bock, K., Maris, M., Ramaekers, M., Nielens, H., Francaux, M.& Hespel, P. (2010). Increased p70s6k phosphorylation during intake of a protein–carbohydrate drink following resistance exercise in the fasted state. *European journal of applied physiology*, 108(4), 791-800.

Dill, D.& Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology*, 37(2), 247-248.

Dreyer, H. C., Fujita, S., Cadenas, J. G., Chinkes, D. L., Volpi, E.& Rasmussen, B. B. (2006). Resistance exercise increases ampk activity and reduces 4e-bp1 phosphorylation and protein synthesis in human skeletal muscle. *The Journal of physiology*, 576(2), 613-624.

Dreyer, H. C., Fujita, S., Glynn, E. L., Drummond, M. J., Volpi, E.& Rasmussen, B. B. (2010). Resistance exercise increases leg muscle protein synthesis and mtor signalling independent of sex. *Acta physiologica*, 199(1), 71-81.

Drummond, M. J., Fry, C. S., Glynn, E. L., Dreyer, H. C., Dhanani, S., Timmerman, K. L., Volpi, E.& Rasmussen, B. B. (2009). Rapamycin administration in humans blocks the contraction-induced increase in skeletal muscle protein synthesis. *The Journal of physiology*, 587(7), 1535-1546.

Eliasson, J., Elfegoun, T., Nilsson, J., Köhnke, R., Ekblom, B.& Blomstrand, E. (2006). Maximal lengthening contractions increase p70 s6 kinase phosphorylation in human skeletal muscle in the absence of nutritional supply. *American Journal of Physiology-Endocrinology and Metabolism*, 291(6), E1197-E1205.

Fang, Y., Vilella-Bach, M., Bachmann, R., Flanigan, A.& Chen, J. (2001). Phosphatidic acid-mediated mitogenic activation of mtor signaling. *Science*, 294(5548), 1942-1945.

Farnfield, M. M., Breen, L., Carey, K. A., Garnham, A.& Cameron-Smith, D. (2011). Activation of mtor signalling in young and old human skeletal muscle in response to combined resistance exercise and whey protein ingestion. *Applied Physiology, Nutrition, and Metabolism*, 37(1), 21-30.

Farnfield, M. M., Carey, K. A., Gran, P., Trenerry, M. K.& Cameron-Smith, D. (2009). Whey protein ingestion activates mtor-dependent signalling after resistance exercise in young men: A double-blinded randomized controlled trial. *Nutrients*, 1(2), 263-275.

Ferrando, A. A., Sheffield-Moore, M., Yeckel, C. W., Gilkison, C., Jiang, J., Achacosa, A., Lieberman, S. A., Tipton, K., Wolfe, R. R.& Urban, R. J. (2002). Testosterone administration to older men improves muscle function: Molecular and physiological mechanisms. *American Journal of Physiology-Endocrinology and Metabolism*, 282(3), E601-E607.

Ferrando, A. A., Tipton, K. D., Doyle, D., Phillips, S. M., Cortiella, J.& Wolfe, R. R. (1998). Testosterone injection stimulates net protein synthesis but not tissue amino acid transport. *American Journal of Physiology-Endocrinology And Metabolism*, 275(5), E864-E871.

Flann, K. L., LaStayo, P. C., McClain, D. A., Hazel, M.& Lindstedt, S. L. (2011). Muscle damage and muscle remodeling: No pain, no gain? *The Journal of experimental biology*, 214(4), 674-679.

Foster, D. A., Salloum, D., Menon, D.& Frias, M. A. (2014). Phospholipase d and the maintenance of phosphatidic acid levels for regulation of mammalian target of rapamycin (mtor). *Journal of Biological Chemistry*, 289(33), 22583-22588.

Fry, A. C. (2004). The role of resistance exercise intensity on muscle fibre adaptations. *Sports medicine*, 34(10), 663-679.

Fuglsang-Frederiksen, A.& Rønager, J. (1988). The motor unit firing rate and the power spectrum of emg in humans. *Electroencephalography and clinical neurophysiology*, 70(1), 68-72.

Gehlert, S., Suhr, F., Gutsche, K., Willkomm, L., Kern, J., Jacko, D., Knicker, A., Schiffer, T., Wackerhage, H.& Bloch, W. (2014). High force development augments skeletal muscle signalling in resistance exercise modes equalized for time under tension. *Pflügers Archiv-European Journal of Physiology*, 1-14.

Glover, E. I., Oates, B. R., Tang, J. E., Moore, D. R., Tarnopolsky, M. A.& Phillips, S. M. (2008). Resistance exercise decreases eif2bε phosphorylation and potentiates the feeding-induced stimulation of p70s6k1 and rps6 in young men. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 295(2), R604-R610.

Goldberg, A. L., Etlinger, J. D., Goldspink, D. F.& Jablecki, C. (1974). Mechanism of work-induced hypertrophy of skeletal muscle. *Medicine and science in sports*, 7(3), 185-198.

Gonzalez, A. M., Hoffman, J. R., Townsend, J. R., Jajtner, A. R., Wells, A. J., Beyer, K. S., Willoughby, D. S., Oliveira, L. P., Fukuda, D. H.& Fragala, M. S. (2015). Association between myosin heavy chain protein isoforms and intramuscular anabolic signaling following resistance exercise in trained men. *Physiological Reports*, 3(1), e12268.

Gonzalez, A. M., Stout, J. R., Jajtner, A. R., Townsend, J. R., Wells, A. J., Beyer, K. S., Boone, C. H., Pruna, G. J., Mangine, G. T.& Scanlon, T. M. (2014). Effects of β-hydroxy-β-methylbutyrate free acid and cold water immersion on post-exercise markers of muscle damage. *Amino acids*, 46(6), 1501-1511.

Goodman, C. A. (2014). The role of mtorc1 in regulating protein synthesis and skeletal muscle mass in response to various mechanical stimuli. *Reviews of Physiology, Biochemistry and Pharmacology*, 166(43-95.

Goodman, C. A., Frey, J. W., Mabrey, D. M., Jacobs, B. L., Lincoln, H. C., You, J. S.& Hornberger, T. A. (2011). The role of skeletal muscle mtor in the regulation of mechanical load-induced growth. *The Journal of physiology*, 589(22), 5485-5501.

Goodman, C. A., Mayhew, D. L.& Hornberger, T. A. (2011). Recent progress toward understanding the molecular mechanisms that regulate skeletal muscle mass. *Cellular signalling*, 23(12), 1896-1906.

Goodman, C. A., Miu, M. H., Frey, J. W., Mabrey, D. M., Lincoln, H. C., Ge, Y., Chen, J.& Hornberger, T. A. (2010). A phosphatidylinositol 3-kinase/protein kinase b-independent activation of mammalian target of rapamycin signaling is sufficient to induce skeletal muscle hypertrophy. *Molecular biology of the cell*, 21(18), 3258-3268.

Goto, K., Sato, K.& Takamatsu, K. (2003). A single set of low intensity resistance exercise immediately following high intensity resistance exercise stimulates growth hormone secretion in men. *The Journal of sports medicine and physical fitness*, 43(2), 243-249.

Gotshalk, L. A., Loebel, C. C., Nindl, B. C., Putukian, M., Sebastianelli, W. J., Newton, R. U., Häkkinen, K.& Kraemer, W. J. (1997). Hormonal responses of multiset versus single-set heavy-resistance exercise protocols. *Canadian Journal of Applied Physiology*, 22(3), 244-255.

Green, S., Salkind, N.& Akey, T. (2000). Methods for controlling type i error across multiple hypothesis tests. *Using SPSS for Windows: Analysing and Understanding Data*, 395-396.

Greenhaff, P. L., Karagounis, L., Peirce, N., Simpson, E. J., Hazell, M., Layfield, R., Wackerhage, H., Smith, K., Atherton, P.& Selby, A. (2008). Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *American Journal of Physiology-Endocrinology and Metabolism*, 295(3), E595-E604.

Griggs, R. C., Kingston, W., Jozefowicz, R. F., Herr, B. E., Forbes, G.& Halliday, D. (1989). Effect of testosterone on muscle mass and muscle protein synthesis. *Journal of Applied Physiology*, 66(1), 498-503.

Gundermann, D. M., Dickinson, J. M., Fry, C. S., Walker, D. K., Volpi, E.& Rasmussen, B. B. (2012). Inhibition of glycolysis and mtorc1 activation in human skeletal muscle with blood flow restriction exercise. *The FASEB Journal*, 26(1076.3.

Gundermann, D. M., Walker, D. K., Reidy, P. T., Borack, M. S., Dickinson, J. M., Volpi, E.& Rasmussen, B. B. (2014). Activation of mtorc1 signaling and protein synthesis in human muscle following blood flow restriction exercise is inhibited by rapamycin. *American Journal of Physiology-Endocrinology and Metabolism*, 306(10), E1198-E1204.

Häkkinen, K., Komi, P. V., Alén, M.& Kauhanen, H. (1987). Emg, muscle fibre and force production characteristics during a 1 year training period in elite weight-lifters. *European journal of applied physiology and occupational physiology*, 56(4), 419-427.

Hakkinen, K.& Pakarinen, A. (1993). Acute hormonal responses to two different fatiguing heavy-resistance protocols in male athletes. *Journal of Applied Physiology*, 74(2), 882-887.

Henneman, E., Somjen, G.& Carpenter, D. O. (1965). Functional significance of cell size in spinal motoneurons. *Journal of neurophysiology*, 28(3), 560-580.

Hisaeda, H., Miyagawa, K., Kuno, S., Fukunaga, T.& Muraoka, I. (1996). Influence of two different modes of resistance training in female subjects. *Ergonomics*, 39(6), 842.

Hoffman, J., Maresh, C., Armstrong, L.& Kraemer, W. (1991). Effects of off-season and in-season resistance training programs on a collegiate male basketball team. *Journal of Human Muscle Performance*, 1(2), 48-55.

Holm, L., Reitelseder, S., Pedersen, T. G., Doessing, S., Petersen, S. G., Flyvbjerg, A., Andersen, J. L., Aagaard, P.& Kjaer, M. (2008). Changes in muscle size and mhc composition in response to resistance exercise with heavy and light loading intensity. *Journal of applied physiology*, 105(5), 1454-1461.

Hornberger, T., Chu, W., Mak, Y., Hsiung, J., Huang, S.& Chien, S. (2006). The role of phospholipase d and phosphatidic acid in the mechanical activation of mtor signaling in skeletal muscle. *Proceedings of the National Academy of Sciences of the United States of America*, 103(12), 4741-4746.

Hornberger, T., Stuppard, R., Conley, K., Fedele, M., Fiorotto, M., Chin, E.& Esser, K. (2004). Mechanical stimuli regulate rapamycin-sensitive signalling by a phosphoinositide 3-kinase-, protein kinase b-and growth factor-independent mechanism. *Biochemical Journal*, 380(795-804.

Hornberger, T. A. (2011). Mechanotransduction and the regulation of mtorc1 signaling in skeletal muscle. *The international journal of biochemistry & cell biology*, 43(9), 1267-1276.

Hornberger, T. A., McLoughlin, T. J., Leszczynski, J. K., Armstrong, D. D., Jameson, R. R., Bowen, P. E., Hwang, E.-S., Hou, H., Moustafa, M. E.& Carlson, B. A. (2003). Selenoprotein-deficient transgenic mice exhibit enhanced exercise-induced muscle growth. *The Journal of nutrition*, 133(10), 3091-3097.

Hornberger, T. A., Sukhija, K. B.& Chien, S. (2006). Regulation of mtor by mechanically induced signaling events in skeletal muscle. *Cell cycle*, 5(13), 1391-1396.

Hubal, M. J., Gordish-Dressman, H., Thompson, P. D., Price, T. B., Hoffman, E. P., Angelopoulos, T. J., Gordon, P. M., Moyna, N. M., Pescatello, L. S.& Visich, P. S. (2005). Variability in muscle size and strength gain after unilateral resistance training. *Medicine & Science in Sports & Exercise*, 37(6), 964-72.

Hulmi, J., Walker, S., Ahtiainen, J., Nyman, K., Kraemer, W.& Häkkinen, K. (2012). Molecular signaling in muscle is affected by the specificity of resistance exercise protocol. *Scandinavian journal of medicine & science in sports*, 22(2), 240-248.

Hulmi, J. J., Tannerstedt, J., Selänne, H., Kainulainen, H., Kovanen, V.& Mero, A. A. (2009). Resistance exercise with whey protein ingestion affects mtor signaling pathway and myostatin in men. *Journal of Applied Physiology*, 106(5), 1720-1729.

Hwang, J. (2011). *Using the milliplex map akt/mtor 11-plex panel (technical report). Billerica, MA: EMD Millipore*,
Inoki, K., Li, Y., Zhu, T., Wu, J.& Guan, K.-L. (2002). Tsc2 is phosphorylated and inhibited by akt and suppresses mtor signalling. *Nature cell biology*, 4(9), 648-657.

Jacobs, B. L., You, J.-S., Frey, J. W., Goodman, C. A., Gundermann, D. M.& Hornberger, T. A. (2013). Eccentric contractions increase the phosphorylation of tuberous sclerosis complex-2 (tsc2) and alter the targeting of tsc2 and the mechanistic target of rapamycin to the lysosome. *The Journal of physiology*, 591(18), 4611-4620.

Jamurtas, A. Z., Theocharis, V., Tofas, T., Tsiokanos, A., Yfanti, C., Paschalis, V., Koutedakis, Y.& Nosaka, K. (2005). Comparison between leg and arm eccentric exercises of the same relative intensity on indices of muscle damage. *European Journal of Applied Physiology*, 95(2-3), 179-185.

Janssen, I., Heymsfield, S. B.& Ross, R. (2002). Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *Journal of the American Geriatrics Society*, 50(5), 889-896.

Joy, J. M., Gundermann, D. M., Lowery, R. P., Jäger, R., McCleary, S. A., Purpura, M., Roberts, M. D., Wilson, S. M., Hornberger, T. A.& Wilson, J. M. (2014). Phosphatidic acid enhances mtor signaling and resistance exercise induced hypertrophy. *Nutrition & metabolism*, 11(1), 29.

Karlsson, H. K., Nilsson, P.-A., Nilsson, J., Chibalin, A. V., Zierath, J. R.& Blomstrand, E. (2004). Branched-chain amino acids increase p70s6k phosphorylation in human skeletal muscle after resistance exercise. *American Journal of Physiology-Endocrinology and Metabolism*, 287(1), E1-E7.

Kim, P. L., Staron, R. S.& Phillips, S. M. (2005). Fasted-state skeletal muscle protein synthesis after resistance exercise is altered with training. *The Journal of physiology*, 568(1), 283-290.

Koopman, R., Zorenc, A. H., Gransier, R. J., Cameron-Smith, D.& van Loon, L. J. (2006). Increase in s6k1 phosphorylation in human skeletal muscle following resistance exercise occurs mainly in type ii muscle fibers. *American Journal of Physiology-Endocrinology and Metabolism*, 290(6), E1245-E1252.

Kraemer, W., Marchitelli, L., Gordon, S., Harman, E., Dziados, J., Mello, R., Frykman, P., McCurry, D.& Fleck, S. (1990). Hormonal and growth factor responses to heavy resistance exercise protocols. *Journal of applied physiology (Bethesda, Md.: 1985)*, 69(4), 1442.

Kraemer, W. J., Aguilera, B. A., Terada, M., Newton, R. U., Lynch, J. M., Rosendaal, G., McBride, J. M., Gordon, S. E.& Hakkinen, K. (1995). Responses of igf-i to endogenous increases in growth hormone after heavy-resistance exercise. *Journal of Applied Physiology*, 79(4), 1310-1315.

Kraemer, W. J., Gordon, S., Fleck, S., Marchitelli, L., Mello, R., Dziados, J., Friedl, K., Harman, E., Maresh, C.& Fry, A. (1991). Endogenous anabolic hormonal and growth factor responses to heavy resistance exercise in males and females. *International journal of sports medicine*, 12(2), 228-235.

Kraemer, W. J., Häkkinen, K., Newton, R. U., Nindl, B. C., Volek, J. S., McCormick, M., Gotshalk, L. A., Gordon, S. E., Fleck, S. J.& Campbell, W. W. (1999). Effects of heavy-resistance training on hormonal response patterns in younger vs. Older men. *Journal of Applied Physiology*, 87(3), 982-992.

Kraemer, W. J., Marchitelli, L., Gordon, S. E., Harman, E., Dziados, J. E., Mello, R., Frykman, P., McCurry, D.& Fleck, S. J. (1990). Hormonal and growth factor responses to heavy resistance exercise protocols. *Journal of Applied Physiology* 69(4), 1442-1450.

Kraemer, W. J., Nindl, B. C., Ratamess, N. A., Gotshalk, L. A., Volek, J. S., Fleck, S. J., Newton, R. U.& Hakkinen, K. (2004). Changes in muscle hypertrophy in women with periodized resistance training. *Medicine & science in sports & exercise*, 36(4), 697-708.

Kraemer, W. J.& Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Medicine*, 35(4), 339-361.

Krieger, J. W. (2010). Single vs. Multiple sets of resistance exercise for muscle hypertrophy: A meta-analysis. *The Journal of Strength & Conditioning Research*, 24(4), 1150-1159.

Kubica, N., Bolster, D. R., Farrell, P. A., Kimball, S. R.& Jefferson, L. S. (2005). Resistance exercise increases muscle protein synthesis and translation of eukaryotic initiation factor 2bϵ mrna in a mammalian target of rapamycin-dependent manner. *Journal of Biological Chemistry*, 280(9), 7570-7580.

Kumar, V., Selby, A., Rankin, D., Patel, R., Atherton, P., Hildebrandt, W., Williams, J., Smith, K., Seynnes, O.& Hiscock, N. (2009). Age-related differences in the dose–response relationship of muscle protein synthesis to resistance exercise in young and old men. *The Journal of physiology*, 587(1), 211-217.

Lamon, S., Wallace, M. A., Léger, B.& Russell, A. P. (2009). Regulation of stars and its downstream targets suggest a novel pathway involved in human skeletal muscle hypertrophy and atrophy. *The Journal of physiology*, 587(8), 1795-1803.

Laplante, M.& Sabatini, D. M. (2012). Mtor signaling in growth control and disease. *Cell*, 149(2), 274-293.

Léger, B., Cartoni, R., Praz, M., Lamon, S., Dériaz, O., Crettenand, A., Gobelet, C., Rohmer, P., Konzelmann, M.& Luthi, F. (2006). Akt signalling through gsk-3β, mtor and foxo1 is involved in human skeletal muscle hypertrophy and atrophy. *The Journal of physiology*, 576(3), 923-933.

Linnamo, V., Pakarinen, A., Komi, P. V., Kraemer, W. J.& Häkkinen, K. (2005). Acute hormonal responses to submaximal and maximal heavy resistance and explosive exercises in men and women. *The Journal of Strength & Conditioning Research*, 19(3), 566-571.

Lüthi, J., Howald, H., Claassen, H., Rösler, K., Vock, P.& Hoppeler, H. (1986). Structural changes in skeletal muscle tissue with heavy-resistance exercise. *International journal of sports medicine*, 7(3), 123-127.

MacDougall, J. D., Gibala, M. J., Tarnopolsky, M. A., MacDonald, J. R., Interisano, S. A.& Yarasheski, K. E. (1995). The time course for elevated muscle protein synthesis following heavy resistance exercise. *Canadian journal of applied physiology*, 20(4), 480-486.

Marcotte, G. R., West, D. W.& Baar, K. (2014). The molecular basis for load-induced skeletal muscle hypertrophy. *Calcified Tissue International*, 1-15.

Masuda, K., Choi, J. Y., Shimojo, H.& Katsuta, S. (1999). Maintenance of myoglobin concentration in human skeletal muscle after heavy resistance training. *European journal of applied physiology and occupational physiology*, 79(4), 347-352.

Mayhew, D. L., Kim, J.-s., Cross, J. M., Ferrando, A. A.& Bamman, M. M. (2009). Translational signaling responses preceding resistance training-mediated myofiber hypertrophy in young and old humans. *Journal of Applied Physiology*, 107(5), 1655-1662.

McCall, G., Byrnes, W., Dickinson, A., Pattany, P.& Fleck, S. (1996). Muscle fiber hypertrophy, hyperplasia, and capillary density in college men after resistance training. *Journal of applied physiology*, 81(5), 2004-2012.

McCall, G. E., Byrnes, W. C., Fleck, S. J., Dickinson, A.& Kraemer, W. J. (1999). Acute and chronic hormonal responses to resistance training designed to promote muscle hypertrophy. *Canadian Journal of Applied Physiology*, 24(1), 96-107.

McCaulley, G. O., McBride, J. M., Cormie, P., Hudson, M. B., Nuzzo, J. L., Quindry, J. C.& Triplett, N. T. (2009). Acute hormonal and neuromuscular responses to hypertrophy, strength and power type resistance exercise. *European journal of applied physiology*, 105(5), 695-704.

McDonagh, M.& Davies, C. (1984). Adaptive response of mammalian skeletal muscle to exercise with high loads. *European journal of applied physiology and occupational physiology*, 52(2), 139-155.

Menon, S., Dibble, C. C., Talbott, G., Hoxhaj, G., Valvezan, A. J., Takahashi, H., Cantley, L. C.& Manning, B. D. (2014). Spatial control of the tsc complex integrates insulin and nutrient regulation of mtorc1 at the lysosome. *Cell*, 156(4), 771-785.

Mitchell, C. J., Churchward-Venne, T. A., Bellamy, L., Parise, G., Baker, S. K.& Phillips, S. M. (2013). Muscular and systemic correlates of resistance training-induced muscle hypertrophy. *PLoS ONE*, 8(10), e78636.

Mitchell, C. J., Churchward-Venne, T. A., Parise, G., Bellamy, L., Baker, S. K., Smith, K., Atherton, P. J.& Phillips, S. M. (2014). Acute post-exercise myofibrillar protein synthesis is not correlated with resistance training-induced muscle hypertrophy in young men. *PloS one*, 9(2), e89431.

Mitchell, C. J., Churchward-Venne, T. A., West, D. W., Burd, N. A., Breen, L., Baker, S. K.& Phillips, S. M. (2012). Resistance exercise load does not determine training-mediated hypertrophic gains in young men. *Journal of applied physiology*, 113(1), 71-77.

Mitchell, W. K., Phillips, B. E., Williams, J. P., Rankin, D., Lund, J. N., Smith, K.& Atherton, P. J. (2015). A dose-rather than delivery profile–dependent mechanism regulates the "muscle-full" effect in response to oral essential amino acid intake in young men. *The Journal of Nutrition*, 114(1-8.

Moore, D. R., Phillips, S. M., Babraj, J. A., Smith, K.& Rennie, M. J. (2005). Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions. *American Journal of Physiology-Endocrinology and Metabolism*, 288(6), E1153-E1159.

Moore, D. R., Young, M.& Phillips, S. M. (2012). Similar increases in muscle size and strength in young men after training with maximal shortening or lengthening contractions when matched for total work. *European journal of applied physiology*, 112(4), 1587-1592.

Morley, J. E., Perry 3rd, H., Kaiser, F., Kraenzle, D., Jensen, J., Houston, K., Mattammal, M.& Perry Jr, H. (1993). Effects of testosterone replacement therapy in old hypogonadal males: A preliminary study. *Journal of the American Geriatrics Society*, 41(2), 149-152.

Nader, G. A., von Walden, F., Liu, C., Lindvall, J., Gutmann, L., Pistilli, E. E.& Gordon, P. M. (2014). Resistance exercise training modulates acute gene expression during human skeletal muscle hypertrophy. *Journal of Applied Physiology*, 116(6), 693-702.

Nosaka, K., Lavender, A., Newton, M.& Sacco, P. (2003). Muscle damage in resistance training. *International Journal of Sport and Health Science*, 1(1), 1-8.

Ogasawara, R., Kobayashi, K., Tsutaki, A., Lee, K., Abe, T., Fujita, S., Nakazato, K.& Ishii, N. (2013). Mtor signaling response to resistance exercise is altered by chronic resistance training and detraining in skeletal muscle. *Journal of applied physiology*, 114(7), 934-940.

Ogasawara, R., Loenneke, J. P., Thiebaud, R. S.& Abe, T. (2013). Low-load bench press training to fatigue results in muscle hypertrophy similar to high-load bench press training. *International Journal of Clinical Medicine*, 4(2), 114.

Oishi, Y., Tsukamoto, H., Yokokawa, T., Hirotsu, K., Shimazu, M., Uchida, K., Tomi, H., Higashida, K., Iwanaka, N.& Hashimoto, T. (2015). Mixed lactate and caffeine compound increases satellite cell activity and anabolic signals for muscle hypertrophy. *Journal of Applied Physiology*,

Paul, A. C.& Rosenthal, N. (2002). Different modes of hypertrophy in skeletal muscle fibers. *The Journal of cell biology*, 156(4), 751-760.

Peterson, M. D.& Gordon, P. M. (2011). Resistance exercise for the aging adult: Clinical implications and prescription guidelines. *The American journal of medicine*, 124(3), 194-198.

Phillips, S. M. (2014). A brief review of critical processes in exercise-induced muscular hypertrophy. *Sports Medicine*, 44(1), 71-77.

Phillips, S. M., Tipton, K., Ferrando, A. A.& Wolfe, R. R. (1999). Resistance training reduces the acute exercise-induced increase in muscle protein turnover. *American Journal of Physiology-Endocrinology And Metabolism*, 276(1), E118-E124.

Phillips, S. M., Tipton, K. D., Aarsland, A., Wolf, S. E.& Wolfe, R. R. (1997). Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *American Journal of Physiology-Endocrinology And Metabolism*, 36(1), E99.

Popov, D., Swirkun, D., Netreba, A., Tarasova, O., Prostova, A., Larina, I., Borovik, A.& Vinogradova, O. (2006). Hormonal adaptation determines the increase in muscle mass and strength during low-intensity strength training without relaxation. *Human Physiology*, 32(5), 609-614.

Popov, D. V., Lysenko, E. A., Bachinin, A. V., Miller, T. F., Kurochkina, N. S., Kravchenko, I. V., Furalyov, V. A.& Vinogradova, O. (2015). Influence of resistance exercise intensity and metabolic stress on anabolic signaling and expression of myogenic genes in skeletal muscle. *Muscle & nerve*, 51(3), 434-442.

Rahbek, S. K., Farup, J., Møller, A. B., Vendelbo, M. H., Holm, L., Jessen, N.& Vissing, K. (2014). Effects of divergent resistance exercise contraction mode and dietary supplementation type on anabolic signalling, muscle protein synthesis and muscle hypertrophy. *Amino acids*, 46(10), 2377-2392.

Rahimi, R., Qaderi, M., Faraji, H.& Boroujerdi, S. S. (2010). Effects of very short rest periods on hormonal responses to resistance exercise in men. *The Journal of Strength & Conditioning Research*, 24(7), 1851-1859.

Rodrigues, B. M., Dantas, E., de Salles, B. F., Miranda, H., Koch, A. J., Willardson, J. M.& Simão, R. (2010). Creatine kinase and lactate dehydrogenase responses after upper-body resistance exercise with different rest intervals. *The Journal of Strength & Conditioning Research*, 24(6), 1657-1662.

Rønnestad, B. R., Nygaard, H.& Raastad, T. (2011). Physiological elevation of endogenous hormones results in superior strength training adaptation. *European journal of applied physiology*, 111(9), 2249-2259.

Roschel, H., Ugrinowistch, C., Barroso, R., Batista, M. A., Souza, E. O., Aoki, M. S., Siqueira-Filho, M. A., Zanuto, R., Carvalho, C. R.& Neves Jr, M. (2011). Effect of eccentric exercise velocity on akt/mtor/p70s6k signaling in human skeletal muscle. *Applied Physiology, Nutrition, and Metabolism*, 36(2), 283-290.

Sandri, M. (2008). Signaling in muscle atrophy and hypertrophy. *Physiology*, 23(3), 160-170.

Sato, T., Nakashima, A., Guo, L.& Tamanoi, F. (2009). Specific activation of mtorc1 by rheb g-protein in vitro involves enhanced recruitment of its substrate protein. *Journal of Biological Chemistry*, 284(19), 12783-12791.

Schoenfeld, B. J., Ratamess, N. A., Peterson, M. D., Contreras, B., Tiryaki-Sonmez, G.& Alvar, B. A. (2014). Effects of different volume-equated resistance training loading strategies on muscular adaptations in well-trained men. *Journal of Strength & Conditioning Research*, 28(10), 2909-2918.

Schroeder, E. T., Villanueva, M., West, D.& Phillips, S. M. (2013). Are acute post-resistance exercise increases in testosterone, growth hormone, and igf-1 necessary to stimulate skeletal muscle anabolism and hypertrophy? *Medicine & science in sports & exercise*, 45(11), 2044-2051.

Schuenke, M. D., Herman, J. R., Gliders, R. M., Hagerman, F. C., Hikida, R. S., Rana, S. R., Ragg, K. E.& Staron, R. S. (2012). Early-phase muscular adaptations in response to slow-speed versus traditional resistance-training regimens. *European journal of applied physiology*, 112(10), 3585-3595.

Seynnes, O. R., de Boer, M.& Narici, M. V. (2007). Early skeletal muscle hypertrophy and architectural changes in response to high-intensity resistance training. *Journal of applied physiology*, 102(1), 368-373.

Sharma, N., Castorena, C. M.& Cartee, G. D. (2012). Tissue-specific responses of igf-1/insulin and mtor signaling in calorie restricted rats. *PloS one*, 7(6), e38835.

Sharma, N., Sequea, D. A., Arias, E. B.& Cartee, G. D. (2012). Greater insulin-mediated akt phosphorylation concomitant with heterogeneous effects on phosphorylation of akt substrates in soleus of calorie-restricted rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 303(12), R1261-R1267.

Shepherd, P., Withers, D.& Siddle, K. (1998). Phosphoinositide 3-kinase: The key switch mechanism in insulin signalling. *Biochemical Journal*, 333(471-490.

Sih, R., Morley, J. E., Kaiser, F. E., Perry III, H. M., Patrick, P.& Ross, C. (1997). Testosterone replacement in older hypogonadal men: A 12-month randomized controlled trial. *The Journal of Clinical Endocrinology & Metabolism*, 82(6), 1661-1667.

Smilios, I., Pilianidis, T., Karamouzis, M.& Tokmakidis, S. P. (2003). Hormonal responses after various resistance exercise protocols. *Medicine & science in sports & exercise*, 35(4), 644-654.

Snyder, P. J., Peachey, H., Hannoush, P., Berlin, J. A., Loh, L., Lenrow, D. A., Holmes, J. H., Dlewati, A., Santanna, J.& Rosen, C. J. (1999). Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age 1. *The Journal of Clinical Endocrinology & Metabolism*, 84(8), 2647-2653.

Solomon, A.& Bouloux, P. (2006). Modifying muscle mass–the endocrine perspective. *Journal of Endocrinology*, 191(2), 349-360.

Spangenburg, E. E., Le Roith, D., Ward, C. W.& Bodine, S. C. (2008). A functional insulin-like growth factor receptor is not necessary for load-induced skeletal muscle hypertrophy. *The Journal of physiology*, 586(1), 283-291.

Spiering, B. A., Kraemer, W. J., Anderson, J. M., Armstrong, L. E., Nindl, B. C., Volek, J. S., Judelson, D. A., Joseph, M., Vingren, J. L.& Hatfield, D. L. (2008). Effects of elevated circulating hormones on resistance exercise-induced akt signaling. *Medicine & science in sports & exercise*, 40(6), 1039-1048.

Sun, Y., Fang, Y., Yoon, M.-S., Zhang, C., Roccio, M., Zwartkruis, F., Armstrong, M., Brown, H.& Chen, J. (2008). Phospholipase d1 is an effector of rheb in the mtor pathway. *Proceedings of the National Academy of Sciences*, 105(24), 8286-8291.

Tang, J. E., Perco, J. G., Moore, D. R., Wilkinson, S. B.& Phillips, S. M. (2008). Resistance training alters the response of fed state mixed muscle protein synthesis in young men. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 294(1), R172-R178.

Tang, W., Yuan, J., Chen, X., Gu, X., Luo, K., Li, J., Wan, B., Wang, Y.& Yu, L. (2006). Identification of a novel human lysophosphatidic acid acyltransferase, lpaat-theta, which activates mtor pathway. *Journal of biochemistry and molecular biology*, 39(5), 626.

Tanimoto, M.& Ishii, N. (2006). Effects of low-intensity resistance exercise with slow movement and tonic force generation on muscular function in young men. *Journal of Applied Physiology*, 100(4), 1150-1157.

Tanimoto, M., Sanada, K., Yamamoto, K., Kawano, H., Gando, Y., Tabata, I., Ishii, N.& Miyachi, M. (2008). Effects of whole-body low-intensity resistance training with slow movement and tonic force generation on muscular size and strength in young men. *The Journal of Strength & Conditioning Research*, 22(6), 1926-1938.

Tee, A. R., Manning, B. D., Roux, P. P., Cantley, L. C.& Blenis, J. (2003). Tuberous sclerosis complex gene products, tuberin and hamartin, control mtor signaling by acting as a gtpase-activating protein complex toward rheb. *Current Biology*, 13(15), 1259-1268.

Tenover, J. S. (1992). Effects of testosterone supplementation in the aging male. *The Journal of Clinical Endocrinology & Metabolism*, 75(4), 1092-1098.

Terzis, G., Georgiadis, G., Stratakos, G., Vogiatzis, I., Kavouras, S., Manta, P., Mascher, H.& Blomstrand, E. (2008). Resistance exercise-induced increase in muscle mass correlates with p70s6 kinase phosphorylation in human subjects. *European journal of applied physiology*, 102(2), 145-152.

Terzis, G., Spengos, K., Mascher, H., Georgiadis, G., Manta, P.& Blomstrand, E. (2010). The degree of p70s6k and s6 phosphorylation in human skeletal muscle in response to resistance exercise depends on the training volume. *European journal of applied physiology*, 110(4), 835-843.

Timmons, J. A. (2011). Variability in training-induced skeletal muscle adaptation. *Journal of applied physiology*, 110(3), 846-853.

Toigo, M.& Boutellier, U. (2006). New fundamental resistance exercise determinants of molecular and cellular muscle adaptations. *European journal of applied physiology*, 97(6), 643-663.

Uchida, M. C., Crewther, B. T., Ugrinowitsch, C., Bacurau, R. F. P., Moriscot, A. S.& Aoki, M. S. (2009). Hormonal responses to different resistance exercise schemes of similar total volume. *The Journal of Strength & Conditioning Research*, 23(7), 2003-2008.

Vander Haar, E., Lee, S.-i., Bandhakavi, S., Griffin, T. J.& Kim, D.-H. (2007). Insulin signalling to mtor mediated by the akt/pkb substrate pras40. *Nature cell biology*, 9(3), 316-323.

Veldhuis, J. D., Roemmich, J. N., Richmond, E. J., Rogol, A. D., Lovejoy, J. C., Sheffield-Moore, M., Mauras, N.& Bowers, C. Y. (2005). Endocrine control of body composition in infancy, childhood, and puberty. *Endocrine Reviews*, 26(1), 114-146.

Villanueva, M. G., Villanueva, M. G., Lane, C. J.& Schroeder, E. T. (2012). Influence of rest interval length on acute testosterone and cortisol responses to volume-load–equated total body hypertrophic and strength protocols. *The Journal of Strength & Conditioning Research*, 26(10), 2755-2764.

Wang, X., Devaiah, S. P., Zhang, W.& Welti, R. (2006). Signaling functions of phosphatidic acid. *Progress in lipid research*, 45(3), 250-278.

Welle, S., Bhatt, K.& Thornton, C. A. (1999). Stimulation of myofibrillar synthesis by exercise is mediated by more efficient translation of mrna. *Journal of Applied Physiology*, 86(4), 1220-1225.

Wernbom, M., Augustsson, J.& Thomeé, R. (2007). The influence of frequency, intensity, volume and mode of strength training on whole muscle cross-sectional area in humans. *Sports Medicine*, 37(3), 225-264.

West, D. W., Burd, N. A., Staples, A. W.& Phillips, S. M. (2010a). Human exercise-mediated skeletal muscle hypertrophy is an intrinsic process. *The international journal of biochemistry & cell biology*, 42(9), 1371-1375.

West, D. W., Burd, N. A., Tang, J. E., Moore, D. R., Staples, A. W., Holwerda, A. M., Baker, S. K.& Phillips, S. M. (2010b). Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor strength of the elbow flexors. *Journal of Applied Physiology*, 108(1), 60-67.

West, D. W., Cotie, L. M., Mitchell, C. J., Churchward-Venne, T. A., MacDonald, M. J.& Phillips, S. M. (2012). Resistance exercise order does not determine postexercise delivery of testosterone, growth hormone, and igf-1 to skeletal muscle. *Applied Physiology, Nutrition, and Metabolism*, 38(2), 220-226.

West, D. W., Kujbida, G. W., Moore, D. R., Atherton, P., Burd, N. A., Padzik, J. P., De Lisio, M., Tang, J. E., Parise, G.& Rennie, M. J. (2009). Resistance exercise-induced increases in putative anabolic hormones do not enhance muscle protein synthesis or intracellular signalling in young men. *The Journal of physiology*, 587(21), 5239-5247.

West, D. W.& Phillips, S. M. (2012). Associations of exercise-induced hormone profiles and gains in strength and hypertrophy in a large cohort after weight training. *European journal of applied physiology*, 112(7), 2693-2702.

Wilkinson, S. B., Tarnopolsky, M. A., Grant, E. J., Correia, C. E.& Phillips, S. M. (2006). Hypertrophy with unilateral resistance exercise occurs without increases in endogenous anabolic hormone concentration. *European journal of applied physiology*, 98(6), 546-555.

Winter, J. N., Fox, T. E., Kester, M., Jefferson, L. S.& Kimball, S. R. (2010). Phosphatidic acid mediates activation of mtorc1 through the erk signaling pathway. *American Journal of Physiology-Cell Physiology*, 299(2), C335-C344.

Yanagita, M.& Shiotsu, Y. (2014). Role of resistance training for preventing frailty and metabolic syndromes in aged adults. *The Journal of Physical Fitness and Sports Medicine*, 3(1), 35-42.

Yarasheski, K. E., Zachwieja, J. J.& Bier, D. M. (1993). Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. *American Journal of Physiology*, 265(E210-E210.

You, J.-S., Lincoln, H. C., Kim, C.-R., Frey, J. W., Goodman, C. A., Zhong, X.-P.& Hornberger, T. A. (2014). The role of diacylglycerol kinase ζ and phosphatidic acid in the mechanical activation of mammalian target of rapamycin (mtor) signaling and skeletal muscle hypertrophy. *Journal of Biological Chemistry*, 289(3), 1551-1563.