DETERMINANTS OF THE MAGNITUDE OF TRAINING MEDIATED MUSCLE

HYPERTROPHY

DETERMINANTS OF THE MAGNITUDE OF RESISTANCE TRAINING MEDIATED MUSCLE HYPERTROPHY

By

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ABSTRACT

It is well established in the exercise science literature that chronic resistance training leads to muscle hypotrophy in a wide range of populations however most resistance training studies are relatively small in sample size. The few larger studies show a wide range in the magnitude of muscle growth among cohorts undergoing the same training regime.

Three studies were conducted to better understand the sources of this variability. The first study employed a unilateral resistance training model to test the effects of relative training load and volume on the magnitude of hypertrophy and strength gains. This study showed that contrary to the recommendations of many professional organizations high relative training loads were no better than low training loads at inducing muscle hypertrophy provided that each set was performed to the point of muscular exhaustion. In agreement with previous finding, strength gains were greatest with the highest loads.

The next two studies attempted to correlate various putative regulators of muscle hypertrophy with the magnitude of hypertrophy after 16 weeks of training in 23 subjects. Study two showed no association between the acute responses of testosterone, GH or IGF-1 and muscle hypertrophy but did show associations with androgen receptor content and acute phosphorylation of p70S6K. This suggests that local rather than systemic processes are the most important regulators of muscle hypertrophy. The third study tested whether the acute post exercise protein synthetic response to a single bout of resistance exercise is related to the magnitude of hypertrophy following training in the same subjects. Although previous work has shown that acute post exercise protein synthetic response is qualitatively similar to the magnitude of hypertrophy after chronic training with similar manipulations in different subjects, we did not see any relationship.

Based on the three studies in this thesis it appears that intrinsic factors rather than resistance training program variables are most important for regulating muscle hypertrophy. A large sample size and an 'omic' approach is the logical next step to explain greater variability in the magnitude of hypertrophy following training.

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FORMAT AND ORGANIZATION OF THIS THESIS

This thesis was prepared in the "sandwich" thesis format as outlined in the McMaster University School of Graduate Studies Thesis Preparation Guide. This thesis is comprised of a general introduction, three original research papers (Chapters 2-4) and a general conclusion. The papers presented in chapters two and three have been published in in peer reviewed journals with the candidate as first author. The paper presented in chapter four has been submitted for peer review prior to publication.

CONTRIBUTION TO PAPERS WITH MULTIPLE AUTHORS

CHAPTER 2

Publication

Mitchell CJ, Churchward-Venne TA, West DW, Burd NA, Breen L, Baker SK, & Phillips SM (2012). Resistance exercise load does not determine training-mediated hypertrophic gains in young men. *J Appl Physiol*. 113(1):71-7

Contributions

CJ Mitchell, NA Burd, TA Churchward-Venne, DW West and SM Phillips planned the study. CJ Mitchell obtained ethics board approval. CJ Mitchell, NA Burd, TA Churchward-Venne, DWD West, SK Baker and SM Phillips collected the data. SM Phillips supervised the study and obtained muscle biopsies. CJ Mitchell performed the MRI and muscle fiber type analyses. Western blot analysis was performed by L Breen, NA Burd and CJ Mitchell. CJ Mitchell drafted the manuscript. All authors contributed to revising intellectual content of the manuscript and approved the final version.

CHAPTER 3

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Contributions

CJ Mitchell, G Parise and SM Phillips planned the study. CJ Mitchell obtained ethics board approval. CJ Mitchell, TA Churchward-Venne, L Bellamy, SM Phillips and many undergraduate research assistants collected the acute blood and muscle samples. The training and testing protocols were designed and supervised by CJ Mitchell and carried out by undergraduate research assistants. The hormonal and cytokine analysis was performed by staff at the McMaster Core Lab. L Bellamy performed the muscle fiber type analysis. TA Churchward-Venne and CJ Mitchell preformed the western blot analysis. CJ Mitchell drafted the manuscript. All authors contributed to revising intellectual content of the manuscript and approved the final version.

CHAPTER 4

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CJ. Mitchell, TA. Churchward-Venne, G. Parise, L Bellamy , K. Baker, K. Smith, PJ Atherton & SM. Phillips (2013) Increases in fed-state post-exercise myofibrillar protein synthesis are unrelated to resistance training-induced muscle hypertrophy. This article was submitted for peer review to PLoS ONE

Contributions

CJ Mitchell, SM Phillips, and G Parise conceived and designed the study. CJ Mitchell, L Bellamy, TA Churchward-Venne, and SM Phillips ran the experiments. CJ Mitchell, TA Churchward-Venne, K Smith, and PJ Atherton ran analyses. CJ Mitchell and SM Phillips prepared the manuscript and all authors provided feedback and input. All authors approved the final version of the manuscript.

CHAPTER 1: INTRODUCTION

1.1 HISTORICAL BACKGROUND

Skeletal muscle mass is maintained through a balance in the magnitude and duration of muscle protein synthesis (MPS) and muscle protein breakdown (MPB). The primary regulators of MPS and MPB in adult skeletal muscle are nutrition and activity. The term 'activity' is used as a generic term referring to increased loading in this thesis. Periodic feeding stimulates MPS and supresses MPB. Increased muscle activity generally increases MPS and MPB; however MPS contributes to a much greater extent. When activity is combined with nutrition MPS is stimulated to an even greater extent and MPB is supressed leading to a net new addition of muscle proteins.

In young healthy adults receiving adequate nutrition muscle mass is relatively stable. However, muscle mass can be lost during periods of diminished activity such as step reduction or periods of immobilization (Glover *et al.*, 2008; Krogh-Madsen *et al.*, 2010). Small increases in whole body lean mass, presumably muscle mass, can also be brought about in addition to fat mass gains via a combination of energy intakes in excess of requirement and increased protein intakes (Bray *et al.*, 2012). Exercise is the primary non-pharmacological means by which adults gain muscle mass, a process termed hypertrophy. There is evidence that traditional exercise modalities that are not strongly associated with high external loads, such as walking, running, and cycling may cause a small amount of muscle hypertrophy especially in untrained or older adults (Harber et al., 2012; Kubo et al., 2008; Ross et al., 2001). Nonetheless, resistance training is the mode of exercise most often employed to promote gains in strength and muscle mass.

Resistance training is defined as a purposeful repeated body movement with a load greater than what would be encountered during normal activities of daily living (Folland et al.,

2007). In this definition, resistance training is distinct from occupational activities or activities of daily living even if they are performed against a high level of resistance. Resistance training encompasses the use of free weight, weight machines, resistance bands as well as exercise with one's own body weight. Olympic style weight lifting as well as strength training, which is training with weights with the specific goals of increasing strength, are both components of resistance training (ACSM, 2009). The main focus of this thesis will be resistance training with free weight or machines with the specific goal of increasing muscle mass.

The idea of using resistance exercise to induce muscle hypertrophy first appeared in the literature in the 1940's as a rehabilitation modality for soldiers injured during World War II (Coffey, 1946). At the time no reliable measurement was available to evaluate muscle hypertrophy so muscle strength was used as a proxy outcome measure for the effectiveness of resistance training in studies at the time. In the 1960's resistance training gained popularity as a form of recreation and as a means of improving athletic performance. This lead to a series of studies by Berger that attempted to identify the optimal resistance training program design for the improvement of muscle strength however during this time hypertrophy following training was still not routinely measured (Berger, 1962; Carpinelli, 2002). As time progressed, more resistance training studies were conducted and studies began to measure changes in muscle size in addition to muscle strength (MacDougall et al., 1980).

1.2 MEASUREMENT OF MUSCLE HYPERTROPHY

Muscle hypertrophy has been measured in a number of ways ranging from rudimentary muscle girths and anthropometry (O'Shea, 1966) to advanced imaging techniques (Mitchell *et al.*, 2012). Today there are four main approaches used to quantify muscle hypertrophy. Firstly, the histological approach. Histochemical evaluation of hypertrophy involves the collection of

muscle biopsies which are then sectioned and mounted on slides, stained for cell borders and sometimes fibre type, and then photographed and quantified (West et al., 2009a). The crosssections of the muscle cells in the resulting images are then measured and an average muscle fiber size can be obtained. The advantage of this technique is it allows for the direct quantification of different muscle fiber types and subtypes. However, since it is a random sampling of only a very small proportion of the muscle it can be confounded by samples with a low number of visible fibers or skewed mounting of the sample resulting in various degrees of oblique- rather than truly cross-sectional measurements. Another popular technique for the measurement of muscle hypertrophy is the use of Dual-energy X-ray absorptiometry (DXA). This method does not directly measure the mass or size of a single muscle, but rather quantifies fat- and bone-free (FBF) or lean mass which includes muscle as well as other protein-containing organs. It is generally assumed that visceral organs do not change in mass with resistance training and therefore changes in FBF mass are thought to solely reflect changes in lean or muscle mass. DXA can be used either to measure changes in whole-body lean mass or changes in a particular body segment such as a legs; however, it cannot be used to measure hypertrophy in a single muscle or muscle group (Nana et al., 2013). Ultrasound technology is sometime used as an inexpensive and non-invasive measure of muscle hypertrophy. Ultrasound can only provide a one dimensional measurement of muscle thickness or depth and because it is sensitive to the skill of the technician and not sensitive to changes in the three-dimensional size and shape of the muscle it is not considered an optimal method by which to measure muscle hypertrophy following resistance training (Abe et al., 2000). The other, and likely the most reliable, method that can be used to measure muscle hypertrophy with resistance training is imaging. The two major types of imaging that are commonly used are magnetic resonance imaging (MRI)

(Esmarck *et al.*, 2001) and computerized tomography (CT) (Jones et al., 1987). Images are taken as coronal sections of a muscle and then an algorithm identifies fat, bone and muscle on the image to come up with the muscle cross sectional area (CSA). Many cross-sectional images which when taken serially from the muscle can be combined to calculate muscle volume which gives a more complete description of changes in muscle size with resistance training. Muscle CSA determination via CT is cheaper than MRI, however, MRI is higher resolution and is therefore considered the gold standard for measures of whole muscle hypertrophy.

1.3 VARIABILITY IN HYPERTROPHIC RESPONSE

In recent years there have been a number of larger scale resistance training studies conducted. A consistent finding from these studies has been that there is a substantial degree of variability in the magnitude of muscle hypertrophy in participants completing the same resistance training program (Hubal et al., 2005; Kosek et al., 2006; Phillips et al., 2013; West et al., 2012). Like many biological processes, the degree of hypertrophy following resistance training is roughly normally distributed (Hubal, et al., 2005). Different statistical approaches such as Z-scores (West, et al., 2012) and k-means cluster analysis (Bamman et al., 2007) have been employed to group subjects according to the magnitude of muscle hypertrophy experienced by the participants. Regardless of the exact statistical method used, subjects are generally classified based on their hypertrophic response as low, moderate, or high-/extreme-responders. In most studies 15-20% of subjects are low or non-responders, 60-70% are moderate responders and 15-20% are high or extreme responders (Bamman, et al., 2007; West, et al., 2012).

Body size is generally positively related to absolute gains in muscle mass following training; however, when gains are expressed relative to baseline body size or muscle mass this relationship disappears (Hubal, et al., 2005). Similarly it is often thought that sex is a major

factor in the magnitude of muscle hypertrophy following resistance training. While it is true that absolute gains in muscle mass are greater in men than in women, when these gains are normalized to body size these differences decrease drastically. Studies of hypertrophy with large sample sizes (i.e., >500) have shown significantly larger absolute and relative gains in muscle size in men than women following the same resistance training program. Nonetheless, the effect size of these differences was very small and the practical significance of sex differences in relative gains in muscle mass following resistance training is probably negligible (Hubal, et al., 2005). Interestingly, relative strength gains are much larger in women than in men (Hubal, et al., 2005).

Differences in nutritional intake could possibly explain some differences in the magnitude of hypertrophy following resistance training. The influence of nutritional factors in hypertrophy have been studied in two ways: intentional manipulation via the addition of supplements to, or meal replacements in, the diet during training; and the analysis of habitual dietary intake during training, to examine if differences exist between high an lower responders. Dietary interventions during resistance training have focused mainly on the addition of protein to the diet (Hartman *et al.*, 2007) and on supplements such as creatine monohydrate (Vandenberghe *et al.*, 1997). The effects of protein timing (Esmarck, et al., 2001), protein source (Hartman, et al., 2007) and quantity (Josse et al., 2010) have been investigated. Some studies have shown slightly greater muscle hypertrophy on average in subjects when ingesting higher quality proteins such as milk or whey as they produce more rapid blood aminoacidemia (Hartman, et al., 2007). A recent meta-analysis has shown that over the course of a moderate duration (i.e., 12-16wk) training study consumption of supplemental protein resulted in the accrual of approximately one extra kilogram of lean mass (Cermak et al., 2012). Slightly larger increases in lean mass have

been observed after supplementation with creatine monohydrate during resistance training. However, strikingly within each supplementation regime the distribution of hypertrophic responses, low, moderate and high responders, is still evident (Nissen et al., 2003). The implication of this observation is thus that the supplement increases the group mean gain in muscle mass but not the variability in response.

Theoretically, habitual dietary intake could have a large effect on hypertrophy with resistance training. For example, if a study participant were in a large energy deficit or were consuming very low amounts of protein and/or certain amino acids there would be limited potential for their accretion of muscle mass (Pasiakos *et al.*, 2010). Conversely very high calorie diets with a normal macronutrient distributions have been shown the result in the accretion of muscle mass under controlled conditions (Bray, et al., 2012). It is possible that large caloric surplus during resistance training could result in an increased accretion of both fat and lean mass. To date studies that have reported habitual dietary intake in relation to hypertrophy have found that most people who volunteer for participation in studies involving a resistance training protocol are consuming sufficient energy and protein to support gains in muscle mass with training. These studies have also found that variations in energy or protein intake do not explain the response variability in resistance training induced muscle hypertrophy (Thalacker-Mercer et al., 2009).

Variations in resistance training-mediated hypertrophy have been attributed to a variety of different physiological variables. For example, multiple attempts to find markers in the blood that can predict and individual's training response as blood sampling is relatively easy and noninvasive (Kraemer et al., 2005; West, et al., 2012). Because of the potent anabolic effect of supraphysiological doses of testosterone on hypertrophy (Sinha-Hikim et al., 2002); responses of

circulating testosterone have been viewed as candidate regulators of training-mediated muscle hypertrophy and have been hypothesized as being causative, and thus explaining variability, in the training-induced hypertrophic response (Kraemer, et al., 2005). Because their serum concentrations increase acutely following resistance exercise, testosterone and other hormones such as growth hormone and insulin-like growth factor-1 (IGF-1) have been proposed to be anabolic and thus hypertrophy promoting. Thus, the response of these anabolic hormones has been measured acutely after resistance exercise with the thesis that the magnitude of this rise is indicative of, or at least related to, the magnitude of muscle hypertrophy which would occur with chronic training (Kraemer, et al., 2005). Previous work from our lab has shown no relationship between the magnitude of the acute rise of testosterone and subsequent muscle hypertrophy after training (West, et al., 2012). Other potentially anabolic hormones such as IGF-1 show the same lack of relationship as is observed with testosterone and hypertrophy (West, et al., 2012). A weak relationship has been observed between type I fiber hypertrophy and growth hormone response to an acute bout of resistance exercise (West, et al., 2012). Because supraphysiological doses of growth hormone do not stimulate muscle protein synthesis (Doessing et al., 2010) the mechanism of action by which the acute increase in growth hormone concentration could induce muscle hypertrophy is unclear (Burd et al., 2010b). It is likely that this isolated finding of a correlation between acute serum rise in growth hormone and type I fiber hypertrophy (West, et al., 2012) is a result of type I error due to correlations which were not corrected for multiple comparisons.

A number of mechanisms have been investigated that are hypothesized to be able to explain the variability in the resistance training-mediated hypertrophic response. The commonality linking all of these mechanisms is that they are intrinsic to the muscle and

measured through the use of muscle biopsies. Satellite cells (SC) are muscle-specific progenitor cells that reside between the basal lamina and sarcolemma of human muscle cells. Satellite cells are activated in response to muscle injury in order to facilitate repair and remodelling. The function of these cells is to donate their nuclei to existing myofibers to either replace nuclei that may have undergone apoptosis (Hawke et al., 2001) or to maintain a relatively constant nuclei to cytoplasmic ratio (Allen et al., 1999). Extreme hypertrophic 'responders' to resistance training appear to demonstrate a greater ability to expand their SC pool following training in comparison to either moderate or non-responders (Petrella et al., 2008). These results indicate that SC may not be an important regulatory factor in modest hypertrophy, however the ability to mobilize a robust SC response may partially separate extreme from moderate hypertrophic responders to resistance training.

Micro RNA (miRNA) are small non-coding fragments of RNA that act as posttranscriptional regulators of gene translation. Expression of certain miRNAs has been shown to impact muscle hypertrophy (Davidsen *et al.*, 2011). The mechanisms through which miRNA modulate hypertrophy is unknown. However, there is evidence that specific miRNAs modulate both anabolic signaling (Small et al., 2010) and MPB in animal models (Xu et al., 2012). Even without a full mechanistic understanding, the differential expression of miRNA has been used to explain the variance in resistance training mediated hypertrophy (Timmons, 2011). Four miRNA transcripts have been found to be differentially regulated in high and low responders to resistance exercise. High responders to training did not exhibit any change in miRNA expression over 12 weeks of resistance training whereas low responders showed a down-regulation of miRNA-378, miRNA-29a, and miRNA-26a and an up-regulation of miRNA-451 (Davidsen, et al., 2011). Changes in expression of miR-378 alone were able to explain 51% of the variance in muscle

hypertrophy in a cohort of high and low responders (Davidsen, et al., 2011). The amount of variance explained in the population as a whole is, however, likely significantly less as the sample only included the upper and lower quartiles from a single study population. The authors of the above study suggested that the miRNA changes within the low responder group may be reflected of a failure to up-regulate growth or remodelling genes and may be compensatory in nature (Davidsen, et al., 2011).

There have been other attempts to explain variability in resistance training-mediated muscle hypertrophy such as using signalling protein phosphorylation (Terzis *et al.*, 2008) and genome-wide transcriptome profiling (Phillips, et al., 2013) which will be discussed later in this document. Although several studies have been successful in explaining some of the variance in resistance training mediated hypertrophy, no single biomarker has been validated and shown to explain a large proportion of the variance. The main common finding between the few large scale studies which have attempted to explain variability in training induced muscle hypertrophy has been a consistent distribution of low, moderate and high responders to training (Bamman, et al., 2007; Davidsen, et al., 2011; Hubal, et al., 2005; Phillips, et al., 2013)

1.4 RESISTANCE TRAINING LOAD

The external load used during resistance exercise is one of the major program variables that can be modulated in exercise prescription (ACSM, 2009) and is thought to have a profound effect on hypertrophy (Holm et al., 2008) . Load can be reported as an absolute mass but relative load is most often reported in the literature (ACSM, 2009). Relative load can be reported as a percentage of the heaviest load an individual can lift once for a given exercise (single repetition maximum – 1RM) or as load which can only be lifted a set number of times but no more such as a ten repetition maximum or 10RM. Relative load is often referred to in the literature as

'intensity'. This terminology may be appropriate when discussing a single repetition, however, when many repetitions are performed a low relative load can still be quite 'intense' at least at the point of fatigue (Steele, 2013). For this reason, relative load will be used in place of intensity throughout this document.

DeLorme published one of the earliest studies on the benefits of higher relative loads in promoting resistance exercise-induced strength gains (Delorme, 1945). However, data produced by Berger has had a heavy influence on the prescription of resistance exercise to this day (Berger, 1962). Berger suggested that training between 4 and 8 repetitions would maximize strength gains in the bench press exercise (Berger, 1962). The study he conducted was underpowered and not well controlled by today's standards, but that doctrine still stands today. There have been other studies which have confirmed that heavier relative loads are superior at producing strength gains yet there have been very few studies that have addressed the effects of relative load on muscle hypertrophy(ACSM, 2009). A single study by Campos and colleagues is often cited as evidence that a heavy load must be employed to produce hypertrophy (Campos et al., 2002). This study demonstrated that training with 3-5RM or 9-11RM for 8 weeks resulted in hypertrophy in the vastus lateralis whereas training with 28-30 RM did not result in detectible hypertrophy. The training program employed by Campos et al (Campos, et al., 2002) has been used by another research group who showed hypertrophy in both the high and low repetition training groups but did not show any difference in hypertrophy between the high and low repetition groups after 8 weeks of training (Leger et al., 2006).

The Campos et al. (Campos, et al., 2002) study along with numerous anecdotal reports, have led to the popularization of training in a so-called 'hypertrophy zone' between 6 and 12RM as the optimal method to achieve resistance training induced muscle hypertrophy (Baechle et al., 2000; Kraemer et al., 2004; Schoenfeld, 2010). This concept is an extension of the strengthendurance continuum model, which states that training with a high relative load and low repetitions maximizes strength gains whereas training with a light relative load and higher repetitions will maximize gains in muscle endurance (Campos, et al., 2002; Robinson et al., 2009). The strength-endurance continuum has strong empirical support and is largely a function of neural adaptations induced by heavy relative loads and metabolic adaptations induced by lighter relative loads (ACSM, 2009). Although the concept of a 'hypertrophy zone' has little empirical support, proponents argue that the size principle of motor unit recruitment implies that light relative loads do not result in recruitment of the large type II motor units. Type II motor units innervate type II muscle fibres that are particularly sensitive to hypertrophy with resistance training (Carpinelli, 2008). The size principal states that motor units are recruited in an orderly fashion from smallest to largest based on motor neuron diameter in order, to generate the required force for a given task (Henneman, 1957). Type I motor units have the smallest motor neuron diameter and type IIx motor units have the largest motor neuron diameter. Based on this theory maximal contractions would require activation of all the motor units in a muscle whereas contractions requiring a low relative load should only activate a portion of the motor unit pool and thus primarily rely on force generation from type I fibers (Henneman et al., 1965). This is taken as evidence that lifting relatively light loads cannot induce hypertrophy in type II fibers because these fibers are not activated. What is missed by promoters of this view is that when light relative loads are lifted repeatedly type I fibers that are originally activated fatigue and therefore to continue to exert a given amount of force the muscle must recruit more fibers. This results in the use of fibers from the type II pool (Carpinelli, 2008). If a load is lifted until the point of failure then theoretically all muscle fibers should be activated; thus, there should be a

potential for all muscle fibers to experience hypertrophy when light relative loads are lifted to the point of failure.

1.5 RESISTANCE TRAINING VOLUME

The volume used during a session of resistance training is often defined as the product of the load used. The number of repetitions preformed and the number of sets completed is often reported as total kilograms lifted (load X reps X sets) (Burd et al., 2010a). Changes in the number of sets which are performed is the most common way to manipulate the volume of resistance training. It is generally agreed that larger volumes of resistance exercise produce larger gains in muscle hypertrophy to a certain point after which additional volume is no longer beneficial for hypertrophy (ACSM, 2009). There is debate over what the exact optimal volume to induce to maximize hypertrophy is. Some researchers believe that a single set to failure is all that is necessary (Carpinelli et al., 1998) and others believe that 4-6 sets or more per muscle group is optimal for maximizing muscle hypertrophy with resistance training (Peterson et al., 2005). Most individual studies comparing single and multiple sets have failed to show a significant difference in hypertrophy gains over studies ranging from 4 to 25 weeks (Carpinelli, et al., 1998; McBride et al., 2003; Ostrowski et al., 1997). Although, a recent meta-analysis lead to the conclusion that more sets (i.e., 3 > 1) are beneficial for maximizing muscle hypertrophy (Krieger, 2010). Multiple sets were recommended; however, no recommendations on the optimal number of sets to maximize muscle hypertrophy could be made. This discrepancy probably arises due to the large individual variation in the magnitude of resistance training induced muscle hypertrophy and relatively small sample sizes used in the majority of studies (Carpinelli, et al., 1998; Timmons, 2011). In fact, only a single study included in the Krieger (2010) meta-analysis independently found greater lean mass or muscle cross-sectional area gains with the performance

of multiple sets rather than a single set (Ronnestad et al., 2007). If this single study is removed from the Krieger (2010) meta-analysis then there is no longer a statically significant benefit of performing multiple rather than a single set for inducing muscle hypertrophy (Ronnestad, et al., 2007). It appears that there are likely small benefits to increased training volumes for resistance training induced hypertrophy and that these benefits are probably more pronounced in more experienced resistance trainers.

1.6 PROTEIN TURNOVER AND HYPERTROPHY

Muscle mass is regulated by the dynamic balance between muscle protein synthesis (MPS) and muscle protein break down (MPB) (Millward et al., 1976). Both processes are ongoing and in constant opposition to each other, however, consumption of protein and/or carbohydrate as well as performance of exercise can alter the balance and shift between MPS and MPB (Bennet et al., 1989; Phillips et al., 1997). In the post-absorptive state, MPB is greater than MPS putting the muscle in a state of net catabolism. When a meal containing amino acids is ingested MPS is up-regulated and MPB is down-regulated resulting in a period of net protein accretion (Glynn et al., 2010). In young healthy individuals who are properly nourished (i.e., in energy balance with protein at least as high as the protein RDA - 0.8g/kg/d), the magnitude of post-absorptive muscle catabolism and postprandial muscle anabolism are roughly equal throughout the day, and thus muscle mass is maintained. When resistance exercise is performed in the post-absorptive state, MPS is increased and MPB is also increased but to a smaller extent. Thus net protein balance (MPS minus MPB) becomes less negative and there is an overall increase in the rate of protein turnover, however the muscle remains in a state of net catabolism (Phillips, et al., 1997). It is only when amino acids are consumed in close temporal proximity after resistance exercise that net protein accretion occurs. Amino acids and resistance exercise

act in a synergistic fashion when the two stimuli are combined which promotes larger increases in MPS and net protein balance than when either stimulus is applied independently (Biolo et al., 1997). When resistance exercise is performed chronically over time (training) and in combination with adequate protein intake, the periods of net protein anabolism are larger than the periods of net protein catabolism and muscle undergoes hypertrophy (Phillips, 2000).

If both MPS and MPB could be measured over the entire course of a resistance training study then it would be theoretically possible to calculate the exact percentage increase in muscle size. MPS is most often measured with acute stable isotope infusion methodology; this technique is both expensive and invasive and is therefore only used for periods of a few hours in duration. MPB can also be calculated using stable isotope methodology, however, it is more technically challenging and cannot be calculated at the same time with the same tracer (Kumar et al., 2009a). Because MPS appears to be more responsive to exercise and nutritional manipulations and because of the technical challenges associated with MPB, may studies measure MPS in the hours after a manipulation as a surrogate for long term muscle protein accretion (Glynn, et al., 2010). In addition, changes in MPS are more than 2-3 times as great as those in MPB (Phillips, 2004), pointing to the fact that a more obvious loci of regulation in determining net muscle protein balance is by regulation of MPS and not MPB. It is possible that changes in MPB are playing some kind of regulatory role, however, in healthy young men or women who are receiving an adequate energy and protein intake it is unlikely that proteolysis is substantially elevated and unlikely that it is adaptively regulated to control muscle protein mass. Previous work from our lab has shown that acute manipulations of exercise-based or nutrition-related variables result in a qualitatively similar pattern of outcomes in acute MPS response and hypertrophy in different groups of subjects. An acute study showed that consumption of both soy and milk protein after

resistance exercise lead to an increase in MPS but that the magnitude of the increases was greater in the group who ingested milk protein (Wilkinson et al., 2007). A 12 week resistance training study showed an identical pattern of results where subjects who were fed either soy or milk protein after their workout gained lean mass but that magnitude of the gain was greater in the subjects who consumed milk protein post exercise (Hartman, et al., 2007). Similarly an acute study by West et al. showed that there was no difference in biceps MPS when subjects preformed the same workout in isolation or with elevated endogenous hormones induced by an intense lower body workout (West et al., 2009b). This study was followed with a training study by the same author who showed that when one bicep was trained in isolation and the other was trained under elevated endogenous hormones induced by an intense lower body workout, there was identical biceps hypertrophy in both conditions (West, et al., 2009a). Together this pair of studies shows a similar pattern of results between acute MPS studies and training studies in different subjects which involved the same manipulated variables.

Acute measurements of MPS have shown a dose-response relationship between relative load and MPS where lower relative loads result in lower post exercise FSR measurements (Kumar et al., 2009b). Holm and colleagues showed when equated for total volume, contractions at 70% of 1RM resulted in a greater stimulation of muscle FSR than contractions at 16% of 1RM (Holm *et al.*, 2010). Similarly, Kumar and coworkers conducted a study testing the MPS responses to loads ranging from 20 to 90% of 1RM where the number of repetitions preformed in each condition was controlled to equate total volume. They found that loads of 60% of 1RM or greater were required to maximize the post exercise FSR response (Kumar, et al., 2009b). Taken together, these studies suggest that heavier relative training loads may be required to maximize post exercise anabolism. Because volume was equated in these studies the higher relative load

conditions resulted in the accumulation of fatigue whereas the low relative load conditions resulted in lower levels of fatigue. In contrast, Burd et al. tested both the effects of an equal volume at a lower relative load and the same low load lifted to fatigue by conducting a study with three conditions; 90% 1RM load preformed to the point of failure, 30% 1RM load preformed to the point of failure and 30% 1RM load with the total work matched to the 90% 1RM condition. The results from this study showed that when both the 30% and 90% loads were lifted to the point of failure there were large and similar increases in acute post exercise FSR. However, in the 30% work matched group there were only small and transient increases in post exercise FSR (Burd et al., 2010c). These results indicate that training to failure regardless of load may be a potent stimulus for hypertrophy.

The optimal number of sets, to maximize the post exercise MPS response, has been investigated in both young and older subjects. Previous work from our lab has shown that in young men, 3 sets of knee extensions resulted in an FSR response that was both greater in magnitude and duration compared to a single set of knee extensions (Burd, et al., 2010a). In this study each set was performed to the point of muscular failure. A study conducted by Kumar and colleagues looked at the effects of higher exercise volumes on post exercise MPS and compared the FSR response of 3 and 6 sets of knee extensions in both young and older men (Kumar *et al.*, 2012). They found that 6 sets produced a larger FSR response than 3 sets in older men, but not in young men. There was, however, a trend towards a larger MPS response with 6 sets in young men. Other than the small sample size used in this study there are two major confounding factors. First, volume was strictly controlled so that none of the sets were performed to the point of failure and second, all MPS measurements were made in the fasted state. In the Kumar et al. (2012) study MPS returned to resting levels 24 hours post exercise in all conditions whereas in

the study by Burd et al. MPS was elevated 24 hours after the performance of 3 sets in the fed state (Burd, et al., 2010a). It is possible that greater exercise volume prolongs a sensitization to feeding but not an elevation in resting MPS (Burd et al., 2011) which may be an underlying mechanism for the greater observed hypertrophy with higher training volumes (Krieger, 2010).

1.7 ANABOLIC SIGNALING PATHWAYS

Protein synthesis is regulated by multiple overlapping pathways that regulate both the initiation of protein translation and the elongation of proteins (Drummond et al., 2009a). The pathway involved in protein synthesis that has received the most attention is the Akt-mammalian target of rapamycin (mTOR) pathway. mTOR is centrally important in this pathway and exists in two complexes (1 and 2) (Drummond, et al., 2009a). mTOR complex 1 (mTORC1) is thought to be the main site that integrates signals from contraction, growth factors, and nutrition, serving to regulate the phosphorylation of downstream proteins such as p70S6K1 and 4EBP-1 (Bodine et al., 2001). One of the most investigated upstream regulatory proteins of mTOR is Akt, also known as protein kinase B (PKB), which can be activated by insulin and other growth factors (Bodine, et al., 2001). Although Akt phosphorylation has been found to increase after many diet and exercise manipulations, there are also reports of mTOR phosphorylation and elevations of MPS in an Akt-independent fashion. This suggests a low degree of concordance between Akt phosphorylation and MPS (Liu et al., 2002).

Nutrients can also regulate mTOR in a number of different ways. First, the muscle cell is unable to mount a large protein synthetic response in the face of low energy availability. The inability to activate mTOR in low energy is likely due to activation of Adeonsine monophosphate kinase (AMPK), which appears to act as an energy sensor and activates tuberous sclerosis complex-2 (TSC2) in the face of low cellular energy status, which serves to inhibit mTOR (Hardie et al., 2012). The essential amino acid leucine is thought to be a primary positive nutritional regulator of mTOR, possibly through direct action of the amino acid on the protein itself, however, the exact mechanism by which leucine interacts with mTOR is yet to be fully elucidated. It also appears that leucine likely acts through multiple redundant mechanisms (Kimball et al., 2001). Leucine's main transporter LAT1 (Drummond et al., 2010) may be involved in the process as well as Vps34 and other endosomal proteins (MacKenzie et al., 2009).

Contraction is known to be a potent activator of mTOR and MPS. However, the mechanism by which the contraction signal is detected is not well understood. Recent evidence suggests that phosphorylation (inactivation) of the mTOR inhibitor TSC2 may be involved in the mechanotransduction process. Recent work by Jacobs and colleagues showed that contraction phosphorylates TSC2 at a different site than Akt (Jacobs et al., 2013) but the upstream contraction sensor that transduces the contractile signal is still unknown. Two potential candidates for a contraction sensor are phospholipase D which catalyzes the formation of the second messenger phosphatidic acids and Focal adhesion kinase (FAK). Phospholipase D inhibition has been shown to prevent the mechanical activation of mTOR signalling (Hornberger, 2011). FAK knockdown C_2C_{12} cells are unable to respond to IGF-1 by increases in protein synthesis (Crossland et al., 2013). More work is needed to determine the exact physiological roles of each of the putative contraction sensors.

Multiple lines of evidence from animal (Bodine, et al., 2001) and cell culture models (Crossland, et al., 2013) to acute protein synthetic measurements in humans (Drummond et al., 2009b) to large scale transcriptomic studies (Phillips, et al., 2013) have clearly implicated the mTOR pathway as important in muscle hypertrophy. For this reason many authors have speculated that downstream targets of this pathway could be used as a proxy measure for protein

synthesis or muscle hypertrophy. The earliest demonstration of this concept is work by Keith Baar which showed a correlation between the acute phosphorylation status of p70S6K after an acute bout of electrical muscle stimulation and the magnitude of hypertrophy after repeated electrical stimulation in a rodent model (Baar et al., 1999). The first human study to show a correlation between p70S6K phosphorylation after an acute bout of resistance exercise and training induced hypertrophy was conducted by Terzis et al. (2008) however, this study only included 8 subjects. A weak correlation was also found between p70S6K phosphorylation after an acute bout of resistance exercise and training mediated hypertrophy by a second group (Mayhew et al., 2009). There have also been other studies including one with a very large sample size, which have not shown any relationship between acute phosphorylation of downstream mTOR targets and training mediated hypertrophy (Fernandez-Gonzalo et al., 2013; Phillips, et al., 2013). There are also multiple reports in the literature of weak correlations between both 4EBP-1 (Burd, et al., 2010c) and p70S6K (Kumar, et al., 2009b) and the acute elevation in MPS after resistance exercise. Because negative or non-significant results are less likely to be published there may be additional findings of no relationship between the acute phosphorylation of downstream mTOR targets and hypertrophy which are not included in the literature (Easterbrook et al., 1991). More work investigating both the time course of phosphorylation of mTOR targets and employing larger samples sizes will be required to better understand how acute signalling might relate to long term adaptation.

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CHAPTER 2

TITLE:	Resistance exercise load does not determine training-mediated
	hypertrophic gains in young men

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Resistance exercise load does not determine training-mediated hypertrophic gains in young men

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Mitchell CJ, Churchward-Venne TA, West DW, Burd NA, Breen L, Baker SK, Phillips SM. Resistance exercise load does not determine training-mediated hypertrophic gains in young men. J Appl Physiol 113: 71-77, 2012. First published April 19, doi:10.1152/japplphysiol.00307.2012.-We have reported that the acute postexercise increases in muscle protein synthesis rates, with differing nutritional support, are predictive of longer-term traininginduced muscle hypertrophy. Here, we aimed to test whether the same was true with acute exercise-mediated changes in muscle protein synthesis. Eighteen men $(21 \pm 1 \text{ yr}, 22.6 \pm 2.1 \text{ kg/m}^2; \text{ means } \pm \text{SE})$ had their legs randomly assigned to two of three training conditions that differed in contraction intensity [% of maximal strength (1 repetition maximum)] or contraction volume (1 or 3 sets of repetitions): 30%-3, 80%-1, and 80%-3. Subjects trained each leg with their assigned regime for a period of 10 wk, 3 times/wk. We made pre- and posttraining measures of strength, muscle volume by magnetic resonance (MR) scans, as well as pre- and posttraining biopsies of the vastus lateralis, and a single postexercise (1 h) biopsy following the first bout of exercise, to measure signaling proteins. Training-induced increases in MR-measured muscle volume were significant (P <0.01), with no difference between groups: 30%- $3 = 6.8 \pm 1.8\%$, 80%- $1 = 3.2 \pm 0.8\%$, and 80%- $3 = 7.2 \pm 1.9\%$, P = 0.18. Isotonic maximal strength gains were not different between 80%-1 and 80%-3, but were greater than 30%-3 (P = 0.04), whereas training-induced isometric strength gains were significant but not different between conditions (P = 0.92). Biopsies taken 1 h following the initial resistance exercise bout showed increased phosphorylation (P < 0.05) of p70S6K only in the 80%-1 and 80%-3 conditions. There was no correlation between phosphorylation of any signaling protein and hypertrophy. In accordance with our previous acute measurements of muscle protein synthetic rates a lower load lifted to failure resulted in similar hypertrophy as a heavy load lifted to failure.

skeletal muscle; protein synthesis; motor unit; loading

HEAVIER LOADING [usually expressed as percentage of a person's maximal strength or single repetition maximum (1RM)] is often recommended as the optimal way to maximize muscle hypertrophy with resistance training (1). However, there is very little empirical evidence to support this supposition and it is unclear as to the physiological mechanisms by which heavier training loads would provide a signal for greater muscle hypertrophy compared with, for example, a lighter load lifted to the point of fatigue; both conditions would result in a large amount of muscle fibers being recruited. As proof-of-principle, we recently tested this idea and demonstrated (9) that a single bout of resistance exercise performed at 30% of 1RM to the point of momentary muscle fatigue (failure) was equally as

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effective in stimulating myofibrillar protein synthesis rates (MPS) as loads lifted at 90% of 1RM (also lifted to fatigue). In addition, the 30%-1RM condition resulted in a more prolonged muscle protein synthetic response with a greater elevation of myofibrillar protein synthesis rates than the 90% of 1RM condition at 24 h after exercise (9). We have proposed that the acute exercise-induced increases in MPS that are further augmented with protein ingestion summate and lead to muscular hypertrophy (27). If such a thesis is valid then acute measures of protein synthesis would be, at least qualitatively if not quantitatively, predictive of long-term gains in muscle protein. There is support for this concept as we have shown that measures of acute postexercise MPS, with differing nutritional support (44), are qualitatively predictive of the training-induced phenotypic outcome (17) between young men consuming milk or soy. Therefore our previous results (9) suggest that resistance training performed at 30% of 1RM to fatigue should result in muscle hypertrophy after chronic resistance training that, based on its prolonged stimulation of myofibrillar protein synthesis, is at least equivalent or greater than the degree of hypertrophy resulting from training with heavy loads. Support for this thesis exists from previous training studies and from a number of studies using low loads with vascular occlusion showing equivalent hypertrophy as that with high loads (36-38).

Other than relative training load, another resistance training variable that is often considered important is the volume of work performed (1, 7, 25). Since fatigue limits the number of repetitions that can be completed in a set at a given load, varying the number of sets is a common way to adjust training volume. There is currently disagreement concerning the benefit of additional sets for increases in muscle hypertrophy (11, 29, 34). We have generated acute protein synthesis data from young men showing that 3 sets performed at 70% of 1RM to failure resulted in a greater and more prolonged myofibrillar protein synthetic rate compared with a single set condition (7). We speculate that if the model of summative changes leading to hypertrophy (27) is correct, then our data (7) would mean a greater hypertrophic response with 3 sets vs. 1 set of resistance exercise.

We also wished to test the thesis that early postexercise signaling responses, in particular that of p7056-kinase (p7056K), would be related to hypertrophy as has been shown in humans (39) and rodents (4). This is of interest since if the phosphorylation of one single protein is truly predictive of hypertrophy and strength gains then it is certainly worthy of great attention as to its exact mechanistic role in muscle hypertrophy. The overall purpose of the present study was to test whether the acute results we had observed previously (7, 9) would be reflected

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with longer-term resistance training adaptations. We employed acute measurements of protein signaling to evaluate the relevance of these variables in predicting phenotype.

METHODS

Subjects. Eighteen healthy young men (21 ± 0.8 yr, 1.76 ± 0.04 m, 73.3 ± 1.4 kg; means \pm SE) volunteered to participate in the study after being informed of the procedures and potential risks involved in the investigation. Subjects were recreationally active with no formal weightlifting experience or regular weightlifting activity over the last year. The protocol was approved by the Research Ethics Board of Hamilton Health Sciences and McMaster University and was written in accordance with standards set by the Declaration of Helsinki.

Experimental design. Participants completed 10 wk of unilateral knee extension resistance training. Each leg was randomly assigned in counterbalanced fashion to one of three possible unilateral training conditions: one set of knee extension performed to voluntary failure at 80% of 1RM (80%-1); three sets of knee extension performed to the point of fatigue with 30% of 1RM (80%-3), each participant trained both legs and was therefore assigned to two of the three possible training conditions. Immediately after each training session subjects consumed a source of high-quality protein (PowerBar Protein Plus, 360 kcal, 3.5 g leucine, 30 g protein, 33 g carbohydrate, 11 g fat; Nestle Nutrition, Florham Park, NJ) in conjunction with ~300 ml of water to standardize the postexercise meal and maximize training adaptations.

Before and after the training program, whole muscle volume was measured using magnetic resonance imaging (described below) and changes in muscle fiber area were determined by fiber planimetry with myosin ATPase histochemistry (described below). Knee extension performance was measured by IRM, maximal voluntary isometric contraction (MVC), rate of isometric force development (RFD), and peak power (described below).

Prior to the first training session, participants fasted overnight and consumed a standard liquid meal (480 kcal, 20 g protein, 82 g carbohydrate, 8 g fat) 2 h prior to having resting bilateral muscle biopsies taken from the vastus lateralis. Biopsies were performed with a Bergström needle that was custom-modified for manual suction under local anesthesia (2% xylocaine). Tissue from this biopsy (~80 mg) was used for myosin ATPase histochemistry and Western blotting analysis. Following these initial biopsies, subjects completed their prescribed training session for each leg and immediately consumed a PowerBar Protein Plus bar (Nestle Nutrition, Florham Park, NJ) and 300 ml of water. One hour following the completion of the exercise, bilateral biopsies were taken from the vastus lateralis, for the measurement of acute changes in anabolic signaling molecule phosphorylation status via Western blot. Following 10 wk of training a third set of muscle biopsies was taken from each vastus lateralis muscle and used for fiber area quantification.

Western blots. A piece of wet muscle (~20 mg) was homogenized by hand on ice using a Teflon pestle in a standard Western blotting homogenization buffer (10 µl/mg): 25 mM Tris (pH 7.2) buffer containing 1 mM Na₃VO₄, 50 mM NaF, 40 mM β-glycerolphosphate, 20 mM sodium pyrophosphate, 0.5% vol/vol Triton X-100, and Complete Protease Inhibitor Mini-Tabs (Roche, Indianapolis, IN) was used. The samples were centrifuged at 1,500 g at 4°C for 10 min. The resulting supernatant was removed and protein content was determined by the Bradford assay. Equal aliquots of protein were boiled in Laemmli sample buffer (250 mM Tris-HCI, pH 6.8; 2% SDS; 10% glycerol; 0.01% bromophenol blue; 5%β-mercaptoethanol) for 5 min. Samples (20 µg of protein) were loaded onto 7.5–10% SDS-polyacrylamide gels and run for 1.5 h at 150 V. Gels were then transferred to a PVDF membrane at 100 V for 1 h. Membranes were blocked with 5% skim milk powder (wt/vol) in Tris-buffered saline with 0.1% Tween (vol/vol) (TBST). Membranes were then incubated overnight in primary antibody at 4°C. The following phosphorylation sites were determined: p7086K-1^{Thr389} (Santa Cruz Biotechnology, Santa Cruz, CA; no. 11759, 1:500 dilution in TBST), mTOR^{Ser2448} (Cell Signaling Technology, no. 2971; 1:1,000 dilution in TBST). Att^{Ser473} (Cell Signaling Technology, no. 4056; 1:1,000 dilution in TBST). After washing in TBST, membranes were incubated in horseradish peroxidase-linked anti-rabbit IgG secondary antibody (GE Healthcare, Amersham Biosciences, Piscataway, NJ; no. NA934VS, 1:15,000 dilution in TBST), washed with TBST, and detected by chemiluminescence (SuperSignalWest Dura Extended Duration Substrate, Thermo Scientific, no. 34075). Images were developed using FluorChem SP Imaging system and quantified by spot densitometry using ImageJ software. All signaling protein phosphorylation responses were normalized to their respective total protein.

Magnetic resonance imaging. Subjects rested in the supine position for 1 h prior to scanning to prevent the influence of fluid shifts on muscle volume. Also, no strenuous activity was allowed within 24 h of the scanning. MR imaging was performed in a 3-T HD scanner (Signa MRI System; GE Medical, Milwaukee, WI) at the Brain-Body Institute, Imaging Research Centre, St. Joseph's Healthcare (Hamilton, Ontario). Image acquisition was carried out using T1 fluid attenuation inversion recovery (FLAIR) in the axial plane with the following parameters: repetition time/echo time = 2,574 ms/6.7 ms; field of view = 25-30 cm; matrix size = range from 320/320 to 512/512 phase/frequency; inversion time = 958 ms; slice thickness = 5 mm. Thigh image acquisition utilized an eight-channel torso coil with two excitations. There was a 10 mm gap between slices. Quadriceps volume was calculated by multiplying the slice area by the distance between slices. Volume was measured from the first slice where the rectus femoris was visible to the first slice where the gluteus maximus was visible. ImageJ software (U. S. National Institutes of Health, Bethesda, MD) was used to determine the area of each slice. Pre- and postscans were performed at the same time of day and joint angle and leg compression was controlled.

Muscle function. Subjects completed two testing sessions to assess knee extensor function before and after the 10-wk training period. The two sessions occurred in randomized order and were separated from each other and the first training session by 3 days. Each session started with two sets of submaximal dynamic knee extensions with a load designed not to be fatiguing. Session one consisted of three 5-s unilateral knee extension MVCs conducted on a Biodex dynamometer (Shirley, NY) with 1 min of rest between contractions. A knee angle of 90° was used. The highest recorded torque for each leg was taken as the MVC. The analog torque signal for the Biodex was sampled at 2,000 Hz with PowerLab 3 data acquisition system (ADInstruments, Bella Vista, Australia) and the RFD was calculated off-line by taking the first derivative with respect to time. The dynamometer was then set in isotonic mode and subjects were instructed to move the load as quickly as possible. Three trials at 20, 30, 40, and 50% of MVC were completed in a random order and the highest instantaneous power achieved in any of the trials was recorded as peak power. Subjects then completed a single set of knee extension to muscle fatigue for each leg. The load used for this test was 30% of 1RM for legs assigned to train with 80% of 1RM and 80% of 1RM for legs assigned to train with 30% of 1RM (to assess the endurance of each subject at their nontrained load). The second visit required subjects to complete a single set to fatigue using the percentage of 1RM which they would train with for the 10-wk study. Both the total number of repetitions completed and the total work (the product of repetitions and load) ompleted were recorded for each test.

 \dot{M} uscle fiber cross-sectional area (CSA). Muscle fibers were oriented vertically by visual inspection and embedded in optimal cutting temperature (OCT) medium. The mounted muscle was frozen in isopentane cooled by liquid nitrogen and stored at -80° C until processing for cross-sectional area analysis. Cross sections (10 μm thick) were cut, mounted on glass slides, and stained using a myofibrillar ATPase histochemisty procedure that uses an acid preincuba-

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tion pH of 4.6 to distinguish type I and type II fibers as described previously (24, 33). Pictures of the stained slides were taken using a light microscope and NIS Elements 3.0 Imaging Software (Nikon, NY). An average of 55 fibers of each type (type I and II) were outlined using ImageJ software (U. S. National Institutes of Health, Bethesda, MD) for each subject. Statistics. Our mixed design did not permit us to make within-

Statistics. Our mixed design did not permit us to make withinsubject comparisons; therefore, between-condition differences (muscle hypertrophy, anabolic signaling, and performance) were tested with a blocked two-factor (condition \times time) analysis of variance with repeated measures on time, where applicable. A Tukey's post hoc test was used to test for significant interactions. For all analyses differences were considered significant at P < 0.05. All results are presented as means \pm SE.

RESULTS

Muscle hypertrophy. Prior to training, quadriceps muscle volume was $1,581 \pm 242$, $1,602 \pm 215$, and $1,529 \pm 207$ cm³ in the 30%-3, 80%-1, and 80%-3 groups, respectively (no differences between conditions at baseline). After 10 wk of training, the quadriceps muscle volume increased significantly in all groups (P < 0.001) to $1,676 \pm 198$, $1,651 \pm 213$, and $1,633 \pm 198$ cm³ in the 30%-3, 80%-1, and 80%-3 groups, respectively. Figure 1 depicts these data expressed as percentage change from baseline. Average type I and type II muscle fiber area increased with training (both P < 0.05), irrespective of training condition with no significant between-group differences (Table 1).

Western blots. Phosphorylation of Akt at Ser473 was not elevated in any of the conditions. Phosphorylation of mTOR at Ser2448 was elevated above rest at 1 h postexercise in all conditions (P = 0.0002). p70S6K phosphorylation at Thr389 was elevated 1 h postexercise in the 80%-1 and 80%-3 groups, but not 30%-3. There was no correlation between the degree of p70S6K phosphorylation at Thr389 and changes in muscle volume within any training condition (all P > 0.3). Overall, there was no correlation between the degree of p70S6K phospphorylation at Thr389 and the magnitude of quadriceps hypertrophy (r = -0.03, P = 0.88; Fig. 2D). There was also no correlation between the degree of p70S6K phosphorylation at Thr389 and the magnitude of fiber hypertrophy for either fiber (data not shown).

Muscle function. Initial unilateral knee extension 1RM was 71 ± 2 kg in the 30%-3 condition, 76 ± 2 kg in the 80%-1



Fig. 1. Percentage change in quadriceps muscle volume following 10 wk of resistance training. The 3 training condition groups differed in contraction intensity [% of maximal strength (1 repetition maximum)] or contraction volume (1 or 3 sets of repetitions): 30%-3, 80%-1, and 80%-3. There was a significant main effect for time (increase in quadriceps volume pre- to post-training, P < 0.0001). N = 12 legs in each condition.

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nsion Peak r, W	Post*	808. ± 805 ± 805 ± vs. post (<i>I</i>
Knee Exter Powe	Pre	589 ± 88 626 ± 113.2 607 ± 113.2 main effect pre-
ge in Fiber	Type II	20 ± 5% 16 ± 5% 18 ± 8% ∗Significant
Percentage Cha Size	Type I	16 ± 7% 17 ± 4% 30 ± 12% uy contraction.
iber Area, µm ²	Post*	7,604 ± 1,019 6,784 ± 1,019 6,782 ± 1,100 C, maximal volunts
Average Type II Fibe	Pre	6,430 ± 869.5 5,873 ± 715.4 6,052 ± 1,189.6 æ development; MV ^v
Fiber Area, μm^2	Post*	5,163 ± 589 4,970 ± 882 5,324 ± 1,167 s. RFD, rate of forc
Average Type I	Pre	4,532 ± 739 4,268 ± 701 4,315 ± 685 r all comparison
s/m·N	Post*	1,259 ± 256 1,110 ± 234 1248 ± 335 ach conditions fo
RFD,	Pre	768 ± 150 817 ± 101 747 ± 186 $V = 12 \log \sin e$
м·ш	Post*	248 ± 52 255 ± 47 278 ± 52 reans \pm SE; <i>h</i>
MVC,	Pre	192 ± 37 188 ± 42 219 ± 37 2resented as n
	Group	0%-1 set 0%-3 sets 0%-3 sets Values are I 05).

vastus lateralis fiber area following 10 wk of resistance training

and

Table 1. Changes in knee extension performance

× 124

Fig. 2. Phosphorylated Akt, mammalian target of rapamycin (mTOR), and P7056K at rest and 1 h following resistance exercise. A: Akt phosphorylated at Ser 473 expressed relative to total Akt. There are no significant differences between conditions. B: mTOR phosphorylated at Ser 2448 relative to total mTOR. "Main effect for greater phosphorylated mTOR 1 h following resistance exercise (P < 0.05). C: p7056K phosphorylated at Thr 389 relative to total p7056K. "Significant increases in phosphorylated p7056K 1 h after 1 or 3 sets at 80% of one repetition maximum (IRM) but not 3 sets at 30% of 1RM (P < 0.05). D: correlation between p7056K phosphorylation and muscle hypertrophy, r = -0.03 (P = 0.88). N = 12 legs in each condition.



condition, and 73 \pm 2 kg in the 80%-1 condition. After the training period all conditions significantly increased in 1RM strength. The increase in 1RM strength was greater in the 80%-1 and 80%-3 conditions compared with the 30%-3 condition (P = 0.04; Fig. 3). MVC force, knee extension maximal power output, and RFD increased in all conditions with no between-condition differences (Table 1). The total work that could be completed with 30% of the subject's 1RM increased with no between-condition differences (Fig. 4). The total work that could be completed with 80% of the subject's 1RM increased in all groups. The magnitude of the increase was significantly less in the 30%-3 condition compared with the other conditions. The number of repetitions that could be performed with 80% of their current 1RM increased in all groups from 10 \pm 1 (30%-3), 10 \pm 1 (80%-1), and 11 \pm 1 (80%-3) pretraining to 12 ± 1 (30%-3), 13 ± 1 (80%-1), and 12 ± 1 (80%-3) with no-between condition differences in the magnitude of the increase. The number of repetitions that could be performed with 30% of 1RM increased in the 30%-3 condition; the other conditions did not increase.

DISCUSSION

We have advocated a model of resistance exercise-induced human skeletal muscle hypertrophy that, when supported by adequate nutrition, arises over time due to summed incremental acute increases in muscle protein synthesis that occur after each training session (27, 28). Thus, as a proof-of-principle of this model, we tested here whether the acute changes we observed in two previous studies comparing different volumes of work (1 set vs. 3 sets of exercise) (7) and divergent intensities of work (high-load vs. low-load lifting) (9) would be borne out in a long-term study. We discovered that there was no difference in the magnitude of quadriceps muscle hypertrophy, as determined by both MRI and muscle fiber area, between legs that trained at 30% or 80% of 1RM after 10 wk of knee-extensor exercise. Interestingly, there was no statistical difference in the degree of quadriceps hypertrophy between the 80%-1 and 80%-3 conditions, despite a mean gain in quadriceps volume of \sim 7% in the 80%-3 condition and only \sim 3% in the 80%-1 condition (P = 0.18). However, the 80%-3 and 30%-3 showed more than double the average hypertrophy of the 80%-1 condition. These results, while not quantitatively congruent with our acute data (7, 9), are, we propose, broadly supportive of the framework we have proposed of how muscle hypertrophy arises (27, 28). Moreover, our results are actually congruent with a number of other lines of evidence showing that lifting lighter loads, so long as fatigue is induced, leads to roughly equivalent hypertrophy and strength gains (23, 36-38). There are of course a number of factors, beyond acute changes in muscle protein synthesis, which contribute to hypertrophy. In fact, when subjects are stratified as high and low responders, 20-25% subjects exhibit a very limited hypertrophic response where as the top 20-25% show robust muscle hypertrophy that is four to five times greater than that seen in low responders (17, 26). To date, factors such as changes in microRNA expression (12), satellite cell number (26), and intramuscular anabolic signaling protein activation (39) have been shown to be related to the variability in training response, but systemic hormonal factors do not play a role (41-43).

Perhaps the most interesting finding from our work is that hypertrophy in the 80%-3 and 30%-3 conditions was equivalent, which is in contrast to the range of lifting intensities usually prescribed to promote muscle hypertrophy (1, 10). However, the current recommendations (1, 10) ignore a large body of evidence showing that lower loads, when combined with vascular occlusion, promote equivalent hypertrophy and

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Fig. 3. Knee extension strength. A: the maximal load that could be lifted prior to training and after 10 wk of training. *Significantly greater than prior to training (P < 0.05). †Significantly greater than the 3-sets a 30% of 1RM condition. B: single leg isometric knee extension torque before and after 10 wk of training. *Significantly greater than prior to training (P < 0.05). N = 12 legs in each condition.

strength gains as that observed with conventional heavy training (36–38). These results indicate that at least in principle lower load are effective at inducing muscle hypertrophy. Moreover, most work that has investigated different relative resistance training loads has focused on muscle function measurements such as 1RM and relative endurance (5, 6, 13, 35). In one study by Campos and colleagues (10), 8 wk of training in a 20- to 28-repetition range did not elicit hypertrophy despite increases in 1RM strength and the number of repetitions that could be completed with 60% of 1RM (10). However, in a subsequent study in which the training methods employed previously by Campos et al. (10) were replicated, equivalent hypertrophy was found in high- and low-load training groups (23). It is often claimed (1, 10) that high relative training loads are necessary to induce hypertrophy as they are associated with full muscle fiber recruitment and activation of the type II fibers which are known to be more "responsive" to hypertrophy (40). This statement is, however, only accurate during a single effort (repetition) since Henneman's size principle of motor unit activation states that motor units are recruited in an orderly fashion from smallest to largest with increasing requirement for force generation (18, 19). Thus it is true that an isometric contraction performed at 30% 1RM will recruit less muscle than a contraction preformed at 80% of 1RM (2). In agreement, it has also been shown that nonfatiguing acute resistance exercise or chronic resistance training results in lower rates of MPS (21) and hypertrophy (20) compared with heavier resistance exercise loads, respectively. However, when a submaximal contraction is sustained, motor units that were initially recruited will fatigue (produce less force) or cease firing completely necessitating the recruitment of additional motor units (15) to sustain force generation. In this way, as the repetitions at lighter loads are repeated, the point of failure/ fatigue ultimately necessitates near maximal motor unit recruitment to sustain muscle tension (16). Thus relatively lighter loads lifted to the point of failure would result in a similar amount of muscle fiber activation compared with heavier loads lifted to failure (18, 31); however, we acknowledge it is difficult to experimentally verify this motor unit recruitment strategy during voluntary dynamic contractions in humans. In the present study, the average area of both type I and II fibers increased equally with heavy and light relative loads, which is suggestive that both fiber types were recruited during training and to a roughly equivalent extent, at least insofar as the phenotypic hypertrophy response is an indication of this.



Fig. 4. Maximal work and repetitions with 30 and 80% of 1RM before and after 10 wk of resistance training. Load product (in arbitrary units) is the product of load in kg and repetitions completed. A: work completed at 80% of the subject's 1RM (determined within 1 wk of the tsubject's 1RM with 80% of 1RM pre- to posttraining (P < 0.05). f Significantly less work at 80% 1RM after training in the 3-sets at 30% of 1RM condition than the other two groups (P < 0.05). B: work completed with 30% of the subject's 1RM. *Significant main effect for an increase in work performed with 30% of 1RM pre- to posttraining (P < 0.05). C: maximal number of repetitions completed with 80% of 1RM (determined within 1 wk of the test). *Significant main effect for an increase in the number of repetitions performed with 80% of 1RM pre- to posttraining P < 0.05. D: maximal number of repetitions completed with 30% of 1RM oco, 0.5), s^3 sets at 30% of 1RM condition completed more repetitions after training than before (P < 0.05), s^3 sets at 30% of 1RM condition completed more repetitions after training than before (P < 0.05).

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The regulation of muscle mass is the result of small changes in net muscle protein balance over time. MPS can be increased by resistance exercise alone; however, adequate and properly timed protein consumption is required for a positive net muscle protein balance and thus muscle hypertrophy (8). It is not completely clear how resistance exercise results in increases in MPS as this process is multifaceted. One potentially important step is the activation of p70S6K, which is a downstream protein target of mTORC1 that when activated upregulates initiation of mRNA translation and subsequently increases muscle protein synthesis rates (14). It has been reported that heavy resistance exercise results in an increase in p70S6K phosphorylation (7, 9, 42); our data, at least in the 80%-1 and 80%-3 conditions, broadly agree with these findings. We did not observe an increase in p70S6K phosphorylation in the 30%-3 condition at 1 h postexercise, but we have observed elevated phosphorylation of p70S6K at 4 h after exercise when subjects have completed the same protocol (9). Taken together these results suggest that heavy and light relative loads lifted until the point of failure may result in a different time course of anabolic signaling, with p70S6K phosphorylation occurring later after exercise with light compared with heavy relative loads. Previously, a significant correlation between early (1 h postexercise) phosphorylation of p70S6K and muscle hypertrophy was observed (39); our results, which include a larger sample size than the previous investigation, failed to support a similar relationship (Fig. 2D).

Although relative training load did not impact the magnitude of the hypertrophic response, it did have a clear impact on voluntary isotonic strength gains. Both the 80%-1 and 80%-3 conditions demonstrated a larger increase in 1RM strength compared with the 30%-3 group. These results suggest that practice with a heavy relative load is necessary to maximize gains in 1RM strength of the trained movement. These observations are in line with previous work that has shown that strength gains are specific to the movement that is trained (30) and strength gains are due to a combination of muscle hypertrophy and neural adaptations (32). It should be noted, however, that similar gains in MVC strength, maximal instantaneous power output, and rate of force development were seen across all three training conditions. These data show that hypertrophy is generally beneficial to all strength and power tests that engage the larger muscle. However, it appears that neural adaptations are largely specific to the movement and load used in training (32).

One potential limitation to the unilateral training model employed in this study is the cross education effect where resistance training of one muscle can lead to neurally mediated strength gains in the untrained contralateral muscle (22). The magnitude of the effect varies widely but averages \sim 7% (22). However, to the author's knowledge there has been no attempt to quantify this effect when both limbs are training with different protocols. We found no correlation between isotonic strength gains in the left and right legs (r = 0.33, NS), suggesting that the cross education effect is minimal or nonexistent when both limbs are training with different protocols.

In summary, we report that similar resistance traininginduced muscle hypertrophy can result from lifting loads to failure with higher (80% of 1RM) and lower (30% of 1RM) loads than are currently recommended for novice lifters (1). The results from our study also suggest that additional training volume in the form of more sets may result in greater muscle hypertrophy; however, due to the inherent variability in the individual response to resistance training, it appears that longer-term training studies may be required to manifest these differences more clearly. Importantly, these data support the concept that acute increases in rates of MPS are reasonable qualitative indexes of the amount of muscle protein gain with similar training as they appear to be with nutrition. However, these gains in muscle mass may be dependent in the adequate provision of amino acids (3). Finally, despite that lack of support for the idea of a hypertrophy-specific load and repetition range, these data confirm the specificity principle of training with regard to muscle strength and endurance.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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CHAPTER 3

- TITLE: Muscular and Systemic Correlates of Resistance Training-Induced Muscle Hypertrophy
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Muscular and Systemic Correlates of Resistance Training-Induced Muscle Hypertrophy

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Abstract

Purpose: To determine relationships between post-exercise changes in systemic [testosterone, growth hormone (GH), insulin like grow factor 1 (IGF-1) and interleukin 6 (IL-6)], or intramuscular [skeletal muscle androgen receptor (AR) protein content and p7056K phosphorylation status] factors in a moderately-sized cohort of young men exhibiting divergent resistance training-mediated muscle hypertrophy.

Methods: Twenty three adult males completed 4 sessions•wk⁻¹ of resistance training for 16 wk. Muscle biopsies were obtained before and after the training period and acutely 1 and 5 h after the first training session. Serum hormones and cytokines were measured immediately, 15, 30 and 60 minutes following the first and last training sessions of the study.

Results: Mean fiber area increased by 20% (range: -7 to 80%; P<0.001). Protein content of the AR was unchanged with training (fold change = 1.17 ± 0.61 ; P=0.19); however, there was a significant correlation between the changes in AR content and fiber area (r=0.60, P=0.023). Phosphorylation of p70S6K was elevated 5 hours following exercise, which was correlated with gains in mean fiber area (r=0.54, P=0.007). There was no relationship between the magnitude of the pre- or post-training exercise-induced changes in free testosterone, GH, or IGF-1 concentration and muscle fiber hypertrophy; however, the magnitude of the post exercise IL-6 response was correlated with muscle hypertrophy.

Conclusion: Post-exercise increases in circulating hormones are not related to hypertrophy following training. Exercise-induced changes in IL-6 correlated with hypertrophy, but the mechanism for the role of IL-6 in hypertrophy is not known. Acute increases, in p70S6K phosphorylation and changes in muscle AR protein content correlated with muscle hypertrophy implicating intramuscular rather than systemic processes in mediating hypertrophy.

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Introduction

It is well established that resistance training can lead to muscle hypertrophy [1], which appears to be the result of accumulated periods of post-exercise increases in muscle protein synthesis that exceed muscle protein breakdown, resulting in net protein accretion over time [2]. We have examined how differences in contractile paradigms affect skeletal muscle protein synthesis (MPS) [3–5]. Subsequently, we have studied the influence of some of these same variables in affecting skeletal muscle hypertrophy following prolonged training [6,7] with general agreement between short-term measurements of MPS and hypertrophy. While some studies

have shown small effects of training variables such as rest periods between sets and relative training load on muscle hypertrophy [7,8], other studies have not [6,9]. A consistent observation is a high degree of heterogeneity in the hypertrophic response to resistance training [10,11]; the underlying causes of this variability in hypertrophic response are unclear.

As potential explanations for this hypertrophic heterogeneity changes in myogenic gene expression [12], microRNA abundance [13], and the capacity for myonuclear addition via satellite cells [14], have been examined. Other research has examined acute post-exercise changes in circulating factors proposed to be anabolic for muscle such as testosterone.

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growth hormone, and IGF-1 [15,16]. However, we have proposed, and provided evidence, that changes in these circulating factors after a single session of resistance exercise are unrelated to the magnitude of the MPS response or to muscular hypertrophy with resistance training [17-19]. In contrast many state that the acute hormonal response to resistance exercise is an important driver of hypertrophy and have used a transient hormonal response to establish efficacy of interventions, including exercise and nutrition-based changes, to ascribe significance on a phenotypic and functional level: for reviews see [20,21]. Importantly, the anabolic action of testosterone in muscle is mediated via androgen receptors (AR), the mRNA for which has been shown to be up-regulated by resistance exercise [22], and that changes in its protein expression have been reported to correlate with the magnitude of muscle hypertrophy [23]; thus, we sought to ascertain whether changes in AR protein played a role in mediating hypertrophy.

The cytokine interleukin-6 (IL-6) has been implicated as a regulator of satellite cell function [24] and its release after resistance exercise correlates well with IL-6 measured in the muscle and in the blood [24]. Circulating IL-6 concentrations have also been taken as an indicator of resting inflammatory status [25]. Interestingly, in post-menopausal women a strong relationship between abdominal adiposity and IL-6 was negatively associated with changes in IL-6 and lean mass gain with resistance training [26]. It is unclear in young healthy men if the IL-6 response to resistance exercise is related to muscle hyportroohy after training.

In a retrospective analysis we reported that the acute response of various hormones (testosterone, GH, and IGF-1), often stated as being key anabolic drivers of hypertrophy [20,21] with resistance training, were unrelated to the hypertrophic response with resistance training [19]. This analysis [19] did, however, involve a nutritional manipulation, which could have obscured the true nature of the hormonal influence on hypertrophy. Thus, the purpose of the present study was to prospectively assess the relationship between the magnitude of the acute rise in circulating hormones testosterone, GH, and IGF-1 - following resistance exercise early and late during a period (16 wk) of training and examine the relationship with muscle hypertrophy. We examined changes in muscle AR protein content and acute changes in p70S6K phosphorylation since they are proteins that have been shown to be related to the hypertrophic response in humans [23,27]. We also examined the relationship between the acute IL-6 response to resistance exercise and the magnitude of hypertrophy following training [24].

Methods

Subjects

Twenty three young healthy adult males (177 ± 8 cm, 84.1 ± 16.9 kg, 26.4 ± 4.4 kg/m², 24 ± 3 yr) participated in the study. Subjects were recreationally active but had not participated in any resistance training for at least one year. All were deemed healthy based on responses to a standard medical screening questionnaire. Strength testing was conducted to determine

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voluntary isotonic strength defined by 1 repetition maximum (1RM) for the leg press and chest press exercise at the start and end of the training period. Subjects refrained from strenuous physical activity for 72 h before the testing sessions.

Ethics Statement

The study was approved by the Hamilton Health Sciences Research Ethics Board and conformed to standards for the use of human participants in research as outlined in the 5th Declaration of Helsinki and with current Canadian Tri-council research agency guidelines for use of human participants in research (<u>http://www.pre.ethics.gc.ca/eng/policy-politique/</u> <u>initiatives/tcps2-eptc2/Default/</u>). Informed written consent was obtained from all participants prior to entering the study.

Resistance Training Protocol

Subjects underwent 16 wk of progressive full body resistance training consisting of four training sessions per week. Each week consisted of two upper body and two lower body training sessions. The upper body exercises were chest press, shoulder press (Hammer Strength, Lake Forest, IL), lat pull down, rhomboid row (Atlantis, Laval, Quebec), bicep curl, and triceps extension (Hur, Kokkola, Finland). The lower body exercises were leg press (Maxam, Hamilton, Ontario), leg extension (Atlantis, Laval, Quebec), leg curl and calf press (Hur, Finland). The program consisted of 4 blocks of 4 weeks each. The first block consisted of 3 sets (2 sets for the first week) of 12 repetitions for each exercise and 60s rest between sets. Block two consisted of 3 sets (4 during the last week) of 10 with 90s rest between sets. The third block consisted of 4 sets (two sets for week 1) of 8 repetitions with 100s rest between sets. The last block consisted of 4 sets (3 sets for week 1) of 6 repetitions with 120s between sets. The last set for each exercise performed was performed to the point of momentary muscular failure. To maximize the potential for strength and lean mass gains as a result of training [28] subjects immediately consumed, after each training session and with breakfast on non-training days, a protein beverage containing 30 g of milk protein, 25.9 g of carbohydrates and 3.4 g of fat (Musahi P30, Notting Hill, Australia).

Hormone/Cytokine Testing

After the strength testing, subjects rested for at least 96 h. Subjects reported to the lab for a resting blood sample and muscle biopsy of their vastus lateralis muscles. Subjects then underwent an acute bout of resistance exercise which consisted of leg press, leg curl, leg extension and call press performed for 4 sets of 8 repetitions with 2 minutes rest between sets. At the end of the resistance exercise session, a blood sample was taken and the protein beverage described above was consumed. Blood samples were then taken 15, 30 and 60 minutes post exercise. Muscle biopsies (~100mg) were also taken 1 hour and 5 hours after the resistance exercise. The acute resistance exercise session were repeated after the training period, the 8RM load was set based on the subjects my recent 1RM test.

Western Blotting

Muscle samples were homogenized on ice in buffer as previously described [29]. Protein concentrations were determined via BCA protein assay (Thermo Scientific, Rockford, IL, USA). Working samples of equal concentration were then prepared in Laemmli buffer [30] and equal amounts of protein (20 µg) were loaded onto 10% precast gels (BIO-RAD Mini-PROTEAN TGX Gels, Bio-Rad Laboratories, USA) for separation by electrophoresis. Proteins were then transferred to a polyvinylidene fluoride membrane, blocked in 5% skim milk (AR) or 5% Bovine serum albumin (p70S6K) for 1 h, and incubated overnight at 4°C in primary antibody against the AR receptor (1:2000, abcam, #ab3510) or phosphorylated p70S6KThr 389. (1:1000 Santa Cruz Biotechnology # sc-11759) Membranes were then washed in TBST and incubated in secondary antibody for 1h at room temperature before protein detection with chemiluminescence (SuperSignalWest Dura Extended Duration Substrate, ThermoScientific, #34075) on a FluorChem SP Imaging system (Alpha Innotech, Santa Clara, CA, USA). Total AR and phosphorylated p70S6K protein was expressed relative to a-tubulin abundance (1:2000, Sigma-Alderich, St. Louis, MO, USA #T6074) and is presented as foldchange from pre- to post-training. Images were quantified by spot densitometry using ImageJ software (National Institute of Health, USA).

Blood Analysis

Serum blood samples were collected into 4 ml evacuated tubes from a 22 gauge polyurethane catheter inserted into the antecubital vein while a 0.9% saline drip was used to keep the catheter patent. Serum was then left to clot at room temperature for one hour before being centrifuged at 4000rpm for 10 minutes. Blood plasma was then collected and frozen at -20°C until further analysis. Plasma samples were analyzed for serum growth hormone, free testosterone, IGF-1, IL-6, TNF-a, CRP and cortisol using solid-phase, two site chemiluminescence immunometric assays (Immulite: Intermedico, Holliston, MA). All intra-assay coefficients of variation for these hormones were below 5% and all assays included external standards and daily quality controls. The hormone concentrations are uncorrected for changes in plasma volume since these are the concentrations to which potential target tissues would have been exposed.

Muscle Fiber CSA

Muscle biopsies were obtained before and after the 16 wk training period from the vastus lateralis muscle using a 5-mm Bergström needle custom modified for manual suction under local anaesthesia (2% xylocaine). Muscle biopsy samples were embedded in optimal cutting temperature (OCT) and frozen in liquid cooled isopentane in preparation for sectioning and analysis via immunofluroescence. Muscle sections 7µm in thickness were cut placed on a glass slide and allowed to cool at room temperature for 30min before being fixed for 10min in 4% paraformaldehyde. Sections were then washed 3x5min in phosphate buffered saline (PBS) with Tween, and blocked for 1hr RT (in PBS containing 2% bovine serum albumin, 5% fetal bovine serum, 0.2% Triton x-100, 0.1% sodium azide, and 2%

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goat serum). Primary antibody incubation in Laminin (1:750, Abcam ab11575), MHCI (neat, DSHB), and MHCII (1:1000, Abcam ab91506) was completed for 2hr RT or overnight at 4°C. Secondary antibody detection included Laminin (Alexa Fluor 488 goat anti-rabbit, 1:500, Invitrogen A11008), MHCI (Alexa Fluor 488 goat anti-mouse, 1:500, Invitrogen A11029), and MHCII (Alexa Fluor goat anti-rabbit, 1:500, Invitrogen A21244), for 2hr at RT, Nuclei were revealed with DAPI (4'.6diamidino-2-phenylindole) (1:20000, Sigma D-8417), followed by cover slipping slides with fluorescent mounting media (DAKO S3023). Images were revealed with the Nikon Eclipse 90i microscope at 200x magnification and captured with a highresolution QImaging fluorescent camera (Nikon, Tokyo, Japan). Fibre CSA was quantified manually in a blinded fashion using the Nikon NIS Elements AR 3.0 software on large scale images consisting of >100 fibres.

Statistics

Pre to post training changes with a single mean were assessed using paired Student's T-tests. Relationships between variables were assessed with Pearson's product moment correlation. Changes in muscle fiber area were assessed with a two way (time by fiber type) repeated measures ANOVA. A stepwise multiple linear regression model was used to assess the contribution of various independent variables to the change in mean fiber area. Only those independent variables that were significantly correlated with changes in mean fiber area. Only those independent variables that were significantly correlated with changes in mean fiber area and had F probabilities less than 0.05 were entered into the model. The model with the highest proportion of variance, which met criteria above, is reported. Alpha was set at $P \sim 0.05$ and results are reported as means \pm SD. All analyses were completed using SPSS version 20 (IBM, Armonk, New York).

Results

Sixteen weeks of resistance training resulted in a 61% increase in isotonic strength as measured on the leg press, and a significant increases in both type I (18%; range -22 to 106%) and II muscle fiber area (23%; range -4 to 67%), with a significantly greater increase in type II fiber area (P=0.011).

As a result of training there were significant reductions in the resting concentrations of free testosterone, GH, IGF-1and cortisol. There were no changes in the resting concentration of IL-6 or TNFa, however, there was an increase in the concentration of CRP (Table 1). The acute post-exercise AUC for free testosterone, GH, and IGF-1 decreased from pre- to post-training. The magnitude of the acute cortisol, IL-6, TNF-a and CRP responses were unaffected following 16 weeks of resistance training (Table 2). Mean muscle AR protein content was unchanged following the training period; however, there was a significant correlation between the change in AR content and the increase in mean fiber area (Figure 1). Phosphorylation of p70S6K was not increased above rest at 1 h following an acute bout of resistance exercise, but was significantly increased at 5 h post exercise (Figure 2a). Phosphorylation of p70S6K (fold change) at 5h was correlated with changes in muscle fiber area (Figure 2b) There were no relationships

 Table 1. Pre- and post-training resting hormone and cytokine concentrations, muscle fibre cross-sectional area, and strength.

	Pre	Post	Р
Free Testosterone (pM)	150 ± 9	144 ± 9	0.000
GH (ug/L)	1.6 ± 0.5	1.4 ± 0.4	0.009
IGF-1 (nM)	38.4 ± 3.9	37.0 ± 4.6	0.005
Cortisol (nM)	593 ± 84	543 ± 74	0.000
IL-6 (pg/mL)	2.2 ± 0.6	2.1 ± 0.7	0.122
TNF-alpha (pg/mL)	0.87 ± 0.34	0.78 ± 0.26	0.186
CRP (nM)	102 ± 9	110 ± 19	0.007
Mean CSA (µm²)	5928 ± 1651	7013 ± 1471	0.000
Type II CSA (µm ²)	6284 ± 1869	7542 ±1736	0.000
Type I CSA (µm ²)	5355 ± 1553	6098 ±1486	0.001
Leg Press 1RM (kg)	236 ± 70	380 ±73	0.000
Androgen Receptor (fold change)	1	1.17 ± 0.61	0.186

GH – growth hormone, IGF-1 – insulin-like growth factor-1, TNF-a – turnour necrosis factor-a, CRP – C-reactive protein, CSA – cross sectional area, 1RM – single repetition maximal strength.

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Table 2. Pre- and post-training area under the curve of acute hormonal and cytokine responses to a single bout of resistance exercise.

	Pre	Post	Р
Free Testosterone	7557 ± 1414	7190 ± 1417	0.000
GH	443 ± 158	420 ± 149	0.000
IGF-1	398 ± 478	335 ± 499	0.043
Cortisol	5103 ± 9157	4613 ± 8019	0.142
IL-6	131 ± 48	131 ± 48	0.261
TNF-alpha	17± 17	18 ± 19	0.119
CRP	572 ± 771	580 ± 1424	0.968

Gr = growin nominite, nor-1 = insum-line growin raction-1, nor-d = turnour necrosis factor-d, CKP = Creature protein. Area under the curve was calculated based on the serum concentrations immediately after exercise and, 15, 30 and 60 min following exercise with baseline (resting) values subtracted. AUC values presented in arbitrary units.

Discussion

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between free testosterone, GH, and IGF-1 post exercise AUC responses measured before (Table 3) or after (free testosterone: r=0.02, P = 0.932, GH: r=0.37, P = 0.079, IGF-1: r=-0.25, P = 0.249) the 16 week training period and changes in mean muscle fiber area CSA (Table 3). There was a significant relationship between the acute response of IL-6 both pre-(Figure 3) and post-training (r=0.47, P = 0.023) and increases in mean muscle fibre CSA. A stepwise multiple linear regression model revealed that only two variables accounted for a significant proportion of variance related to the traininginduced change in mean fiber area: change in AR protein content and the magnitude of p70S6K phosphorylation at 5h (Table 4). The R² of the model after adjustment for multiple variables was 0.46. Although IL-6 AUC was significantly correlated with changes in mean fiber area it was not included because the F probability when included in the model was greater than 0.05.

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[19].

As with many phenotypic changes with exercise training

muscular hypertrophy in response to resistance training shows

a high degree of variability, the source of which is unknown

[10,11]. We discovered that the increase in AR protein content

with resistance training, the magnitude of acute increase in

p70S6K phosphorylation, and the response of IL-6 to an acute

bout of resistance exercise were significantly correlated with

the magnitude of muscle fiber hypertrophy in a moderate-sized

cohort of young men following 16 weeks of resistance. Using

multiple regression analysis we found that only changes in AR

content and phosphorylation of p70S6K were significant

variables in a model that accounted for 46% of the variance in the hypertrophic response. Our findings also corroborate

previous work from our lab showing no relationship between

the acute increase in circulating free- testosterone, IGF-1, or

GH and the magnitude of muscle hypertrophy following training



Figure 1. Correlation between the fold change in muscle AR protein content and changes in skeletal muscle fibre area following 16 weeks of resistance training. r = 0.60, P=0.003. doi: 10.1371/journal.cone.0078636.0001

The exact role of the AR in muscle hypertrophy has yet to be elucidated. The abundance of the muscle AR mRNA does not increase acutely following resistance exercise but does tend to increase 48h following an acute bout of resistance exercise [22,23]. This pattern of AR mRNA up regulation persists for at least three training sessions but does not seem to be preserved with longer term training [31]. Our study corroborates previous findings that mean AR protein expression was not increased following resistance training; however, the response had marked heterogeneity, with some subjects showing a marked (1.5 - 2.5 fold) increase in AR protein content [23]. Despite no statistically significant change in AR receptor protein content, there was a correlation between AR protein content with fibre hypertrophy. Our results suggest that changes in AR content may be part of a muscle-specific response present to a greater degree in responders and responsible for some (~25%) of the variation in muscle fibre hypertrophy [32].

The protein kinase B (Akt)-mammalian target of rapamycin (mTOR) pathway is thought to be critically important in regulating muscle protein synthesis and hypertrophy. Although the mTOR complex is an important regulator of protein synthesis, simple phosphorylation of mTOR has never been shown to correlate with muscle protein synthesis or hypertrophy, thus it was not measured in this study. The protein kinase p7056K is a target of mTOR and its acute

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phosphorylation following resistance exercise has been reported to correlate with muscle hypertrophy following training

[27,33]; however, not all studies have found a relationship

[6,34]. We saw no increases in p70S6K phosphorylation 1h

after exercise, however, phosphorylation was elevated 5h post-

exercise. The 5h phosphorylation was correlated with muscle

hypertrophy suggesting that individuals who showed a later

(5h) phosphorylation of p70S6K may exhibit greater

hypertrophy. In this study basal fasting phosphorylation status

was compared to the phosphorylation status of each target

after both an acute exercise bout and the ingestion of a drink

containing 30g of protein. It is possible that the observed

results were partly due to the effects of nutation alone [35]. We

chose to examine the acute phosphorylation of proteins to the

response to combined exercise and nutrition because the

provision of post exercise protein results in greater hypertrophy

following training [28] and subjects were given an identical

hypertrophy is in agreement with previous work from our

laboratory [19,36]. Supraphysiological doses of testosterone

dramatically enhance resistance training-induced muscle

hypertrophy [37]; in contrast, the current, and previous [19,36],

findings of no relationship between the acute rise in testosterone and muscle hypertrophy could be explained in one

of two ways. First, other local factors are far more important in

The lack of correlation between free testosterone and muscle

protein supplement after every workout.



Figure 2. Phosphorylation of p70S6K following an acute bout of resistance exercise before 16 weeks of resistance training and the percentage change in skeletal muscle mean fibre area following the training * P < 0.05. A) Fold change in P70S6K phosphorylation. B) Correlation between 5h fold change in P70S6K phosphorylation and the percentage change in skeletal muscle mean fibre area following the training period. r = 0.54, P = 0.007. doi: 10.1371/journal.pone.078636.g002

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Table 3. Pearson correlations between pre-training hormone, cytokine, and androgen receptor (AR) response and changes in muscle fibre cross-sectional area (CSA) following training.

Variable		Change in Mean CSA	Change in Type II CSA	Change in Type I CSA
Fold change AR	r	0.60	0.60	0.47
	P	0.003	0.002	0.023
Free Testosterone AUC	r	0.06	0.07	0.10
	Р	0.771	0.760	0.639
IL-6 AUC	r	0.48	0.42	0.51
	Р	0.019	0.047	0.013
GH AUC	r	0.39	0.40	0.38
	Р	0.069	0.061	0.073
IGF-1 AUC	r	-0.30	-0.27	-0.23
	Р	0.165	0.219	0.292
Cortisol AUC	r	-0.02	0.00	-0.09
	Р	0.919	0.990	0.670
TNF-alpha AUC	r	0.02	-0.05	0.10
	Р	0.919	0.812	0.652
CRP AUC	r	0.18	0.13	0.06
	Р	0.420	0.567	0.784

AR – androgen receptor, IL-6 – interleuking-6, GH – growth hormone, IGF-1 – insulin-like growth factor-1, TNF-α – turnour necrosis factor-α, CRP – C-reactive protein, CSA – cross sectional area, 1RM – single repetition maximal strength

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regulating muscle hypertrophy, and testosterone is thus merely a permissive hormone within its normal physiological range and only has affects during hypo- or hyper-testosteronemic states. The other possible explanation is that the intramuscular concentration of testosterone, where it is more active and bound to its receptor protein, is poorly related to its concentration in circulation. Thus, measurement of circulating testosterone concentrations following exercise is not a marker for, or in any way related to, skeletal muscle hypertrophy; however, intramuscular testosterone, or testosterone-AR complex formation, may be important regulators of protein synthesis and muscle hypertrophy.

IGF-1 is thought to be a potential regulator of muscle hypertrophy acting directly through the Akt/mTOR pathway [38] as well as co-localizing with satellite cells following resistance exercise [39]. It is proposed that local autocrine or paracrine IGF-1 plays an important role in hypertrophy [40]. There is no evidence, however, that the hepatic-derived IGF-1, mediated through release via the GH-IGF-1 axis, and measured in circulation is related to levels in the muscle. In fact, a recent study by Nindl and colleagues showed that there was no relationship between IGF-1 measured in the blood and IGF-1 protein content within the muscle [41]. The lack of correlation between muscle hypertrophy and IGF-1 measured in the blood observed in this study is not surprising in light of the work by Nindl et al. showing no relationship between IGF-1 protein in the muscle or interstitial space and IGF-1 in the blood [41].

We observed a correlation between the magnitude of the IL-6 response post-exercise and fibre hypertrophy. We also found that pre-training resting IL-6 was inversely correlated with fibre hypertrophy. IL-6 is associated with both muscle protein breakdown and JAK/STAT signalling in satellite cells, which are linked to muscle hypertrophy [24]. High resting levels

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associated with greater levels of visceral adiposity [25,42,43]. Conversely IL-6 is released from muscle following exercise and although there is a pronounced rise in the systemic circulation, the main post-exercise effects are likely to be autocrine or paracrine within the muscle [24,44]. Despite the observed correlations of the acute serum IL-6 response and hypertrophy we report here, when added to a multiple regression model the influence of circulating IL-6 is minor by comparison to measures of muscle-specific factors: AR and p70S6K. The serum IL-6 response immediately following exercise may be a 'diluted' version of the response within the exercising muscle; thus, measurement of IL-6 in the muscle itself would be preferable to blood concentrations.

of IL-6 are seen with aging, systemic inflammation and are

The multiple linear regression model showed that the significant model terms for the magnitude of the increase mean muscle fiber area were the change in AR protein content (Beta=0.480) and the degree of p70S6K phosphorylation 5 hours after an acute bout of resistance exercise (Beta=0.404). Although the acute IL-6 response was correlated with changes in mean fiber area it did not add to the variance in hypertrophy explained by the model and so was not included. Our model shows that intrinsic factors within the muscle explain more unique variance in the hypertrophic response compared with circulating factors. The model also showed that p70S6K phosphorylation and AR protein content explained unique variance in mean fiber area gains and thus they are likely acting though different pathways or they represent acute and chronic effects in a linked pathway since p70S6K phosphorylation was an acute measure whereas AR receptor changes represent a change over the 16 week training period.

During the study period subjects consumed a drink containing 30 g of protein after each training session and with



Figure 3. Correlation between the AUC of the acute IL-6 response to resistance exercise before 16 weeks of resistance training and the percentage change in skeletal muscle mean muscle fibre area following 16 weeks of resistance training. r=0.48, P=0.019. doi: 10.1371/journal.pone.0078636.g003

Table 4. Stepwise Multiple Regression Model.

Model	Unstandardized Coefficients		Standardized Coefficients		Р
	В	SE	β	t	
Constant	-10.6	7.5		-1.4	0.174
AR	13.4	4.6	.48	2.9	0.008
P70S6k	10.4	4.2	.40	2.4	0.023

AR - Dependant variable was the percentage change in mean fiber area

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breakfast on non-training days. Subjects refrained from consuming any additional supplements and we told to eat the foods that made up their normal diet *ad libitum*. It seems unlikely that total energy or protein intake were substantial contributors to between subject variability in muscle hypertrophy following the resistance training; previous work has shown no difference in self-reported dietary intake between extreme- and non- responders to resistance training [45]. It would be ideal for future studies to tightly control dietary intake, by providing subjects with food to eliminate any potential effect of dietary intake, however, this would be logistically difficult,

expensive, and, we propose, unlikely to allow a 'non-responder' to become a 'responder' in terms of hypertrophy.

In the current study we chose to measure muscle hypertrophy with the analysis of muscle fiber area. The pattern of results was identical for both type I and type II muscle fibers for this reason we used mean fiber area for our multiple regression model. We were not able to differentiate between fiber sub types (IIa or IIx) or hybrid fiber which is a small limitation of this study. A whole muscle homogenate was used for the AR and p70S6K analysis to further the mechanistic understanding of fiber type specific hypertrophy future studies

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could compare homogenates isolated from specific fiber type with hypertrophy in only fibers of the same type.

Muscle hypertrophy is mainly, if not entirely, a muscle-drive process that is regulated primarily by mechanisms intrinsic to the muscle rather than by systemic factors, which appear permissive until extreme hyper- or hypo-states are present. Changes in AR content and p70S6K phosphorylation, or as we have reported previously expression of the muscle-specific microRNA (miRNA) mi451 [13], within the muscle account for more variability in training induced muscle hypertrophy as compared to measuring multiple putative anabolic hormones (T, GH, IGF-1) in systemic circulation. While we observed a significant correlation between circulating IL-6 and hypertrophy when this was added to a model that contained muscle protein measurements IL-6 did not account for any additional variance in the hypertrophic response. The ability to predict a phenotypic response to training based on variables measured following a single session of resistance exercise has some support when

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measuring muscle-specific variables [13,14]; however, future studies should focus on quantifying factors intrinsic to the muscle or systemic molecular signatures, not of single or multiple hormones, that could act as a surrogates for relevant muscle intrinsic drivers of hypertrophy. Markers intrinsic to skeletal muscle such as AR, p70S6K, miRNA, or transcriptomic profiles will explain much more variance in muscle hypertrophy following resistance training than systemic factors such as T, GH, cortisol or IGF-1.

Author Contributions

Conceived and designed the experiments: CJM GP SMP. Performed the experiments: CJM TACV LB GP SKB SMP. Analyzed the data: CJM TACV LB GP SKB SMP. Contributed reagents/materials/analysis tools: CJM TACV LB GP SKB SMP. Wrote the manuscript: CJM SMP. Edited and approved final manuscript: CJM TACV LB GP SKB SMP.

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CHAPTER 4

TITLE: Increases in fed-state post-exercise myofibrillar protein synthesis are unrelated to resistance training-induced muscle hypertrophy

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Increases in fed-state post-exercise myofibrillar protein synthesis are unrelated to resistance training-induced muscle hypertrophy

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Running Title: Myofibrillar Protein Synthesis and Hypertrophy

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KEY POINTS SUMMARY

- Skeletal muscle hypertrophy is observable following regular resistance exercise (training) and involves the expansion of the myofibrillar protein pool.
- The degree of muscle hypertrophy after a resistance exercise training program is, however, highly variable between individuals and may be related to an individual's capacity to activate the process of myofibrillar protein synthesis.
- We measured signalling protein phosphorylation as well as myofibrillar protein synthesis (MPS) after an acute bout of resistance exercise in the untrained state and muscle hypertrophy following 16 weeks of resistance training; however, there was no relationship between acute MPS and hypertrophy.
- There was a significant, albeit weak, correlation between phosphorylation of the signaling protein 4EBP-1^(Thr37/46) one hour after an acute bout of resistance exercise and muscle hypertrophy after 16 weeks of training.
- Acute measures of either MPS and/or signalling protein phosphorylation are unrelated, or only weakly related, to measures of hypertrophy

ABSTRACT

Muscle hypertrophy following resistance training (RT) involves activation of myofibrillar protein synthesis (MPS) to contribute new proteins to the myofibrillar protein pool. The degree of hypertrophy following RT is, however, highly variable. We sought to determine the relationship between the acute activation of MPS and RT-induced hypertrophy. We measured MPS and signalling protein activation after the first session of resistance exercise (RE) in untrained men (n=23) and then examined the relation between these variables with magnetic resonance image-measured hypertrophy. To measure MPS, young men (24 \pm 1yr; body mass index = 26.4 \pm 0.9 kg • m⁻²) received a primed constant infusion of L-[ring- ${}^{13}C_6$] phenylalanine and MPS was measured at rest and for 6 h, in the fed state, following their first bout of RE prior to 16wk of RT. Rates of MPS were increased $235 \pm 38\%$ (P < 0.001) above rest 1-3 h post-exercise and $184 \pm 28\%$ (P = 0.037) 3-6 h post exercise. Quadriceps volume increased $7.9 \pm 1.6\%$ (range: -1.9-24.7%; P < 0.001) with RT. There was no correlation between changes in quadriceps muscle volume and rates of MPS measured over 1-3 h (r = 0.02), 3-6 h (r = 0.16) or the aggregate 1-6 h post-exercise period (r = 0.10). Hypertrophy with RT was correlated (r = 0.42, P =0.05) with phosphorylation of $4\text{EBP-1}^{\text{Thr}37/46}$ at 1 h post RE. We conclude that acute measures of MPS following an initial exposure to RE in untrained novices are not correlated with muscle hypertrophy following chronic RT.

Abbreviations: 1RM, one repetition maximum; DXA, dual-energy x-ray absorptiometry; FSR, fractional synthetic rate; MPB, myofibrillar protein breakdown; MPS, myofibrillar

protein synthesis; FSR, fractional synthesis rate; MRI, magnetic resonance image; RT, resistance training; RE, resistance exercise; and mTOR, mammalian target of rapamycin.

INTRODUCTION

Skeletal muscle hypertrophy with resistance training (RT) requires the net addition of new myofibrillar proteins; thus, myofibrillar protein synthesis (MPS) must exceed myofibrillar protein breakdown (MPB). Using phosphorylation as a proxy for activation and activity, signalling pathway proteins in the Akt (PKB)-mTOR pathway have been measured in humans and some (Terzis *et al.*, 2008), but not all (Mitchell *et al.*, 2012), have reported correlations between the phosphorylation state of certain proteins and hypertrophy. In larger samples, measures of protein phosphorylation in multiple signaling proteins are unrelated to the hypertrophic response seen with RT (Phillips *et al.*, 2013); however, it is unknown if the same is true for a relationship between MPS and hypertrophy.

Rates of MPS in the fed-state, in combination with resistance exercise (RE), have been used assess potential for induction of muscle hypertrophy with RT (Wilkinson *et al.*, 2007; Tang *et al.*, 2009). Responses of post-exercise MPS with ingestion of milk or soy protein (Wilkinson *et al.*, 2007) or carbohydrate (Tang et al., 2007) are congruent with RT-induced hypertrophy seen, in different groups of subjects, with RT utilizing similar post-exercise nutrition (Hartman *et al.*, 2007). Similarly, the MPS response with fatiguing heavier or lighter load RE (Burd *et al.*, 2010b), or with differing volumes of RE (Burd *et al.*, 2010a) are aligned with muscle hypertrophy seen in different subjects following RT with similar RE programs (Mitchell *et al.*, 2012). Taken together, the relative congruence between MPS responses and chronic RT-induced hypertrophy suggests that measures of post-exercise MPS appear to vary similarly and thus may be correlated with muscle hypertrophy; however, such a possibility has not been tested.

There is a high degree of variability in the hypertrophic response to RT. Typical coefficients of variation of the hypertrophic response measured using muscle fibre size changes in young and old men and women can exceed 100% (Hartman et al., 2007; Petrella et al., 2008; Mitchell et al., 2012; Phillips et al., 2013). There have been attempts to explain the variability in hypertrophy using gene expression (Davidsen et al., 2011; Phillips et al., 2013), satellite cell enumeration (Petrella et al., 2008), measurement of signaling protein phosphorylation (Terzis et al., 2008; Mitchell et al., 2013), and measures of systemic hormonal responses to RE (West & Phillips, 2012). Both gene expression (Davidsen et al., 2011; Phillips et al., 2013) and satellite cell continent (Petrella *et al.*, 2008) appear related to hypertrophy whereas acute post-exercise systemic hormonal responses show no relationship to RT-induced hypertrophy (West & Phillips, 2012). In humans, protein signaling appears aligned but only weakly to hypertrophy (Mayhew et al., 2009). In small samples stronger correlations between phosphorylation of p70S6K and hypertrophy have been observed (Terzis *et al.*, 2008), but this is not consistently seen (Mitchell et al., 2012; Mitchell et al., 2013). To date, however, there have been no attempts to assess the relationship between acute measures of MPS and hypertrophy following RT, in the same subjects. Thus, the purpose of this study was to determine if MPS measured in training-naïve subjects after their first bout of RE, with

protein consumption, was related to muscle hypertrophy following 16 weeks of RT. Our hypothesis was that these measures would be related.

METHODS

Subjects. Twenty-three healthy young men ($177 \pm 2 \text{ cm}$; $84.1 \pm 3.5 \text{ kg}$; body mass index = $26.4 \pm 1.0 \text{ kg} \cdot \text{m}^{-2}$; $24 \pm 1 \text{ yr}$, means $\pm \text{SD}$) participated in the experiment. Subjects were recreationally active but had not engaged in RT within the last year. The protocol was approved by the Research Ethics Board of Hamilton Health Sciences and McMaster University and complied with all ethical standards for research involving human participants set by the Declaration of Helsinki and by the Canadian Tri-Council statement on ethics in human research (<u>http://www.ethics.gc.ca/eng/policy-</u>

politique/initiatives/tcps2-eptc2/Default/).

Experimental Design. Participants underwent a magnetic resonance imagining (MRI) scan of their right thigh to determine muscle volume and a dual-energy x-ray absorptiometry (DXA) scan to assess whole body fat- and bone-free mass (lean mass). Subjects were then strength tested to determine their maximal isotonic strength, traditionally termed one repetition maximum (1RM) for all training exercises. At least 5 d following strength testing participants reported to the lab after a 10 h overnight fast for stable isotope infusion to measure MPS using measures we have described extensively previously. Resting MPS was measured, subjects then completed four sets of 8 repetitions of leg press, leg extension, leg curl and calf press. They then ingested a protein beverage containing 30 g of milk protein, 25.9 g of carbohydrates and 3.4 g of fat (Musahi P30, Notting Hill, Australia). Muscle biopsies were then taken at 1, 3 and 6

hours post exercise to measure MPS. Subjects then completed 16 weeks of RT while ingesting the protein beverage immediately after their exercise session and with breakfast on non-training days, as previously described (Mitchell *et al.*, 2013). Briefly, participants trained four times weekly with two upper and two lower body workouts. Lower body exercises are described above in the acute exercise session. Upper body exercises consisted of chest press, shoulder press, seated row, seated pulldown, bicep curl and tricep extension. The program was progressive in linear manner moving from 3 sets of 12 repetitions to 4 sets of 6 repetitions. At the end of the training period, MRI, DXA scans, and strength testing were repeated.

Infusion protocol. On the trial day, participants reported to the lab after an overnight fast having refrained from any strenuous physical activity for at least 3 days. A 20-gauge plastic catheter was inserted into an antecubital vein and a baseline blood sample was obtained. Following the start of a primed constant infusion of L-[*ring*-¹³C₆] phenylalanine (prime: 2 µmol kg⁻¹; infusion: 0.05 µmol kg⁻¹ min⁻¹), participants rested for 3 h before a muscle biopsy was obtained to determine their resting (basal) rate of MPS. Subjects then completed the lower body exercise protocol described above and ingested their protein beverage (described above). They then rested in bed for the next 6 h while biopsies (*vastus lateralis*) were taken 1, 3 and 6 h after cessation of the exercise bout.

The drink containing 30 g of milk-based protein was enriched to 6% of the protein-contained phenylalanine content with free $[^{13}C_6]$ phenylalanine tracer to minimize disruptions in isotopic steady state, which is an approach we have used numerous times before with good maintenance of isotopic steady-state (Burd *et al.*, 2012c; Churchward-

Venne et al., 2012). Biopsies were obtained with a 5 mm Bergström biopsy needle modified for manual suction under local anaesthesia (2% xylocaine). Biopsy samples were blotted and freed of any visible fat and connective tissue, frozen in liquid nitrogen (within ~ 20 s of being taken from the muscle) and stored at -80° C until further analysis. *Imaging*. After arriving at the site of the MRI scanner, subjects rested in a supine position for 1 h prior to scanning to prevent fluid shifts from influencing measurements of muscle volume. Subjects were instructed not to engage in any strenuous activity for at least 24 h prior to the scan. MRI scans were performed in a 3-T HD scanner (Signa MRI System; GE Medical, Milwaukee, WI) at the Brain-Body Institute, Imaging Research Centre, St. Joseph's Healthcare (Hamilton, Ontario). Image acquisition was carried out using T1 fluid attenuation inversion recovery in the axial plane with the following parameters: repetition time/echo time = 2,100 ms/23.8 ms; field of view = 25-30 cm; matrix size = 512/512slice thickness = 5 mm. Thigh image acquisitions utilized an eight-channel torso coil with two excitations. There was a 10 mm gap between slices. Quadriceps volume was calculated by multiplying the slice area by the distance between slices. Volume was measured from the first slice where the rectus femoris was visible to the first slice where the gluteus maximus was visible. ImageJ software (U. S. National Institutes of Health, Bethesda, MD) was used to determine the area of each slice. Pre- and post-scans were performed at the same time of day and joint angle and leg compression was controlled using a custom built foot frame to suspend the heel of the subject.

Whole-body DXA scans (QDR-4500A; Hologic, software version 12.31) were carried out pre and post training to determine total body weight, fat mass, and (fat and bone free) lean mass.

Western Blotting. Muscle samples (~40-50 mg) were homogenized on ice in buffer (10 μ l mg⁻¹ 25mM Tris 0.5% v/v Triton X-100 and protease/phosphatase inhibitor cocktail tablets (Complete Protease Inhibitor Mini-Tabs, Roche, Indianapolis, IN; PhosSTOP, Roche Applied Science, Mannhein, Germany). Samples were then centrifuged at 15,000 g for 10 minutes 4°C. The supernatant was removed and protein concentrations were determined via BCA protein assay (Thermo Scientific, Rockford, IL). Working samples of equal concentration were prepared in Laemmli buffer. Equal amounts (20 µg) of protein were loaded onto 10% or gradient precast gels (BIO-RAD Mini-PROTEAN TGX Gels, Bio-Rad Laboratories, Hercules, CA) for separation by electrophoresis. Proteins were then transferred to a polyvinylidene fluoride membrane, blocked (5% skim milk) and incubated overnight at 4°C in primary antibody: phospho-Akt^{Ser473} (1:1000, Cell Signalling Technology, #4058) phospho-mTOR^{Ser2448} (1:1000, Cell Signalling Technology, #2971), phospho-4E-BP1^{Thr37/46} (1:1000, Cell Signalling Technology, #2855), Phospho-S6^{Ser240/244} Ribosomal protein (1:2000, Cell Signalling Technology, #2215). Membranes were then washed and incubated in secondary antibody (1 h at room temperature) before detection with chemiluminescence (SuperSignalWest Dura Extended Duration Substrate, ThermoScientific, #34075) on a FluorChem SP Imaging system (Alpha Innotech, Santa Clara, CA). Phosphorylation status was expressed relative to α -

tubulin (1:2000, Cell Signalling Technology, #2125). Images were quantified by spot densitometry using ImageJ software (US National Institutes of Health). *Isotopic analyses*. As described previously (Burd *et al.*, 2010b) approximately 20 mg (wet weight) of muscle was used to isolate free intracellular amino acids. A separate piece of muscle (\sim 30 mg) was used to isolate, hydrolyse, purify, derivatize and analyse the myofibrillar protein fraction enrichment. The rate of myofibrillar protein synthesis was calculated using the standard precursor–product method as previously described (Burd *et al.*, 2010b):

FSR
$$(\% \bullet h^{-1}) = [(E_{p2} - E_{p1}) / (E_{ic} \bullet t^{-1})] \bullet 100$$

Where, FSR is the fractional synthetic rate, E_{p2} and E_{p1} are the protein bound enrichments from muscle biopsies at time 2 (E_{p2}) and plasma proteins or the previous muscle biopsy at time 1 (E_{p1}) and thus their difference is the change in bound protein enrichment between two time points; Eic is the mean intracellular phenylalanine enrichment from biopsies at time 2 and time 1; and t is the tracer incorporation time. The utilization of "tracer naïve" subjects allowed us to use the pre-infusion blood sample (i.e., mixed plasma protein fraction) as the baseline enrichment (E_{p1}) for the calculation of resting MPS. This approach makes the assumption that the baseline ¹³C enrichment ($\delta^{13}C_{PDB}$) in the blood reflects that of muscle protein; this is an assumption that has been previously (West *et al.*, 2009) and shown to be valid in allowing calculation of a reliable rate of MPS in the fasted state (Burd *et al.*, 2012a; Burd *et al.*, 2012b).

Statistics. Differences in means from pre to post training were compared with paired Student's t-tests. Temporal differences in the phosphorylation of signalling proteins and

FSR were compared with one-way repeated measures ANOVA. Relationships between variables were assessed using the Pearson's product moment correlation. All analyses were conducted using SPSS version 20 (IBM Armonk, New York, USA). Alpha was set at $P \le 0.05$. Means are reported \pm SE unless otherwise indicated.

RESULTS

Plasma and muscle intracellular free phenylalanine enrichment. Intracellular freephenylalanine precursor enrichments were 0.046 ± 0.003 at rest and 0.066 ± 0.004 throughout the fed-state post exercise incorporation period. The slope of a linear regression lines fit through the intracellular enrichments was not significantly different from zero during the post-exercise period (P > 0.05). Plasma enrichments at 60, 180 and 360 min were 0.070 ± 0.002 , 0.075 ± 0.003 and 0.076 ± 0.003 , respectively. Linear regression analysis indicated that the slopes of the plasma enrichments were not significantly different from zero (P > 0.05) and thus an isotopic plateau was achieved and that the use of the steady-state precursor product equation was appropriate.

Muscle Size and Strength. Quadriceps muscle volume increased from 1837 ± 195 to $1970 \pm 71 \text{ cm}^3$ (Figure 1A), while whole body fat- and bone-free mass increased from 62.6 ± 2.0 to 64.8 ± 2.1 kg (Figure 1B). Maximal isotonic strength, expressed as 1 RM, increased from 236 ± 15 to 380 ± 15 kg and from 77 ± 4 to 96 ± 4 kg in the leg press (Figure 1C) and chest press (Figure 1D) exercises respectively.

Western Blotting. Phosphorylation of mTOR^{Ser2448} was increased above rest at 1 and 3 h post-exercise but had returned to baseline by 6 h post-exercise (Figure 3A). Phosphorylation of Akt^{Ser473} was increased above resting at 1 h post-exercise then
returned to baseline by 3 h post-exercise (Figure 3B). Phosphorylation of 4EBP-1^{Thr37/46} was not significantly increased at any time post-exercise (P=0.142; Figure 3C). Phosphorylation of rpS6^{Ser240/244} was elevated above rest at 1,3 and 6 h post-exercise; however, at 6 h post exercise the phosphorylation was reduced compared to 1 and 3 h (Figure 3D). There was a significant correlation between the phosphorylation of 4EBP-1 phosphorylation at 1 h post exercise and the RT-induced change in muscle volume (r = 0.42, P=0.047, Figure 4B).

Myofibrillar Protein Synthesis. Rates of MPS following RE were increased compared to rest from 1 to 3 h post-exercise (P < 0.005) and from 3 to 6 h post-exercise (P=0.034; Figure 2). There was no statistically significant difference between the 1-3 h and the 3-6 h rates (P=0.159). The aggregate MPS response over the entire post-exercise period (1-6 h) was $0.052 \pm 0.04 \% \cdot h^{-1}$, which was significantly greater than resting MPS rates. There was no correlation between MPS at any of the time periods measured and the change in muscle volume as measured by MRI. Figure 4a shows the correlation between MPS measured over the full post exercise infusion period and change in muscle volume (r = 0.01). This comparison is highlighted because it should best reflect the full MPS response after exercise and nutrition. In addition, there was not a significant correlation between the change in fat- and bone-free (lean) mass and the aggregate response of myofibrillar protein synthesis measured over 1-6 h post exercise (r = 0.13).

DISCUSSION

We examined the relationship between the response of the fed-state RE-induced rise in MPS and muscle hypertrophy in the same subjects hypothesizing that these variables

would be related. Contrary to our hypothesis, we observed no relationship between these variables. We measured the response of MPS following the subjects' first exposure to leg RE and nutrition, which we subsequently had subjects follow throughout their training protocol, and MRI-measured muscle volume or DXA-measured lean body mass. Our finding is in agreement with one previous report from Mayhew et al. (Mayhew et al., 2009) who observed no relationship between mixed muscle protein synthesis and hypertrophy in a group of 8 young and 7 older men. However, in this study (Mayhew et al., 2009), mixed muscle FSR was measured 24 h after the first bout of resistance training. The present study differed from the previous report (Mayhew et al., 2009) since we measured myofibrillar protein, not mixed protein, synthesis rates, we made measurement of MPS immediately post-exercise, and our subjects were fed. The protein supplement was ingested during training in order to maximize muscle hypertrophy (Cermak et al., 2012) and was thus consumed during the measurement of MPS so conditions would be as similar as possible to that seen in training. Outside of the protein supplementation, we did not control the subjects' diets during the training period; however, previous work has shown that subjects' diets does not explain variation in post training hypertrophy (Thalacker-Mercer *et al.*, 2009). It is possible that contraction, and not nutrition, is the primary driver of the MPS response and hypertrophy and, hence, that measurements of MPS made in the fasted-state would be more reflective of hypertrophic potential.

In previous work, acute responses of MPS to differing nutrition (Hartman *et al.*, 2007; Wilkinson *et al.*, 2007), contraction intensity (Burd *et al.*, 2010b; Mitchell *et al.*,

2012), and contraction volume (Burd *et al.*, 2010b; Mitchell *et al.*, 2012) were found to align with chronic training-mediated changes in hypertrophy in studies employing roughly equivalent nutritional and/or contractile conditions preformed but in different sets of subjects. Thus, the absence of a significant correlation between MPS and hypertrophy, in the present study, was unexpected. The lack of a relationship could, however, be due to subject-specific changes in the MPS response in terms of: magnitude at times later during the training program, specificity of the protein fraction MPS response (i.e., myofibrillar vs. non-myofibrillar MPS), duration of the response of MPS during the course of training, and/or variations in net muscle protein balance due to differential responses in muscle proteolysis. Clearly, however, acute early measures of MPS are not proxy measures for hypertrophy or hypertrophic potential within the same individual.

Cross-sectional comparisons of trained with untrained persons show that increases in mixed muscle protein FSR are smaller in magnitude, as were increases in mixed muscle proteolysis, in response to RE (Phillips *et al.*, 1999). Tang et al. (Tang *et al.*, 2008) showed that fed-state mixed muscle protein FSR with RE, performed at the same relative intensity pre- and post-training, produced a slightly higher FSR immediately post exercise (90-270 min post exercise) in the trained state, however, the duration of the response was reduced. In contrast, a lower mixed muscle protein FSR was observed with RT when the same absolute intensity was utilized (Phillips *et al.*, 2002). When examining myofibrillar as opposed to mixed muscle FSR, there were no differences in acute MPS between the trained and untrained conditions after exercise in the fasted state (Kim *et al.*, 2005). In sum, RT appears to reduce the duration, but not the amplitude, of the protein

synthetic response (Tang *et al.*, 2008). Nonetheless, one adaptation with resistance training appears to be a 'refining' of the synthetic response to RE to emphasize synthesis of myofibrillar proteins with concomitant reductions in sarcoplasmic and mitochondrial protein synthesis (Kim *et al.*, 2005; Wilkinson *et al.*, 2008). Further research aimed at delineating the role of MPS in hypertrophy would perhaps need to include an extended time course of the MPS response and determine whether those gaining more lean mass were able to sustain a greater duration of their MPS response during RT. A difference in muscle satellite cell content and the extent of myonuclear addition have been shown to relate to the magnitude of RT-induced hypertrophy (Petrella *et al.*, 2008). It is conceivable that the individual variation in the change in FSR throughout the training period could be related to the degree of myonuclear addition.

A possibility is that subjects gaining more muscle mass with resistance training had a greater suppression of proteolysis as it is net muscle protein balance (i.e., MPS minus MPB) that would, strictly speaking, determine gains in muscle mass. Work by Glynn and associates shows that there are increases in MPS and reductions MPB in response to a combination of feeding and RE, however, the magnitude of the changes in MPS is ~4-5 fold greater than the change in MPB (Glynn *et al.*, 2010). Similar differences in magnitude of the response of MPS relative to MPB have been seen with RE alone (Phillips *et al.*, 1997). These data suggest that changes in MPS is the main locus of control and is far more responsive to nutritional and contractile stimuli in regulating, changes in muscle size than MPB.. In addition, when measures of mixed muscle protein synthesis and breakdown have been made in the post-exercise period in the same subjects a reasonably good correlation exists between the two variables (Phillips *et al.*, 1997; Phillips *et al.*, 1999), which does not indicate a measurable divergence in regulation but rather a link between the two processes. However, we cannot rule out the possibility that changes in protein breakdown could regulate gains in lean mass with resistance training.

The Akt-mTORC-1 pathway is an important regulatory pathway for muscle hypertrophy and is considered necessary for protein synthesis and growth (Bodine et al., 2001). There are multiple reports of correlations between P70S6K and hypertrophy in the literature, however, these relationships tend to be weak (Mayhew et al., 2009) or are based on small sample sizes (Terzis *et al.*, 2008). Our lab has shown that hypertrophy is possible with RT that is unrelated to phosphorylation of P70S6K 1 h after the first exercise bout (Mitchell *et al.*, 2012). We have also recently shown that in one study P70S6K phosphorylation six hours after the first exercise bout is only weakly related to training mediated hypertrophy (Mitchell et al., 2013). Previous work from our lab has shown a correlation between acute phosphorylation of 4EBP-1 and MPS after RE (Burd et al., 2010b). In the current study we did not, however, see a relationship between MPS and 4EBP-1 phosphorylation but did see a relationship between hypertrophy and 4EBP-1 phosphorylation. We propose, at least in humans, that the relationship between REstimulated phosphorylation of various Akt-mTORC-1 proteins and RT-induced hypertrophy explains, at best, a small degree of the variance in the hypertrophic response. In fact, phosphorylation of multiple proteins in the Akt-mTOR pathway have been shown to be unrelated to RE-induced changes in lean body mass (Phillips *et al.*, 2013). Phosphorylation events in signaling are usually transient and may not reflect activity it is

doubtful that phosphorylation of a single protein could explain a large proportion of the variance in muscle hypertrophy (Phillips *et al.*, 2013).

The results from this study indicate that acute measurements of MPS over 6 hours following exercise and nutrition are not predictive of muscle hypertrophy following 16 weeks of resistance training and supplement ingestion in the same subjects. It is possible that the measures of MPS at later time-points following acute exercise may demonstrate a correlative relationship with muscle hypertrophy. However, the magnitude and duration on the MPS response measured within the same individual appears to change with training (Kim *et al.*, 2005; Tang *et al.*, 2008). It is possible the some subjects may maintain a robust MPS response throughout the training period whereas some subjects may show a diminished MPS response after training. Because data from the present study does not show a relationship between acute measures of MPS and skeletal muscle hypertrophy, it is likely that changes in MPS with training are not uniform between subjects. A systems biology approach incorporating proteomics, genomic, or transcriptomics may be required prospectively to estimate hypertrophy or hypertrophic potential.

Competing Interests: The authors declare that they have no competing interests financial or otherwise

Author Contributions: CJM, SMP, and GP conceived of and designed the study. CJM, LB, TAC-V, SKB, and SMP ran the experiments. CJM, TAC-V, KS, and PJA ran analyses. CJM and SMP prepared the manuscript and all authors provided feedback and input. All authors approved the final version of the manuscript.

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Figure 1. Muscle volume, muscle mass, and strength changes following resistance training. The absolute increase in A) Quadriceps muscle volume determined by MRI, B) Fat free bone free mass determined by DXA, C) Leg press 1RM and D) Chest press 1RM. Each circle represents a single subject, the longer centre line shows the mean change and the error bars indicate the standard deviation of the mean. All increases were significantly different from zero (i.e., an increase from pre training P<0.05).

Figure 2. Myofibrillar Protein synthesis. FSR is calculated at rest and after an acute bout of resistance exercise and protein ingestion prior to the start of the resistance training period. The other rates were calculated from 1 to 3 h and 3-6 h after RE. Each circle represents a single subject, the longer centre line shows the mean change and the error bars indicate the standard deviation of the mean. * Significantly different than rest P<0.05

Figure 3. Phosphorylation of anabolic signalizing proteins. The results are expressed as fold changes from rest at 1, 3 and 6 hours after an acute bout of RE prior to the training period. All proteins are normalized to alpha tubulin. A) mTOR phosphorylation at Ser2448, B) Akt phosphorylation at Ser473, C) 4EBP-1 phosphorylation at Thr37/46 and D) rpS6 phosphorylation at Ser240/244. * Significantly different from rest P<0.05. †

Figure 4. Relationship between muscle hypertrophy, MPS, and 4EBP-1 phosphorylation. A) Relationship between % changes in muscle volume as measured by MRI and MPS measured from 1-6 h after RE (r=0.10, P=0.67). B) The relationship between % changes in muscle volume as measured by MRI and 4EBP-1 phosphorylation at Thr37/46 at 1 h after RE (r=0.42, P=0.05).

Figure 1.



Figure 2.



Figure 3.



Figure 4.



CHAPTER 5: DISCUSSION AND CONCLUSIONS

The studies included in this thesis present new data on potential mechanisms regulating muscle hypertrophy. These findings could inform resistance exercise prescription as well as present new paradigms. The aim of this chapter of the thesis is to provide an overall discussion of the findings of the studies presented in chapters two, three and four with the goal of: providing a simplified recommendation for resistance training prescription, discussing local versus systemic regulation of resistance training induced muscle hypertrophy, and clarifying the relationship between muscle protein synthesis and muscle hypertrophy. Additionally, I will present an examination of some underappreciated factors in resistance training study design, limitations of techniques currently used to assess muscle protein synthesis and hypertrophy, and how future studies may be better able to quantify the variability in resistance training mediated hypertrophy and advocate for a systems biology approach.

5.1 OPTIMAL LOADING PARADIGMS FOR MUSCLE HYPERTROPHY

Guidelines for resistance training exercise prescription are released by many different professional organizations and have been published in various sources (ACSM, 2009; Baechle et al., 2000). One of the most trusted and established sources of sciencebased exercise prescription advice is the American College of Sports Medicine (ACSM). The ACSM commissions and publishes evidence-based position stands on a number of issues, such as hydration during exercise (Sawka et al., 2007), and guidelines for nutrition

for athletes (Rodriguez et al., 2009) which are widely cited by scientists and practitioners alike. The 2009 ACSM-sanctioned position stand entitled "Progression models in resistance training for healthy adults" includes a section on load and volume-based program design recommendations for increasing muscle hypertrophy. In this stand it states that novice exercisers should perform 1-3 sets at 70-85% of 1RM for 8-12 reps in order to maximize muscle hypertrophy. This information is classified as 'category A' evidence, which is defined as "...evidence is from well-designed randomized controlled trials RCTs that provide a consistent pattern of findings in the population for which the recommendation is made and requires a substantial number of studies involving a substantial number of participants" (ACSM, 2009). The authors cite multiple studies to support their recommendation, however, only a single study by Campos et al. in which differing programs of resistance exercise were studied actually supports the idea that training with heavier loads is required to induce muscle hypertrophy (Campos et al., 2002). The authors of the same ACSM position stand also neglected to cite work by Leger et al. who, using the same program as that employed by Campos et al, showed no difference in muscle hypertrophy between groups training with light or heavy relative loads (Leger et al., 2006). Clearly, the ACSM position stand represents a belief and not an evidence-based recommendation insofar as the prescription of load is concerned

The results presented in chapter two of this thesis, as well as previously published work (Leger, et al., 2006), calls into question the current recommendations for prescription of resistance training loads in driving hypertrophy as they are not supported by the available empirical data and can likely be greatly simplified. Instead, I would

propose the following guidelines for resistance training load-selection: 1) chose a load which you feel you can lift 30 times or less and 2) continue to lift it until it is not possible to lift the load without the use of muscle groups which are not desired in the performance of the exercise. This recommendation is simply stated and easy to understand. It also better reflects a synthesis of the literature, which, with the exception of the single study by Campos et al (2002), indicates no difference in the hypertrophic response to lower versus higher load resistance exercise when weights are lifted to volitional failure. The inclusion of a 30RM cut off may seem somewhat arbitrary and it is likely that loads lighter than 30RM may induce similar amounts of muscle hypertrophy (Van Roie et al., 2013). However, it is also likely that there is a minimum intensity cut-off such that if a load can be lifted 1000 times before fatigue (the equivalent of ~10 min cycling to exhaustion) it is unlikely that appreciable hypertrophy will occur (Ross et al., 2001). Because no dose-response studies have been conducted, in which hypertrophy was assed when loads less than ~30RM are lifted to failure., I choose this cut-off as the recommendation for minimum relative load based on the results presented in chapter two.

The ACSM position stand also makes the recommendation that more experienced resistance trainees use loads of up to 100% of their 1RM (ACSM, 2009). The data presented in this thesis did not included experienced resistance exercisers and there is very little published primary literature on this topic. However, there are two studies which may help in the formation of a general recommendation for advanced trainers. Firstly, an acute muscle protein synthesis (MPS) study conducted by Burd et al. showed that in resistance trained subjects there was a similar rise in MPS after lifting loads at either 30%

and 90% of 1RM (Burd et al., 2010), which is at least suggestive that a lighter relative load may be able to induce hypertrophy even in trained individuals. It is important to remember however that most resistance trained individuals train with high relative loads, so it is possible that the MPS response to a light relative load lifted to failure was partly a stress response to unaccustomed exercise which may be attenuated in subsequent workouts (Wilkinson et al., 2008). In another study conducted by Goto et al., the authors showed that in a group of experienced exercisers that multiple sets performed at 3-5RM loads did not induce muscle hypertrophy, whereas the addition of a single set to failure in the 25-35RM rep range resulted in significant hypertrophy (Goto et al., 2004). Thus, based on the available data it does not appear possible to make a category 'A' level recommendation for higher training load and hypertrophy in experienced resistance trainers. However, based on the body of evidence it seems that either short phases of training with lighter relative loads or the addition of a single set of a light relative load performed to failure may be effective for maximizing muscular hypertrophy in resistance trained individuals.

The recommendation set forth in the ACSM position stand of 1-3 sets per exercise for novice exercisers (ACSM, 2009) seems to have greater research support than their relative load recommendations. Many published studies, including the one presented in chapter 2, have not, however, shown a significant benefit of multiple compared to a single set on the magnitude of muscle hypertrophy following resistance training (Baker et al., 2013; Carpinelli et al., 1998). A meta-analysis of resistance-exercise volume comparing single to multiple sets confirmed, from a hypertrophic standpoint, that multiple sets are

superior to a single set (Krieger, 2010). There was also a trend for greater hypertrophy with 4-6 sets versus 1 set, but not 3 sets (Krieger, 2010). This suggests a diminishing returns relationship between volume and hypertrophy where more volume results in greater hypertrophy; however, the incremental benefit of each additional set is small and decreased with the number of sets performed. The results presented in chapter two are in agreement with many other studies in showing that there was no statistically greater hypertrophy in the 3 set, compared to the single set, condition, which is in agreement with the findings of others (Baker, et al., 2013; Carpinelli, et al., 1998). Nonetheless, the mean percentage gain in the three set condition was more than double that which was observed in the single set condition. The lack of statistical significance observed was due to the high degree of variability in the hypertrophic response observed in the 3 set conditions (% change in quadriceps volume: -1.3% to 29.0%). Although this degree of variability in hypertrophic responses is consistent with the results of many other resistance training studies, it makes it very difficult to assess the effect of resistance training program variables (Timmons, 2011).

All of the studies included in this thesis have employed a standard statistical procedure based on hypothesis testing and a critical probability of 95% confidence of a difference. This approach works well for many applications and is a prerequisite for publication within most physiological journals (Drummond et al., 2011). The primary weakness of this approach is that in populations that tend to yield highly variable responses (such as in humans undergoing resistance training) it is often impractical to power studies appropriately to detect relatively modest effects (Hopkins et al., 2009).

Thus if asked to make a recommendation on the effect of a single set versus three sets to maximize muscle hypertrophy based on the data and statistical method used in chapter two, one would conclude that a single set would be sufficient and that the difference from 3 sets is not statistically significant but is double in magnitude. Therefore if one looks at the difference in the magnitude of hypertrophy observed with one set versus three sets then it is clear there may be some benefit to multiple sets. A magnitude based inferential approach in this case could allow for a practitioner to make an evidence-based recommendation to their clients or patients without being constrained by the statistical conventions of physiology journals. In the case of the data presented in chapter 2 it would be appropriate to set a minimum meaningful difference in hypertrophy between groups at 2% because the coefficient of variation was less than 2% for each MRI image analysed (Batterham et al., 2006). When this is done, a magnitude based inferential approach suggests that there is more than an 88% chance of a benefit of preforming 3 sets compared to 1 and only a 0.02% chance that a single set would be more beneficial than 3 sets (Batterham, et al., 2006). Based on this analysis any practitioner or clinician concerned with maximizing hypertrophy should be prescribing at least three sets per muscle group. Viewed alternatively, because a single set resulted in significant hypertrophy and because of the wide variability in the hypertrophic response many practitioners may choose the more time efficient strategy of prescribing only a single set to beginner resistance exercisers. Strength gains were also similar between the one and three set groups over the 10 week training period thus there does not appear to be greater functional benefit from additional training volume in the early stages of training.

5.2 CONSIDERATIONS FOR FUTURE RESISTANCE TRAINING STUDIES

Designing studies to test the effects of a single resistance training program variable can be very difficult because of the interrelationship between resistance training program variables. Two of the main program variables, volume and relative load have already been discussed at length in this thesis. There are many other variables such as lifting tempo (Westcott et al., 2001) and between set rest periods (de Salles et al., 2009), which can also be manipulated; however, what is often not considered is how these manipulations effect what I believe to be the third primary program variable, effort. There are a wide range of possible effort levels which an individual will require for various resistance training situations. For example a set of 10 repetitions with 30% of 1RM will be very easy whereas 10 repetitions with 90% of 1RM would be impossible for most. Thus it is very difficult to quantify submaximal efforts or intensity as it is sometimes incorrectly referred to. So for practical purposes concentric effort can only be defined as submaximal, maximal and supra-maximal (assisted reps after the point of concentric failure). The following example will illustrate why an understanding of effort is critical to the design and interpretation of resistance training studies. A study by Holm et al. (2008) concluded that higher relative loads resulted in greater hypertrophy than lighter relative loads. In the study, one leg completed 10 sets of 8 repetitions at 70% of 1RM and the load was increased when 8 repetitions could be completed. The subjects' contralateral leg completed the same volume of work as the high-load leg but lifted a load of 16% of 1RM (Holm et al., 2008). The authors of this study were able to manipulate relative load and

strictly control volume, however effort would have been drastically different between the conditions. In the Holm et al. (2008) study the 16% of 1RM condition completed a total of 36 reps per set in a pattern of one rep every 5th second for three minutes whereas the 70% 1RM group performed eight continuous reps in approximately 25 seconds.

The study presented in chapter 2 of this thesis concluded that both heavier (80% 1RM) and lighter (30% 1RM) relative loads can induce similar magnitudes of muscle hypertrophy. In this study load was manipulated and effort was equated in that each group lifted a load to failure, which meant that volume varied between groups. Interestingly, by the end of the study, the volume completed was very similar between the two conditions. The findings presented in chapter 2 in conjunction with the Holm et al. (2008) study suggests that relative load, volume, and effort are three primary resistance training variables but that are highly interwoven. When designing a resistance exercise/training study one variable of these three primary variables (relative load, volume and effort) can be manipulated, one can be clamped and the third must be allowed to vary. If researchers choose to manipulate relative load and clamp volume then effort will very between conditions such as in the Holm et al (2008) paper. If instead researchers choose to manipulate relative load but clamp effort (such as in chapter 2) then volume will be different between conditions. Authors must acknowledge that it is not possible to control for both effort and volume in standard resistance training studies and should acknowledge differences in effort or volume that result due to limitations in study design.

The physiology behind relative load, volume, and effort is still not well understood because there remains a lack of understanding about the process of mechanotransduction (West et al., 2013). It is hypothesized that effort is directly related to motor unit activation and thus, based on the size principal, maximal effort should result in near maximal motor unit activation (Henneman, 1957). This hypothesis is difficult to test in human muscle undergoing dynamic contractions. Surface electromyography has been used in attempts to quantify motor unit activation when different force levels are maintained until fatigue, however, this method is confounded by the potential of neuromuscular propagation failure (Fuglevand et al., 1993). Potentially the development of multiple electrode arrays coupled with advanced processing techniques or improved fine wire EMG technology will allow for a more accurate test of the size principle in humans during dynamic contractions performed to the point of fatigue.

5.3 LOCAL VERSUS SYSTEMIC CONTROL OF MUSCLE HYPERTROPHY

When investigating the mechanisms that contribute to muscle hypotrophy there has been much debate over the locus of the primary regulator(s). In a physiological process that involves multiple organs and organ systems it seems unlikely that a single 'master' regulator exists. Thus, it is overly simplistic to assign to a single factor the label of primary regulator of muscle hypertrophy. Instead it may be useful to discuss situations where hypertrophy may be regulated from different loci. The first and most obvious situation where muscle hypertrophy is regulated is via systemic versus local factors is after the administration of exogenous testosterone, muscles undergo hypertrophy. The

doses of exogenous testosterone to achieve this effect are supraphysiological but when administered subjects exhibit large gains in muscle mass independent of resistance training and even larger gains when resistance training and these supraphysiological doses of testosterone are combined (Bhasin et al., 1996). Similarly, individuals with hypogonadism (i.e., hypotestosteronemia) exhibit a very low muscle mass phenotype (Brodsky et al., 1996). Additionally, pre-pubertal boys have a much lower proportion of muscle mass compared with men, and gains in muscle mass appear to be related to drastic shifts in the hormonal milieu which occur around puberty (Rogol et al., 2002). These are all examples of situations where systemic factors are clearly the primary site of regulation of muscle mass changes. In these cases systemic factors do not act independently of local mechanisms but instead through pathways such as the androgen receptor (Ahtiainen et al., 2011), to increase anabolic signalling and muscle protein synthesis to ultimately promote muscle growth (Drummond et al., 2009a).

Another situation where systemic factors may play a role in regulating muscle hypertrophy is in situations of inflammation. For example, situations of extreme inflammation, such as burns, sepsis, and cancer cachexia result in muscle wasting (Lang et al., 2007). In these situations hypercytokinemia (IL-6, TNF-alpha, CRP) as well as hypercortisolemia are significant factors in promoting muscle wasting. Much less is known about the effects of chronic low-grade inflammation on muscle mass gains/losses, however, such an inflammatory state can be observed in aging and has been termed 'inflammaging' (Schaap et al., 2006). Data presented in chapter three suggests that even in young, apparently healthy men, interleukin-6 (IL-6) measured in the serum

immediately after resistance exercise may be positively related to resistance training mediated muscle hypertrophy. Conversely, data presented in appendix A shows a negative relationship between resting serum concentrations of IL-6 and muscle hypertrophy after training. As such, it is possible that resting inflammatory status may modulate the hypertrophic response. Such a thesis has some support from murine data that shows that serum from young mice can reverse age related defects in satellite cell proliferation, a potentially important mechanism for hypertrophy, in old mice (Conboy et al., 2005). As low-grade, chronic inflammation is common in various populations and conditions (i.e. older adults, obesity, type II diabetes), more work needs to be done to determine how low-grade inflammation effects resistance training mediated muscle hypertrophy. Taken together the data from chapter 3 and appendix A suggest a complex and contradictory role for IL-6 in resistance training mediated hypertrophy.

Data presented in chapter three shows, in agreement with a study by West & Phillips (2012), there was no relationship between the acute post exercise rise in any systemic hormone and resistance training-induced muscle hypertrophy. There was, however, a relationship between the magnitude of the acute IL-6 response and the magnitude of training mediated hypertrophy. It is probable that the relationship between the acute IL-6 response and hypertrophy is not an example of IL-6 exerting systemic control of hypertrophy, but rather that systemic IL-6 concentration reflects local production by the muscle and then is being released into the systemic circulation (Febbraio et al., 2002). Future research should look to measure IL-6 in other

compartments such as within the muscle and within the interstitial fluid to test this hypothesis (McKay et al., 2009).

The results presented in chapter three are in agreement with those presented by West & Phillips (2012), which showed that there is no relationship between the acute post exercise rise in purportedly anabolic hormones (GH, T, and IGF-1) and muscle hypertrophy. It is important to note that the pre- and post-training increases in hormone concentration are within the order of magnitudes seen in normal diurnal variation of these hormones and that they are very transient in nature (Diver et al., 2003). Despite no correlation between anabolic hormone concentrations and hypertrophy, a relationship appears to exist between the increase in muscle androgen receptor content during the training period and the magnitude of muscle hypertrophy. It is interesting to note that there was no significant time-dependent change in androgen receptor content, but the increases in androgen receptor content was correlated to muscle mass gains. These findings suggest that, like observations which have been alluded to in studies of satellite cells and microRNA, there may be different regulatory mechanisms that separate low, moderate, and high responders in terms of muscle mass gains (Davidsen et al., 2011; Petrella et al., 2008).

A correlation between the change in androgen receptor content and the magnitude of resistance training mediated muscle hypertrophy has been reported by Ahtiainen and colleagues (Ahtiainen, et al., 2011). Specifically, they found a correlation between the change in androgen receptor content and hypertrophy, but did not observe a significant

pre to post change in androgen receptor content (Ahtiainen, et al., 2011). This finding, in conjunction with the findings reported in chapter three, support a hypothesis that increases in androgen receptor content may only occur in higher responders to resistance training. To confirm this hypothesis larger studies would need to be conducted which would allow for the examination of non-linear effects.

Both chapters three and four present anabolic signalling data showing there were significant increases in the phosphorylation status of many of the upstream proteins within the Akt-mTOR- pathway. The acute phosphorylation status of more proximal pathway proteins, such as Akt and mTOR, showed no correlation with muscle hypertrophy following resistance training (unreported observations from chapters two, three and four). It has been shown that mTOR has a central role in signalling in this pathway since blocking mTOR results in an acutely blunted protein synthetic response in humans (Drummond et al., 2009b) and also an attenuated hypertrophic response to resistance training in rodents (Bodine et al., 2001). It is likely that activation of this pathway is necessary for muscle hypertrophy, but that small variations in the magnitude of mTOR phosphorylation are not linearly related to muscle hypertrophy. The extent to which acute mTOR phosphorylation reflects actual mTOR 'activity' is also unclear. Since mTOR forms a complex with many other regulators and must translocate to the lysosome (Drummond, et al., 2009a), it is unlikely that measuring phosphorylation on a single site of a single protein would yield much insight into hypertrophy unless that protein were the rate-limiting protein for the critical step in hypertrophy. To my knowledge, no published study has attempted to show a relationship between mTOR phosphorylation and

hypertrophy, however, it is possible that a number of non-significant associations have simply not been published.

There were statistically significant but relatively weak relationships observed between the acute phosphorylation status of both 4EBP-1 and p70S6K, proteins that are distal to mTOR and closer to final steps associated with translation initiation, and the magnitude of muscle hypertrophy observed following resistance training. These observations are in agreement with similar published reports and suggest that activation of downstream components of the mTOR pathway may be important in the regulation of muscle hypertrophy following resistance training (Mayhew et al., 2009; Terzis et al., 2008). However, the data in chapter two did not show a correlation between acute p70S6K phosphorylation and hypertrophy. It is unlikely that future studies will be able to show stronger relationships between hypertrophy and downstream members of anabolic signalling pathways because it is doubtful that a single protein, even if very precisely measured, would explain a large component of the variance in muscle hypertrophy as redundancy and cross-talk exist between pathways (Phillips et al., 2013; Timmons, 2011). There is also a major limitation in how frequently the phosphorylation status of anabolic signalling proteins can be determined because a muscle biopsy must be taken at each time point. The invasive nature and expense associated with analyzing muscle biopsies limit how many can be performed on an individual. Thus, what we obtains are 'snapshots' of the anabolic signally pathway activity at discrete time points and we have no information on what is occurring in the minutes or hours between biopsies. Little is known about the time course of activation of anabolic signalling proteins. Small variations in study design

also seem to lead to large variations in anabolic signalling response. The study presented in chapter two did not show an acute increase in p70SK6 phosphorylation one hour after exercise whereas work by Burd et al (2010) showed phosphorylation four hours after a very similar exercise stimulus. It is also unclear the exact meaning and significance of small changes in the phosphorylation status of anabolic signalling proteins.

Phosphorylation status is often thought of as a proxy measure for activity, however, there does not seem to be any relationship between acute phosphorylation status and activity assays run on the same protein from the same sample (Sekulic et al., 2000). Also, because the Western blotting technique commonly employed does not often use a standard curve, there is no evidence that a completely linear relationship exists between blot intensity quantified though densitometry and acute phosphorylation status by a given protein (Gassmann et al., 2009; Mollica et al., 2009). Thus, at best Western blotting is likely semi-quantitative and is very useful in situations such as determining the purity of cellular isolations (Smith et al., 2013) or large differences in protein content such as those that would occur in a knockdown models (Crossland et al., 2013). However, it is a mistake to assume the small changes in optical density are a linear reflection of changes in protein concentration or activity (Mollica, et al., 2009; Sekulic, et al., 2000).

Work from our lab shows that in young and healthy adults that hypertrophy is regulated primarily through local factors rather than changes in systemic hormones (West et al., 2009a; West et al., 2009b; West et al., 2012). Nonetheless, it appears that there are situations such as aging or hypogonadism, where systemic factors play some role in the regulation of muscle mass (Brodsky, et al., 1996; Schaap, et al., 2006). However, in

healthy young subjects undergoing a resistance training program with adequate nutrition, it appears the factors intrinsic to the muscle are regulating the magnitude of muscle hypertrophy. The results from this thesis show that there may be a weak relationship between the acute phosphorylation of certain anabolic signalling proteins and hypertrophy following resistance training. However, the regulation of muscle hypertrophy is a complex and multi-faceted process and a more comprehensive examination of the time course of signalling protein phosphorylation and as well as more comprehensive measures of enzyme activity, gene expression and satellite cell activity may be required to achieve a more complete picture of the local regulation of muscle mass.

5.4 THE RELATIONSHIP BETWEEN MUSCLE PROTEIN SYNTHESIS AND HYPERTROPHY

Stable isotope tracer methodology is often used to measure the rate of muscle protein synthesis in response to nutritional and/or exercise manipulations (Biolo et al., 1997; Phillips et al., 1997). Although it is not always explicitly stated, it is implied, that differences in the MPS response to exercise or nutritional manipulation will be related, at least to some degree, to long-term phenotypic changes in muscle (i.e.hypertrophy). There have been multiple reports from our lab showing qualitatively similar group responses of MPS and hypertrophy between acute tracer studies and resistance training intervention studies performed in different subjects (Hartman et al., 2007; West, et al., 2009a; West, et al., 2009b; Wilkinson et al., 2007). This pattern of results appears to be unique to our lab group. This is not due to the inability of other groups to replicate the findings of acute studies following a chronic intervention, but rather due to the relative lack of investigations that have followed up on acute tracer study results with chronic intervention studies in which the conditions from the acute study are mimicked and repeated chronically. Because of the qualitatively similar pattern of results between acute MPS studies and chronic measurements of hypertrophy in different groups of subjects (Holm, et al., 2008; Holm et al., 2010), it was reasonable to hypothesize that an acute MPS measurement might be related to the magnitude of hypertrophy in the same subjects if they are chronically repeated with the same exercise and nutritional stimulus.

Prior to the results presented in chapter four there had only been a single report of acute MPS being measured before chronic training in the same subjects. In this study a relatively small subgroup of subjects was used and mixed muscle MPS measurements were made in the fasted state 24 hours after the first exercise bout (Mayhew, et al., 2009). Due to the relatively delayed time point at which the FSR measurement was made, the highest magnitude portion of the acute MPS response was not captured (Phillips, et al., 1997). Also because mixed muscle, rather than myofibrillar, protein synthesis was measured, the non-contractile proteins within the muscle would have contributed to the response (Wilkinson, et al., 2008). Since the study presented in chapter four had a larger subject number, measured myofibrillar protein synthesis and determined the acute fed-state peak rate of MPS, the design was, I propose, stronger and more likely to see a potential relationship between acute MPS and hypertrophy in the same subjects than that of Mayhew et al. (2009).

As the data presented in chapter four clearly showed no relationship between acute MPS and hypertrophy, it is necessary to reconcile our current understanding of the regulation of muscle mass with this finding. There are four likely explanations for the lack of relationship between acute MPS and hypertrophy which may expound the observations in chapter 4. Firstly, it has been previously demonstrated that a single bout of resistance exercise can increases rates of MPS for up to 24 to 48 hours (MacDougall et al., 1995; Phillips, et al., 1997), and potentially even longer, after the completion of the exercise (Phillips, et al., 1997). The highest rates of MPS are observed within the immediate post exercise period (Tang et al., 2008), however, the average MPS response in the days following a bout of resistance exercise may be important in regulating long term muscle hypertrophy. Secondly, there appear to be changes in the magnitude and duration of the MPS response to a bout of resistance exercise after completion of multiple bouts of exercise spread over a period of weeks (training) (Tang, et al., 2008). The mixed muscle MPS response has been shown to be of a slightly greater magnitude acutely, but reduced in duration, in the trained state (Tang, et al., 2008). Less is known about changes in the myofibrillar MPS response (Kim et al., 2005), but it is possible that hypertrophic responders to resistance training may be able to sustain a robust MPS response throughout the training period whereas lower responders may show a diminished MPS response to subsequent training sessions. Thirdly, we know that muscle mass is regulated by the dynamic balance between MPS and muscle protein breakdown (MPB). In the study presented in chapter three, only MPS was measured because of the technical challenges that go along with the measurement of MPB (Phillips et al., 1999). It is possible that

differences in MPB resulted in changes in net muscle protein balance that could not be explained by MPS. In the fasted state, changes in MPS and MPB following exercise have been shown to be correlated and thus do not appear to be divergently regulated (Phillips, et al., 1999). Similarly, the magnitude of change in MPS is much higher (2-4 times) than the magnitude of change in MPB with feeding (Glynn et al., 2010), which would seem to indicate a greater degree of regulation and net flux-controlling capacity of MPS over that in MPB. Accordingly, it is unlikely that MPB is a major regulator of resistance training mediated hypertrophy; however the possibility cannot be ruled out. Finally, there is very little information on the reproducibility or between-day variability of MPS measured at rest or after resistance training in the same subject (Smith et al., 2011). It is possible that daily variations in MPS potentially caused by small changes in diet, sleep or physical activity could result in day-to-day changes in the MPS response to a bout of resistance exercise. It is also possible that variability may be inherent in the methodology used. To better understand the magnitude of day-to-day variability in the MPS response repeated measures of the same subjects' response to the same resistance exercise stimulus would need to be conducted. Importantly, a washout period would need to be included between measurements to account for the potential confounding effect of repeated exercise bouts (training). Future work should both attempt to quantify day-to-day variability in measurements of MPS and measure much longer post exercise incorporation periods. There is potential for the use of deuterated water tracers which would allow for the measurement of MPS over periods of days to weeks (Gasier et al., 2010).

5.5 CONCLUSIONS AND FURTHER DIRECTIONS

The studies presented in chapters two through four of this thesis have the common thread of trying to account for variance in the magnitude of hypertrophy following resistance training. Chapter two explored the effects of resistance training program variables, training volume, and relative load, on muscle hypertrophy and showed that even drastic changes in program design, such as a trebling of volume or an almost 4 fold difference in relative load did not significantly affect the magnitude of training mediated hypertrophy. Chapter three presented data in which responses to resistance training were studied to account for variability in resistance training mediated hypertrophy through the measurements of both local and systemic factors. In general, the results showed that systemic factors with the exception of IL-6 explain very little, whereas two local factors explain a substantial portion of the variance in hypertrophy. The fourth chapter of this thesis reported data from a study designed to explain the variance in training mediated hypertrophy through the measurement of the acute early MPS response. Since MPS is a dynamic measurement and is mechanistically necessary for muscle hypertrophy it was hypothesized that there would be a relationship. The results, however, did not agree with our hypothesis and there was no relationship between the variation in acute MPS and the variation in training mediated hypertrophy. Taken together the results from chapters two through four show that resistance training mediated hypertrophy has a high degree of inter-subject variability, which appears to be regulated primarily through local mechanisms and that modifications to most resistance training program variables have
modest effects on hypertrophy. As an underappreciated program variable, effort appears to be the most important as when resistance training is not performed until the point of failure, hypertrophy may not be maximized. It has been shown that short term gains in muscle strength are greater when training is performed to the point of failure (Folland et al., 2002; Rooney et al., 1994) however; there is only limited research on the direct effect of failure on hypertrophy.

To date, most lines of research attempting to understand the regulation of muscle hypertrophy have been done with reductionist approaches, such as the creation of genetically modified rodent models or the administration of inhibitors of protein kinases (Bodine, et al., 2001). This type of research has been useful in demonstrating the importance of the mTOR pathway in muscle hypertrophy; however, attempts to simply correlate the activation of components of this pathway with training induced muscle hypertrophy have been met with only limited success in human models (Terzis, et al., 2008). Further research into the variability in training-induced muscle hypertrophy should employ a systems biology approach, which attempts to discover emergent properties created by the interaction of the many complex and redundant pathways (Greenhaff et al., 2011). This type of approach can utilize many different 'omic' strategies to quantify a molecular signature of responders and non-responders at different levels of organization such as genomic, transcriptomic, and metabolomic. A 'road map' of the way forward can be found in recent work which has used gene expression profiling to determine the transcriptomic signature of responders and non-responders to aerobic training (Timmons et al., 2010). From the transcriptomic signature, candidate genes can be identified and

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potentially a group of single nucleotide polymorphisms (SNP), which relate to trainability, could be identified. When applied to aerobic training a group of 11 SNP were able to explain ~23% of the variance in VO_2 max gains in a large cohort of individuals undergoing aerobic training (Timmons, et al., 2010). Recently, the same research group has published data showing a transcriptomic signature of resistance training (Phillips, et al., 2013). Interestingly this work implicated genes related to the regulation of mTOR as being differentially regulated between responders and non-responders. Remarkably, responders showed an attenuation of mTOR and related gene network activation signature. Thus, as opposed to what one might hypothesize, responders to resistance training are those that do not up regulate the expression of genes coding for proteins in the mTOR pathway. Further research should focus on understanding this finding and quantifying the genetic basis for variation in muscle hypertrophic response to resistance training. A better understanding of the expression of genes that are correlated with muscle hypertrophy could allow for subjects to be block randomized based on a genetic predisposition score in future studies assessing resistance training program variables such as relative load and volume. By reducing inter-individual variability more subtle effects of other variables could be identified.

The work included in this thesis contributes to the body of resistance training literature by showing that light loads lifted to the point of muscular fatigue can result in a similar magnitude of hypertrophy to those observed after following traditional recommendations for exercise prescription. These results can help to inform future guidelines for resistances training. Chapter 3 adds to the body of literature which shows the importance of local factors in the regulation of muscle hypertrophy. The final study presented in this thesis demonstrates the limitations of conventional tracer methodology in predicting chronic adaptations to training. Together these studies highlight the importance of individuality in response to resistance training stimulus.

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APPENDIX A

The Relationship between Interleukin 6 and Hypertrophy Following Resistance Training Cameron J. Mitchell, Daniel W. West, Tyler A. Churchward-Venne, Stuart M. Phillips. Exercise Metabolism Research Group- Department of Kinesiology, McMaster University, Canada

Interleukin 6 is a cytokine which has acute anti-inflammatory effects when released following exercise but pro-inflammatory when resting levels are high. It is possible that high resting Interleukin 6 concentration could negatively affect the ability of an individual to hypertrophy in response to resistance exercise. 23 young men $(23 \pm 3 \text{ years}, 84.1 \pm 16.6 \text{ kg}, 178 \pm 9 \text{ cm}, \text{Means} \pm \text{SD})$ completed 16 weeks of 4x/week full body resistance training. Magnetic resonance imagining was conducted before and after the training period to quantify muscle volume (pre: 1837 ± 395 , post: $1970 \pm 399 \text{ cm}^3$; P<0.000001). Resting serum Interleukin 6 ($2.1 \pm 0.6 \text{ pg/mL}$) was negatively correlated with the percentage change in muscle volume (r = -0.45, p=0.029). Small increases in Interleukin 6 may interfere with the muscle's ability to undergo hypertrophy in response to resistance training.

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