Skeletal Muscle Growth Responses to Voluminous Resistance Training in Young Men with or without Graded Dosage of Protein Supplementation

by

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A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy in Kinesiology

Auburn, Alabama
August 4, 2018

Keywords: skeletal muscle, hypertrophy, graded protein, whey protein, resistance training

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ABSTRACT

Purpose: We examined hypertrophic outcomes of weekly graded whey protein dosing (GWP) versus whey protein (WP) or maltodextrin (MALTO) dosed once daily during 6 weeks of resistance training (RT). Methods: College-aged resistance-trained males (training age=5±1 yrs; mean±SE) were assigned to WP (25g/d; n=10), MALTO (30g/d; n=10), or GWP (25-150 g/d from weeks 1-6; n=11). RT occurred 3d/wk (2 upper- and 2 lower-body exercises/d, 10 repetitions/set), and RT volume increased from 10 sets/exercise (week 1) to 32 sets/exercise (week 6). The 6-week RT program implemented was designed to involve higher RT volumes than ever investigated in this timeframe. Tests performed prior to training (PRE) and after weeks 3 (MID) and 6 (POST) included dual-energy x-ray absorptiometry (DXA), vastus lateralis (VL) and biceps brachii ultrasounds, and bioelectrical impedance spectroscopy (BIS). VL biopsies were also collected for immunohistochemical staining. Repeated-measures ANCOVAs were performed, although emphasis was also placed on effect size calculations. Results: The GWP group experienced the greatest PRE to POST reduction in DXA fat mass (FM) (-1.00 kg, d=-0.24, $p<0.05$) and increase in DXA lean body mass (LBM) (+2.93 kg, d=0.33, $p<0.05$). DXA LBM increases (ΔLBM) occurred from PRE to MID (+1.34 kg, $p<0.001$) and MID to POST (+0.85 kg, $p<0.001$) across all groups. However, when adjusting ΔLBM for extracellular water changes, a significant increase occurred from PRE to MID (+1.18 kg, $p<0.001$), but not MID to POST (+0.25 kg; $p=0.131$). Conclusions: Larger effects on FM and LBM in GWP subjects indicates a need for longer-term investigations with greater sample sizes examining graded WP

intakes and RT. Additionally, ECW-corrected LBM gains were largely dampened, but still positive, in resistance-trained subjects when RT exceeded ~20 sets/exercise/wk.

ACKNOWLEDGMENTS

The love of my life, Courtney. Your support and compassionate care throughout our marriage and during my professional development is astonishing. Your understanding and kindness surpass my ability to express gratitude. The steadfast encouragement and emotional stability you have provided throughout my academic and professional endeavors have catalyzed this moment. I hope that I can become half of the person that you are. I love you. Forever, no matter what.

My loving mother, Marie. You deserve much of the credit for this achievement. Without you, none of this would have been possible. I am so thankful to call you Mom, and I am wrought with sincere thankfulness for your provision and support throughout my life. I am at a loss for words, which I know that you know is rare for me, when trying to express all you mean to me. I love you, Mom.

My stepfather, Tim. Your example and accountability have served as pivotal guiding variables in my development as a man. Frankly, I don't know where I'd be without you. You are an inspiration and I admire the consistency and discipline by which you lead your life. Your influence has instilled character traits that I strive to embody every day, and I am so thankful for you.

My principal mentors, Dr. Michael Roberts and Dr. Michael Stone. Dr. Roberts, you are an amazing man. Your kindness and willingness to listen to my ramblings surpass my ability to comprehend. The integrity and work-ethic throughout our time together has served to influence

my development as an academician in ways that typeset words are incapable of parlaying. I hope to become a fraction of the scientist, and, perhaps more importantly, the husband and father that you are. I thank you from the depths of my being for giving me the opportunity to attain my PhD under your guidance. Dr. Stone, your scientific rigor and mentorship were requisite in the completion of this degree. You instilled in me the critical importance of seeing commitments through to their completion. Your persuasive example as a scientist in my intellectual pursuits and philosophy of experimentation has proven critical to my academic success. The opportunities acquired as a result of my education under your supervision deserve my deepest thank you.

My colleagues and friends in the MASL platoon. This project would have not been completed without you. I sincerely thank each of you for all of your laborious days with me, and for your friendship. It has been an absolute honor and pleasure to work alongside you.

I am deeply indebted to my dissertation committee members, friends, and family for which this section could house story after story of your love and support. Please consider this terse acknowledgement my most sincere thank you.

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RT	resistance training
MPS	muscle protein synthesis
MPB	muscle protein breakdown

CHAPTER 1: INTRODUCTION

Resistance training (RT) and consumption of dietary protein are well-known adjuvants of skeletal muscle growth (i.e., hypertrophy). Early on, humans recognized the power of RT to significantly improve skeletal muscle size and strength (1). At least as far back as the 1800s, a critical relationship between dietary protein and skeletal muscle growth was also observed (2). First, I provide a brief overview of the history of RT and scientific inquiry thereof; particularly as it pertains to skeletal muscle hypertrophy. This is intended to set the stage for the structure of the training paradigm and experimental design for this dissertation. Next, I discuss key events and findings pertaining to dietary protein and protein supplementation and how this relates to my specific research question.

Brief History of Resistance Training

Physical training involving external loads is ubiquitous throughout human history for improving both the size and strength of skeletal muscle. Writings discussing RT paradigms in order to realize specific muscular adaptations dates back to as far as 6th century B.C., but certainly no further in the past than second century A.D. Mention of tests of strength and RT can be traced back to the Chou Dynasty in China and Milo of Croton in 5th and 6th century B.C., while discussion of exercise with external loads (e.g., halteres) is more thoroughly described in writings related to the physical culture of the ancient Greeks (3). Consideration of the optimal

methods for resistance exercise (RE) in order to realize specific muscular adaptations is at least as old (4).

Reportedly, the calf-carrying Milo of Crotona, frequently touted as the father of the RT principle of progressive overload, was using specifically designed weighted implements for training purposes during 6th century B.C. (3). Milo is also famously believed to have hoisted and shouldered a calf from infancy to full-grown on a daily basis, whilst also consuming kilograms of meat and wine per day. In second century A.D. ancient Greece, the physician, Galen, published *De Sanitate Tuenda* while philosopher, Philostratus, also published writings promoting structured models of load and exercise manipulation for Olympic preparation and the lifting of heavier implements upon being strong enough to lift lighter implements for muscular strength adaptation (i.e., progressive overload) (3,5). Although prevalent throughout human history, a milieu of approaches to RT have resulted in much debate and, in the modern World, scientific research attempting to elucidate optimal methods for maximizing human strength and skeletal muscle size is ongoing. By the 1900s, with the advent and success of the United States, formal scientific investigations into RT approaches in humans ensued. In 1946, Dr. Thomas DeLorme published "Heavy Resistance Exercises" where he argued that lifting heavy weights to improve muscle strength after injury prior to building endurance through low resistance, high repetition modes was a superior rehabilitative strategy compared to the opposite sequence (i.e., promoting muscular strength development prior to endurance development) (6,7). Dr. DeLorme continued to publish work in the area of RE during the 1940s and 1950s suggesting the effectiveness of RE to significantly improve strength and muscle size using loads corresponding to the heaviest that

could be lifted with reasonable technique 10 times or fewer, for multiple sets (e.g., 3 sets of 10 repetitions), beginning an era of a more thoughtful pursuit of muscle hypertrophy and strength in the Western world.

In 1962, Dr. Richard Berger published a manuscript reporting effects of specific RE dose configurations for improving maximum strength (8). Dr. Berger's dissertation research, which employed the bench press exercise due to the ease of teaching and standardization, suggested that 3 sets of 6 repetitions resulted in greater strength improvements than sets of 2 reps or 10 reps per set were performed for a total of either 1 total set, 2 total sets, or 3 total sets. That is, 3 sets of 6 repetitions resulted in the greatest strength improvements when compared to 1-3 sets of 2 or 10 repetitions. Dr. Patrick O'Shea also published work in the area of RT in the 1960s demonstrating the power of RT to significantly improve skeletal muscle size and strength (9,10). In the early 1960s, the emergence of the skeletal muscle biopsy technique, still prevalent in skeletal muscle physiological research today (11), paved the way for critical discoveries in the 1970s. The 1970s are considered a nexus point in the history of RT science (4). In 1970, Ikai and Fukunaga were apparently the first to measure muscular hypertrophy from RT in humans using ultrasound technology (12). Ikai and Fukunaga reported increases in bicep muscle thickness of 23.0 % after 100 days of maximum isometric elbow flexion for a total of 30 seconds per day. Through the 1970s, electromyography (EMG) investigations and analysis of skeletal muscle fiber property alterations (e.g., biochemical, structural) in humans undergoing RT further clarified effects of specific RT paradigms. Costill (13), Thorstensson (14), MacDougall (15), Hakkinen (16), and others provided pivotal data in the study of neural, structural, and biochemical adaptations to RT

through the 1970s-1980s. For example, Costill et al. showed significant increases in glycolytic and mitochondrial enzymes after a period of 7 weeks of RT indicating specific, short-term biochemical alterations of skeletal muscle to RT in humans (13). This decade served as a foundation for more critical discovery through the 1980s and 1990s by Hakkinen (16), Tesch (17), Staron (18), and others clarifying relationships between specific dosages of RT (e.g., intensity and volume) and resultant adaptations. Data from this era led Dr. Andrew Fry to conclude that the regular employment of loads corresponding to 80-95 % one-repetition maximum (1RM) seemed to explain greater cross-sectional areas of muscle fibers biopsied from powerlifters and weightlifters, compared to those of bodybuilders (19); sparking further scientific discussion of distinct dose-response relationships of RT parameters and resultant adaptations. Dr. Robert Staron's group also produced seminal work in the area of specific muscle fiber adaptations to RT in the 1990s. For example, Dr. Staron and colleagues were apparently the first to report reductions in the amount of type IIb (now known as type IIx) myosin isoforms with only 8 weeks of RT, indicating early phase differential phenotypic alterations in skeletal muscle fibers from short-term RT (20).

This work served as the foundation for ongoing RT investigations in the 21st century. Although specific relationships to RT paradigms and resultant hypertrophy will be further discussed in Chapter 2 (see *Resistance Training for Hypertrophy)*, a terse discussion of important meta-analytical findings follow in this section. In 2004 (21), Kreiger published a meta-analysis demonstrating significantly greater effect sizes for muscle hypertrophy when programs included 3 sets per week for a specific muscle, compared to 1 set. Peterson and Rhea also published meta-

analytical data demonstrating the same phenomenon for strength, to a point (i.e., greater volume = greater strength improvement) (22). This data agrees with work from Dr. Stuart Phillips laboratory demonstrating greater myofibrillar protein synthesis responses from 3 sets of RT compared to 1 set (23). This led Dr. Brad Schoenfeld et al. to conduct a more recent meta-analysis of RT studies wherein hypertrophy was measured to further clarify relationships between RT volume and skeletal muscle hypertrophy (24). This meta-analysis clearly revealed greater hypertrophy with greater training volumes (e.g., >10 sets per muscle per week). In fact, Schoenfeld et al. suggest almost a doubling in the hypertrophic response when comparing less than 5 sets executing specific exercises emphasizing specific musculature per muscle per week compared to 10 or more sets per muscle per week (i.e., 5.5 % vs 8.6 %) (24). However, as will be discussed later, insufficient data exists to understand hypertrophic responses beyond 10 sets per muscle, per week. Theoretically, this observed increase in hypertrophy with greater training volumes would continue up to a certain point, at which the hypertrophic response would exhaust and further increases in training volume would result in non-functional overreaching and eventually overtraining (see *Human Adaptation*). The lack of data beyond these previously investigated training volumes served as a catalyst in the development of this dissertation's primary research question, namely: What are the hypertrophic responses to RT in previously trained young men beyond 10 sets per muscle per week?

Brief History of Protein Supplementation

The increased consumption of higher amounts of dietary protein by ancient *Homo* genus ancestors is thought to be a primary cause of our human existence today (25). Archaeological evidence dated at ~2.5 million years old suggests a correlation between skull size (i.e., proxy of brain volume) and the concurrent prevalence of animal fossils and weapons pointing to the increased procurement and consumption of animal protein sources in proximity to the emergence of *Homo* skeletons. This suggests an intimate relationship between the increased consumption of dietary protein and our human emergence. More recently, scientific research throughout the past century has revealed an explicit relationship between the amount and quality of dietary protein and consequent impacts on human health and adaptations to RT.

Protein was first isolated in the 1830s by Dutch Chemist Gerhard Mulder. Mulder and colleague Jöns Jakob Berzelius quickly recognized the importance of this discovery and developed the term based on the Greek word *proteios* meaning "of the first order" (26). Protein is a nitrogenous organic compound comprised of amino acids linked by peptide bonds (27). Although specific amounts depend on body fatness, hydration status, and muscle mass, the human body is typically composed of ~ 20 % protein, ~60 % water, and ~15 % lipid; while minerals and other trace elements make up the remaining percentage (28). Around 90 % of the dry weight of human skeletal muscle tissue is composed of protein, immediately presenting a plausible relationship between dietary protein and skeletal muscle tissue function and size (29, 30). Upon consumption of dietary protein and digestion in the human gut, constituent amino

acids are utilized primarily in their free form to accomplish a host of critical physiological processes in the human body necessary for development, growth, and repair (31). In reference to skeletal muscle, amino acid availability is necessary for significant increases in MPS at rest, and as a means to augment MPS in response to RT (32). Moreover, evidence from the past century of dietary protein research suggests that: a) relatively high dietary protein intake is critical for *maximizing* human muscle growth, particularly in context of ongoing RT, and b) both the amount and composition of consumed protein can influence muscle hypertrophic responses (33,34).

In way of convenience and economic gain, commercial protein supplements began to appear in the 1950s (35). Protein supplements were first promoted on a broad scale for health and muscle-building properties boasting many anecdotal benefits without empirical support (35). Consequently, scientific investigations into the effectiveness and supposed underlying mechanisms of protein supplementation followed. Some of the earliest studies on protein supplementation coupled with RT indicated no significantly different effects on muscle hypertrophy or strength compared to a control group (36). However, these findings were likely due to the fact that only ~3g of supplemental protein was provided in capsule form. As shown by more recent evidence, this serving size is insufficient to instigate significant enhancement of MPS and a net positive protein balance leading to eventual hypertrophy in neither trained nor untrained humans (37). Additionally, research since the 1960s has clearly demonstrated that not only the amount, but also the source of supplemental protein is important for sought-after hypertrophic effects (34). Nevertheless, specific dose-response relationships between protein serving sizes and adaptive responses, optimal sources, and effective timing strategies for

maximal muscle hypertrophy in response to specific RT paradigms remains incompletely resolved.

Recent meta-analytical data, and evidence from original investigations, suggests the following: a) in comparison to other prevalent commercially-available protein supplements, whey protein generally results in higher MPS responses on a per-unit serving basis (38), b) humans undergoing RT supplemented with whey protein typically gain more muscle mass after a short-term training period when compared to non-supplemented or control groups (37), c) consuming up to ~0.24 g/kg of protein per meal in the post-RT period results in comparatively higher MPS responses than lower doses, d) spreading doses into servings of ~0.24 g/kg every 3-5 hours rather than smaller, more frequent, or larger, less frequent doses seems to result in superior MPS responses in a 24 hour span, and e) dietary protein intakes beyond ~1.6 g/kg/day do not seem to result in further augmentation of RT-induced increases in lean body mass (LBM) (37). Also, research in the last few decades has consistently demonstrated that although an excess of amino acid availability (hyperaminoacidemia) at rest tends to increase MPS above resting levels, the combination of RT and hyperaminoacidemia results in more pronounced increases in MPS by comparison (39). These data have persuaded the adoption of field-wide dogma encouraging the practices described above to maximize the hypertrophic response.

In spite of this evidence, data from recent investigations challenge the non-skeptical acceptance of these practices for maximal hypertrophic responses to RT, particularly in humans with previous RT experience (i.e., “resistance-trained”). In 2006, Hoffman et al. published data from 23 strength/power athletes wherein subjects were stratified into one of three groups, based

on protein intakes from self-reported dietary logs over the course of 12 weeks of RT: 1) below recommended levels (BL; 1.0-1.4 g/kg/day), 2) recommend levels (RL; 1.6-1.8 g/kg/day), and 3) above recommended levels (AL; >2.0 g/kg/day). Although not reaching statistical significance, subjects in the AL group gained, on average, 1.2 kg more lean body mass (LBM) than the BL group, and 0.33 kg more LBM than the RL group. Furthermore, AL subjects experienced 22 % and 42 % greater improvements in 1RM squat and 1RM bench press than subjects in the RL group. In 2014, Antonio et al. presented findings from 30 resistance-trained individuals continuing their normal RT practices and consuming either: a) 1.8 g/kg/d, or b) 4.4 g/kg/d of dietary protein over the course of an 8-week exploratory study. Although not reaching statistical significance, subjects consuming 4.4 g/kg/d gained, on average, 0.6 kg more fat-free mass (FFM) and lost 0.2 kg more fat mass than subjects consuming 1.8 g/kg/d. Antonio et al. then conducted a follow-up investigation in 2015 wherein a total of 31 subjects consumed ≥ 3 g/kg/d, and 17 subjects consumed their normal amount of dietary protein (1.8-2.3 g/kg/d) for 8 weeks, while undergoing 5 days of RT per week. After the 8-week treatment period, both groups gained statistically equivalent amounts of FFM (+1.5 kg), however, the three highest hypertrophic responders in the study consumed ≥ 3 g/kg/d. Additionally, subjects consuming ≥ 3 g/kg/d lost significantly more fat mass (-1.6 kg vs -0.3 kg). Further, in this follow-up investigation, Antonio and colleagues demonstrated no significant alterations in a basic metabolic panel completed on blood samples derived from subjects before and after the 8-week treatment period. This suggests that the consumption of dietary protein ≥ 3 g/kg/d in young, healthy individuals completing regular RT is a safe practice.

More recently, having employed the tracer infusion technique to objectively measure MPS responses in the post-RT recovery period, Macnaughton et al. published data in 2016 derived from 30 resistance-trained males consuming either 20g or 40g of supplemental whey protein during recovery from a single bout of RE demonstrating significantly greater MPS responses after consumption of 40g of whey compared to 20g. Notwithstanding, the bulk of the available evidence does seem to indicate a saturable MPS response to dietary protein intake with maximal effects seeming to occur at 1.8 g/kg/d (34). However, with the above data in mind, absence of evidence is not evidence of absence regarding effects of significantly higher protein intakes and more elegant supplemental strategies. In a 2012 literature review titled: "Is there a maximal anabolic response to protein intake with a meal?", Dr. Nicolaas Deutz and pioneer of protein metabolism research Dr. Robert Wolfe suggest that many of the studies examining effects of various intakes over the past few decades neglected the measurement of protein breakdown (40). Further, Deutz and Wolfe argue that higher protein intakes when protein synthesis is seemingly maximized is characterized by suppressed muscle protein breakdown (MPB) leading to an overall greater anabolic response (e.g., Net Muscle Protein Balance = MPS – MPB). This occurrence, as noted by the authors, explains why *net* protein balance measurements remain linear without an apparent plateau at higher levels of amino acid availability. Interestingly, Deutz and Wolfe conclude no practical upper limit to the anabolic response to protein intake in context of a single meal. Considering the above, the second research question of this dissertation can be framed as: Is the hypertrophic response to increasing volumes of RT augmented by concomitant increases in dosages of whey protein?

CHAPTER 2: LITERATURE REVIEW

I first provide a brief overview of scientific literature related to human adaptation in particular reference to adaptive processes relevant to RT. Next, I review select concepts and scientific research describing skeletal muscle hypertrophy in response to RT, and specific underlying mechanisms critical to this process. Lastly, the efficacy of whey protein supplementation to augment skeletal muscle hypertrophy in response to RT is discussed, leading to the presentation of data from the original research conducted for this dissertation in Chapter 3.

Human Adaptation

In context of physical exercise and training, recovery can be defined as a reestablishment of the initial state, while adaptation expands upon recovery processes by including the concepts of growth or supercompensation (41). Necessary in the discussion of training adaptation, fatigue is an inherent occurrence of the training process. Although defined in different ways, the practical definition of fatigue is a reduction in performance capability (e.g., decreased power output), which is reversible with recovery. By definition, fatigue is required prior to recovery, from which adaptation may proceed. Adaptation to training has been defined as change in structure and function that results from repeated bouts of exercise that prepare the body to better cope with exercise (42). Stated another way by Sands et al.: “Recovery is getting back what was

lost or simply bringing an athlete's performance back to where it was. Adaptation deals with the process of long-term adjustment or alterations related to a specific training program." In a sense, recovery can be thought of as replenishment, while adaptation can be characterized as the superseding of some previous measure or improvement of a previous performance characteristic. Inherently, the training process aims for adaptation beyond recovery alone. Consequently, various models of human adaptation to physical training have been proposed dealing with adaptive processes and their time courses in way of maximizing the effectiveness of training stimuli. To stay on course, I first discuss what I perceive to be three of the most relevant biological concepts to training-induced adaptation (i.e., Homeostasis, General Adaptation Syndrome, Supercompensation) and then segue into a brief description of two models of adaptation (i.e., Fitness-Fatigue Model, Secondary Signal Model) posited to explain the qualitative pattern of training adaptation and eventual performance.

The biological concept of homeostasis is credited to Walter Cannon in 1929 and based largely on experiments of Claude Bernard (43). Simply stated, homeostasis refers to the phenomenon of the human body tending to maintain a steady state; and, upon perturbation (i.e., stress), mounting physiological responses to return to or establish a new steady state (e.g., set point) (44). It seems that Cannon considered the human body as an open system with reactive mechanisms able to correct large fluctuations (e.g., negative feedback) in order to maintain homeostasis. Since Bernard and Cannon's historical contributions, homeostasis has been extended to the cellular and protein level (e.g., proteostasis) and is currently considered a robust theory of human physiology (45). However, more recently, terms like rheostasis, allostasis, and

others have been proposed to better reflect the human body's capability to *change* the steady state of regulated parameters to adjust to changing environments or regularly encountered stressors (e.g., training stressors) (44). Regardless of nomenclature, the concept of homeostasis suggests a dynamic reactivity to stress based upon the capacity of the human body to adjust physiological parameters as necessary for survival or improved fitness in anticipation for potential future stressors (i.e., adaptation). Consequently, a failure to defend homeostasis when faced with excessive stress leads to death at the cellular, tissue, organ, or whole-body level. In context of RT, the discussion above underpins the rationale for inclusion of the term homeostasis in this section, since this concept is central to the process of human adaptation to RT. Since RT causes a disruption in homeostasis (e.g., elevated heart rate, respiration, muscle protein turnover, etc.), appreciating the magnitudes by which disruption of homeostasis occurs during various RT paradigms is central to the appropriate dosage for desired adaptations. Practically, stratifying training stress categorically into adaptable or unadaptable, functional or nonfunctional, etc., is particularly intriguing in relation to the RT process. Furthermore, clarifying appropriate individual RT dosages to avoid excessive or unnecessary perturbations in homeostasis is of paramount importance to a productive RT process.

Based on Cannon's original work, De Luca et al. suggest Cannon's original definition of homeostasis as, "…steady states maintained by mechanisms peculiar to living beings.", and argue that the essence of homeostasis is related to the constancy of the extracellular fluid (ECF) (i.e., blood and lymph) within narrow limits or homeostatic ranges (e.g., plasma glucose, plasma sodium, etc.) (46). Dysregulation of the ECF (e.g., hypoglycemia, hyponatremia, etc.) has

obvious physiological consequences, with the most extreme example being death. Currently, in support of these homeostatic ranges, nearly all common clinical measurements allow ranges wherein measurements are considered normal (e.g., blood pressure, plasma electrolytes, etc.), while measurements outside these ranges generally indicate pathology or correlate with the presentation of symptoms. For example, plasma glucose levels slightly below the normal range (~65 mg/dL) cause secretion of counterregulatory hormones (e.g., epinephrine, glucagon); where values significantly below this point can result in severe cognitive dysfunction, seizure, and even death (47). Following the theme above, recurrent hypoglycemic events result in characteristic adaptations to better respond to reduced plasma glucose in the future (e.g., alterations in hepatic glucose production and potentiated counterregulatory hormone secretion). An abundance of examples in human physiology could be provided illustrating this qualitative pattern. To be concise, it seems rather clear that many processes in the body are tightly regulated within homeostatic ranges and perturbation of these ranges (i.e., stress) elicits both general and specific responses that can lead to adaptation, and potentially the establishment of a new steady state or "set-point" (48). Critical to the overarching point of this section, disruption in homeostatic ranges, to a tolerable extent, is the catalyst of RT-induced adaptation. Clear examples in skeletal muscle specifically are the significant alterations in MPB and MPS in response to a variety of RT loading scenarios; where the basal status of skeletal muscle (i.e., skeletal muscle homeostasis) is disrupted by the generation of muscular tension spurring alterations in breakdown and synthetic processes. This thesis was beautifully borne-out by demonstrative experiments performed by pioneer of stress research, Dr. Hans Selye.

Dr. Selye, termed the qualitative pattern by which organisms respond to stress as the *General Adaptation Syndrome* (GAS). Selye proposed two types of physiologic stress: 1.) eustress and 2.) distress (49). Eustress is considered favorable and would elicit a positive adaptive outcome (e.g., appropriately dosed RT resulting in muscle hypertrophy), where distress is considered unfavorable and would elicit maladaptation (e.g., inappropriately dosed RT resulting in muscle atrophy or significant injury) (49,50). Selye separated the GAS into three stages: 1.) the alarm reaction, 2.) the stage of resistance, and 3.) the stage of exhaustion. Selye also coined the *Local Adaptation Syndrome* (LAS) where a similar qualitative response can occur to a highly specific, localized stressor (e.g., specific muscle damage from training) (51). This model and its applicability to RT has been recently critiqued and reviewed extensively elsewhere (52,53). Importantly, the nature of the GAS was intended to characterize, as it states in its name, a *general* response to stress and highlight the fact that stress elicits characteristic responses from which adaptations can emerge. Additionally, the GAS model highlighted the fact that too much stress too suddenly, or for too long, can result in maladaptation. Selye also noted the importance of "conditioning factors" in the general response to stress; one of which, nutrition, being of particular relevance to this dissertation. However, given the ubiquitous nature of stress, defined herein as a perturbation in homeostasis, quantifying stress input and adaptive output from a whole-body perspective is an arduous endeavor. Nevertheless, to move toward a true predictive model of RT dose-response, it is a necessary one. Recall that recovery is simply a return to baseline and, although a requisite step for adaptation, is a means to an end in relation to RT. Rather, adaptation is the logical pursuit of all RT (e.g., improved strength, hypertrophy,

etc.). Selye pointed to this distinction by discussing "adaptation energy" and noted that adaptation is an energetically costly process. As an example, we are now aware that muscle protein breakdown, synthesis, chaperoning, and deposition in tissue is indeed an ATP-costing phenomenon. Furthermore, a number of investigations have demonstrated significant improvements in FFM when subjects are overfed, particularly if combined with RT (54–56). However, these investigations have also shown significant accrual of fat mass, in some cases. Of course, the desire of most resistance trainees is to maximize muscle gain while minimizing fat accrual. Therefore, understanding the balance between training stress and resultant recovery-adaptive responses are central to effective dosing of training parameters. To summarize, stress perturbs homeostasis, resulting in fatigue. Stressors within a tolerable spectrum result in physiologic responses (i.e., alarm stage) that can lead to adaptation. Adaptations occur to prepare the organism for future encounters with a stressor (i.e., resistance stage). However, if a stressor is excessive, being either too severe too suddenly, or applied for too long, maladaptation will result (i.e., exhaustion stage). This phenomenon has been termed nonfunctional overreaching or overtraining in the RT literature (57). With the above in mind, the concept of supercompensation deals with the resistance stage of Selye's model, and is a conceptual proposition offered to explain the biological phenomenon of RT-induced adaptation.

Borrowing from the historic research of physiologist Dr. Carl Weigert, supercompensation is related to a biological phenomenon involving the compensatory replacement or overproduction of new tissue during the process of regeneration or repair (i.e., Weigert's Law) (58,59). One of the most well demonstrated supercompensatory responses

caused by training is that of muscle glycogen supercompensation. Briefly, exercise significantly reducing muscle glycogen stores followed by a high intake of carbohydrate tends to result in a significantly increased storage of muscle glycogen in the exercised muscle upon execution (60,61). Hallmark adaptations of RT like increased strength and muscle hypertrophy can also be characterized as supercompensatory responses to training. The growth of muscle tissue in response to appropriately dosed RT aimed at increasing muscle size quite literally adheres to the concept of supercompensation since a greater amount of tissue exists upon adaptation than was present previously. Therefore, the RT process can be conceptualized in light of the above concepts. It logically follows that models have been proposed to help visualize these processes and better understand dose-response relationships of RT.

Two popularized models are: 1.) The Fitness-Fatigue Model, and 2.) The Secondary Signal Model (42,62,63). The Fitness-Fatigue Model is a two-component model proposed by Bannister et al. suggesting that an individual's preparedness to perform is the summation of both fatigue and fitness after-effects due to training. The appeal of this model, beyond consideration of supercompensation alone, is related to the fact that the fatigue generated from training is considered to influence subsequent training or competition performances and thereby impacts preparedness. This model has served to form the basis for modern periodization strategies like tapering or unloading periods wherein attempts are made to reduce fatigue potentially masking fitness, while fitness is largely maintained through appropriate recovery and training strategies to better potentiate performance in subsequent events or training sessions. The Secondary Signal Model builds upon the original tenets of the Fitness-Fatigue Model by distinguishing the acute

fatigue from training, maladaptation to excessive training loads, and positive adaptive responses as secondary signals to the primary training stimulus. A detailed discussion of these models and supporting evidence, along with other proposed models, is outside the scope of this section. The overarching point of their inclusion is to appreciate the balance between training stress, consequent fatigue and adaptive responses, and how these factors interact to influence resultant adaptation and eventual performance. In particular reference to the RT process as it pertains to maximizing skeletal muscle growth, these models suggest that training sessions be organized in such a way to allow subsequent, productive training sessions (e.g., overloading sessions) rather than considering their original intent with keen focus on performance in sport alone. Stated differently, these models provide conceptual rationale for the organization of training sessions from which skeletal muscle growth is the sought-after outcome into concentrated periods where training stimuli, and the secondary signals thereof, are structured to avoid unnecessary signal interference. Sensibly, the ideal hypertrophy-based RT paradigm instigates appropriate programming manipulations to avoid regression of muscle tissue, respectively, and maximizes tissue growth. The pursuit of such a model is related to the research question for this dissertation, since the available evidence insufficiently explains at what point short-term (e.g., 6-8 weeks) hypertrophy-based RT dosage surpasses adaptive capacity. That is, it remains unclear at what point RT aimed at increasing muscle size surpasses the effective dose range and humans move into the "exhaustion stage" of Selye's model during such an RT program. In light of these concepts, the following sections serve to provide an explanation of skeletal muscle hypertrophy and underlying mechanisms, including an analysis of suggested RT practices surmised from the

available evidence highlighting the limited data beyond certain training loads. Chapter 2 finishes with consideration of how whey protein may augment RT-induced skeletal muscle hypertrophy, before the original work from this dissertation is presented in Chapter 3.

Skeletal Muscle Hypertrophy

Etymology of the term hypertrophy reveals both English and Greek roots where hyper-, from English, denotes "beyond or exceeding" and –trophia, from Greek, denotes "nourishment". Thus, hypertrophy indicates an excess of nourishment required to maintain muscle fiber size, and intends to describe fiber growth. Consequently, skeletal muscle hypertrophy has been defined as an enlargement of contractile elements and expansion of the extracellular matrix, indicative of sufficient nourishment to support cell growth (64,65). Skeletal muscle hypertrophy is an integrative process involving a milieu of signals. These signals can result in post-translational modifications to various proteins, transcription of various types of RNA, and/or an enhanced translation of mRNA into proteins which can eventually serve to expand cell size. This section serves to provide a conceptual overview of hypertrophy, describe some of the nuances worthy of consideration for its measurement in humans, and intends to lay a foundation for a more mechanistic discussion in following sections.

The definition provided above offers more of a macroscopic description of hypertrophy, while the technicalities pertaining to which contractile elements enlarge and what exactly is meant by contractile element is left subject to interpretation. For clarity, whole skeletal muscle is

organized into the following structures, in order from largest to smallest: a) fascicles of muscle fibers, b) individual muscle fibers, c) myofibrils composing muscle fibers, and d) sarcomeres composing myofibrils. These structures are suspended in a fluid medium of mostly water (e.g., ~75 % of muscle is water in vivo), while muscle also contains typical cell components and organelles. For example, mitochondria, ribosomes, lysosomes, and a phospholipid membrane. However, skeletal muscle cells are unique to many cell types by their cylindrical, tube-like shape, direct innervation of alpha motor neurons, relatively abundant capillary supply, specialized "sarcoplasmic" reticulum, and multiple nuclei per cell. Furthermore, skeletal muscle represents ~50 % of body mass in most humans and is characterized as post-mitotic. For these reasons, among others, skeletal muscle is a particularly plastic tissue and represents an important tissue for health, and, of course, exercise performance. In regard to health, skeletal muscle is the primary site of glucose disposal, immediately presenting a role in pathological conditions related to glucose handling (e.g., diabetes). For performance, muscle's role is obvious in locomotion and in the performance of advanced athletic skills.

As stated prior, ~90 % of the dry weight of skeletal muscle tissue is protein. Since 5,341 non-redundant proteins have been discovered to be present in human skeletal muscle, the definition of hypertrophy above provides little clarity regarding specific proteomic changes during hypertrophy from RT. Approximately 85-90 % of a skeletal muscle cell is occupied by myofibrils, by volume, and basic myofibrillar arrangement (e.g., sarcomere spacing) is largely conserved across taxa. Importantly, nearly the entire volume of a skeletal muscle cell is occupied by three constituents: a) myofibrils, b) mitochondria, and c) sarcoplasmic reticulum (66,67).

Only ~1-3 % of skeletal muscle cells seem to be occupied by other constituents (e.g., glycogen, sarcoplasm, nuclei, organelles, etc). This evolutionarily conserved structure is worthy of mention as an expansion of muscle cell size would seemingly still result in the typically observed components, with these components simply increasing or decreasing in amounts constrained to a relatively tightly controlled concentration spectrum. Stated differently and excluding genetic anomaly, environmental stimuli (e.g., exercise, nutrition, etc.) would seemingly alter amounts of cell constituents rather than result in separate constituents altogether. Considering this, hypertrophy seems primarily due to the difference between MPS and MPB rates, since skeletal muscle is primarily composed of myofibrillar protein. Specifically, an increase in fiber size is thought to be due primarily to the magnitude of MPS outpacing MPB for a sufficient amount of time. Conversely, a decrease in fiber size is thought to be due primarily to the magnitude of MPB outpacing MPS. Importantly, although adequate to parlay the general outcome of RT-induced skeletal muscle growth, the term "hypertrophy" and the definition provided above insufficiently describe *what* exactly within skeletal muscle tissue increases during hypertrophy and *where* specifically this increase occurs. Additionally, although generally understood to refer to an increase in fiber number, etymology of the term skeletal muscle hyperplasia (-plasis from Greek meaning "formation") similarly allows a variety of interpretations to the foreign student of skeletal muscle. For example, myofibril number possibly increases or decreases with training or detraining (68), which could be interpreted as a form of hyperplasia. Notwithstanding, a clear observation of an increase in whole fiber number in adult humans in response to RT is evidently absent, yet debated (69,70). Theoretically, packing density (mass/volume) of myofibrils could

increase without an observed change in cross-sectional area measured by histological methods (67,71). In this case, hypertrophy of the cell measured by cross-sectional area (CSA) would be denied. Clearly, however, since myofibrils are composed of sarcomeres (i.e., the basic functional unit of a skeletal muscle fiber [contractile elements in the above definition]) and more myofibrils would necessarily increase the capacity of force production since more myosin and actin cross-bridges could be formed, it is inappropriate to conclude a lack of hypertrophy in this case. Conversely, RT has been shown to increase intracellular water concentration and this could hypothetically expand the measurement of CSA without a concomitant increase in myofibrillar protein and resultant force production (72). Unless water or ion concentration are considered "contractile elements", this observation would be inappropriately termed hypertrophy based on the definition provided above. For these reasons, this calls into question the various methods used to measure hypertrophy, and, in particular, CSA measured by immunohistochemistry. Of course, a multi-compartment model of analysis where multiple measurement techniques are used can provide more insight and are commonly employed (e.g., DXA, Ultrasound, etc). Still yet, these measurements do not and cannot for that matter provide direct insight into proteomic changes in skeletal muscle. This warrants a reconsideration of how the presupposed physiological response of hypertrophy to RT is scientifically detected moving forward into the 21st century. Semantics aside, the overarching point so far is that the current state of the skeletal muscle hypertrophy literature provides little clarity in regard to the specific extent to which contractile elements are altered in response to specific RT paradigms. Presupposition based upon skeletal muscle structure and function fairly allows an expected increase in sarcomeric protein

content directly involved in force generation in response to RT, since most physiology obeys the concept of form following function (e.g., increase in contractile protein content [e.g., symmorphosis]). Indeed, mechanistic data indicate an increase in both sarcomeres and myofibrils in parallel in response to RT (17,73). For this reason, terms like sarcomerogenesis and myofibrillogenesis have been coined to better describe the process of muscle hypertrophy (74,75). However, based on this review of the literature, scant evidence allows confident inference of this process in human skeletal muscle in response to RT wherein hypertrophy data are provided. This is due to the fact that the majority of investigations provide *indirect* measurement of the previously described definition of hypertrophy since contractile elements are not *directly* assessed. Contractile elements, in this case, can be thought of as sarcomeric proteins involved with the formation of myosin and actin cross-bridges. With this in mind, ultrasound measurement of muscle thickness, muscle fiber cross sectional area (fCSA) assessed by histological staining, functional magnetic resonance imaging (fMRI), and dual-energy x-ray absorptiometry (DXA) data are often reported in RT investigations as measurements of hypertrophy. Although these measurements can be confidently assumed to corroborate increases in skeletal muscle protein content, they do not *directly* measure an increase in contractile protein content, or extracellular matrix protein content. Consequently, it remains unclear if proxies of hypertrophy like the ones mentioned above strongly correlate to a true increase in contractile protein content per fiber in response to RT. Or, if an increase in muscle size suggested by these measurements is rather associated with an expansion of the sarcoplasm due to an increased volume of fluid, sarcoplasmic protein content (e.g., glycolytic enzymes), or some other factor.

Stated differently, hypertrophy in response to short-term RT, according to the measurement methods above, insufficiently clarifies if an increase in muscle size is due to an increase in myofibrillar protein content, sarcoplasmic protein content, or some other component (e.g., increased glycogen storage). Indeed, a recent review in 2016 by Petriz et al. on the skeletal muscle proteome response to exercise concluded: "studies concerning the proteomic modulations upon resistance and strength training are still poorly explored" (76). Therefore, future research can provide important clarification in this area of skeletal muscle physiology and novel methods allowing more sensitive measurement of the skeletal muscle proteome in response to RT are warranted. As a follow-up to the primary analysis of this dissertation described in Chapter 3, we intend to measure myofibrillar and sarcoplasmic protein concentrations using plate-based assays, along with muscle glycogen content, in vastus lateralis biopsy samples from subjects undergoing RT over a 7-week period. We intend to also collect both wet and dry weights of samples (after dehydration of tissues) to characterize fluid alterations. This is a first step to elucidate which fraction of the skeletal muscle proteome (i.e., sarcoplasmic vs myofibrillar) is more affected from the training paradigm employed and intends to clarify alterations in light of the other more indirect measurements of hypertrophy described above and presented in Chapter 3. This can also allow targeted mass spectrometry analyses probing either the myofibrillar or sarcoplasmic fraction for specific proteomic alterations, depending on the fraction most affected by RT. These aspects of skeletal muscle hypertrophy in response to RT are further discussed below. Additionally, effects of specific training paradigms involving different intensities, volumes, or frequencies on the human muscle proteome deserve future research. Notwithstanding, a

conceptual relationship between RT-induced changes in muscle mass have been clarified and are further discussed below.

Recently, the human skeletal muscle proteome was "reappraised" (77,78). Interestingly, most of the proteins in skeletal muscle, by percentage, are involved in regulation, transport, cell cycle, and metabolism. This runs counter to the assumption that most of the proteins in skeletal muscle serve a direct contractile role (e.g., actin, myosin). To be more specific, around 40 % of the total number of proteins in skeletal muscle are enzymes whereas under 10 % seem to be contractile. Furthermore, ~20 % of the proteins in human skeletal muscle are characterized as mitochondrial, apparently serving critical roles in oxidative metabolism. Notably, these percentages are relative to the total number of proteins in human skeletal muscle, and not the concentration of proteins within skeletal muscle. Of the mixed muscle protein pool, ~60-70 % of proteins are myofibrillar, whereas ~30 % are characterized as sarcoplasmic. According to this review of the literature, and a recently published review of the literature (79), the formal measurement of myofibrillar protein turnover in response to RE began in the 1990s (80,81). A comprehensive discussion of each study investigating the synthetic and breakdown responses to RT is outside the scope of this section. However, strategically selected examples in the literature and a discussion of meta-analytical data capturing the current state of the MPS and MPB science follow. Notably, proteomic investigations and fractional breakdown and synthetic responses to exercise types other than RT in humans are not discussed.

It was quickly evident that both MPS and MPB increase in response to RT upon conclusion of some of the earliest experiments in humans (82). As alluded to above, which

proteins are specifically synthesized and broken down in response to RT is still being unraveled, although recent progress has been made. In 2011, Hody et al. reported reductions in MHC isoform abundance and glycolytic enzyme abundance after subjects completed 3 sets of 30 maximal contractions of the quadriceps over the course of two weeks compared to a control group completing no training (83). Clearly, this runs counter to the concept suggesting an increase in MHC protein abundance during skeletal myocyte growth. However, based on the methodology employed by Hody et al. where standard amounts of muscle samples were analyzed using mass spectrometry, this does not unequivocally indicate a true reduction in *total* MHC isoforms. To clarify, it is possible that total MHC isoform number could have increased with concomitant increases in muscle volume. This would result in an equal concentration, or, if volume increases outpaced MHC isoform synthesis, a potential reduction in MHC isoform abundance per unit volume. This could explain the findings of Hody et al., where standard volumes of sample were analyzed before and after the intervention where fiber volume increased and MHC isoform number increased, but MHC abundance to a lesser extent (i.e., an apparent reduction in abundance).

Although in rodents, Tibana et al. reported muscle proteomic data suggesting that higher volumes of RT resulted in no additional increase in muscle CSA compared to a lower volume RT model (i.e., CSA was similarly increased in both conditions), wherein the authors reported a "significant disturbance" of other proteins in the higher volume group and suggested that the higher volume condition induced excessive protein breakdown (84). The authors speculated that breakdown processes matched synthetic after a certain training dose, indicating an ineffective

point of training for hypertrophy after a certain point. In 2017 (85), Camera et al. implicated the importance of measuring both breakdown and synthesis of muscle proteins reporting significant responses of 28 proteins in response to RT and highlighted that the most common response observed was an increase in turnover succeeded by an increase in protein abundance but no detected increase in protein synthesis. Camera et al. suggest that protein-by-protein turnover responses should be considered and, particularly, that degradation responses deserve more attention in response to RE. Interestingly, based on this review of the literature, the above investigations are the only proteomic-based investigations in apparently healthy humans employing sub-chronic RT paradigms and analyzing the proteomic effects thereof. This implicates a severe lack of data regarding which skeletal muscle proteins are significantly affected by RT beyond acute bouts, and provides a ripe area of inquiry for future experiments.

Along the line of reasoning related to the importance of measuring MPB responses to RT, in a review article published in 2006 prominent protein metabolism researcher Dr. Robert Wolfe argues that net muscle protein balance is negative in the fasted state and remains negative for ~24-48 hours after a RE stimulus completed in the fasted state with no provision of amino acids post-exercise (39). However, Wolfe argues that while ingestion of amino acids alone increases MPS slightly and thereby results in a transient positive muscle protein balance, RE combined with post-exercise consumption of amino acids results in a more pronounced increase in muscle protein balance than either practice alone. This contention has been challenged by other researchers in the field who have argued that MPB changes comparatively less than MPS in response to RE and feeding, and that the alteration in MPB does not match the more pronounced

increase in MPS after RT, particularly when RT is combined with a post-exercise consumption of amino acids (79). A recent review from Tipton et al. highlights our limited understanding of MPB in response to RT given the disparate findings up to this point. Since muscle protein balance is equal to the difference between MPS and MPB, the importance of understanding MPB responses to RT are obvious (86). Unfortunately, most of the evidence to date has focused on measurements of MPS, with comparatively less attention paid to MPB. Practically, this is due to the more technical nature of directly measuring MPB. However, of the evidence available, it seems that MPS and MPB are positively correlated in the post-absorptive state (81,87,88). This indicates that both processes decrease or increase together, and that they are related rather than exclusive. As stated by Tipton et al., MPB seems to primarily increase to facilitate the supply of amino acids for MPS processes. Logically, if amino acids are supplied from exogenous sources, the apparent need to increase MPB after RT is reduced. Indeed, when amino acids are consumed before and/or just after RT, measurements of MPB are comparatively lower than those collected in fasted conditions (39,86). This agrees with the thesis that supply of exogenous amino acids reduces the need for increases in MPB to provide amino acids for increases in MPS. That is, the supply of exogenous amino acids when combined with RT results in comparatively greater muscle protein balance due to reductions in MPB and significant increases in MPS. In addition to these points, Damas et al. recently argued that muscle damage and resultant increases in MPB in response to RT deserve a reexamination regarding their role in RT-induced hypertrophy (89). Specifically, Damas et al. point out that significant increases in CSA were realized only after significant damage to the vastus lateralis was attenuated during 9 weeks of RT. The authors

noted significant reductions in muscle damage after the third and tenth week of RT, compared to the acute damage response to bout 1, while MPS was still significantly elevated above resting levels at the third and tenth time points. Damas et al. indicate that the MPB responses to RT near the beginning of an RT program are directed to tissue remodeling and approximately match elevations in MPS, while regular presentation of a similar RT stimulus for a period of ~8 weeks results in reductions in MPB with continued elevations in MPS and thereby an increase in CSA. Hence, although eliciting damage through an RT stimulus early on in an RT program is likely requisite to a degree in order to result in eventual hypertrophy (i.e., remodeling followed by growth), the extent to which damage is necessary in following weeks seems to be reduced and potentially a net negative if too severe (e.g., non-functional overreaching or overtraining).

To summarize these points, evidence indicates that: a) MPS increases in response to RT, although the specific proteins synthesized are not entirely clear, b) MPB increases in response to RT, although the specific proteins broken are not entirely clear, and c) consumption of amino acids post-exercise can promote a positive muscle protein balance after RT. The nuances of skeletal muscle hypertrophy in response to various RT paradigms remain suspicious. While an acceptable operational definition was proposed by Schoenfeld (64), it remains to be determined which specific proteins in skeletal muscle tissue increase in response to various RT paradigms. However, it seems logical enough that hypertrophy results from a positive muscle protein balance. This positive balance is the consequence of MPS outpacing MPB for a sufficient duration for notable changes in cell size. Evidentially, this positive balance is best achieved through the combination of RT and consumption of essential amino acids. To this end, specific

mechanisms involved in RT-induced hypertrophy and augmentation through protein consumption are discussed below. First, a brief analysis of RT dosage parameters and relationships to skeletal muscle hypertrophy is provided.

Resistance Training for Hypertrophy

It was clear early on in the history of formal RT research that skeletal muscle hypertrophy was a common adaptation (90). Naturally, certain dogma regarding RT dosing parameters and expected adaptive outcomes emerged. For example, for many years heavy loads and specific repetition per set values were promoted for realizing hypertrophy from RT (4), yet, recent evidence has suggested that a wide spectrum of loads can elicit similar short-term hypertrophic responses from RT (91–93). Continued debate centers around optimal training practices for maximizing hypertrophic outcomes to RT (94). Notwithstanding, certain relationships between training parameters and adaptive outcomes have been well characterized. A more detailed discussion of the physiological processes underlying hypertrophy follow this section, while this section primarily serves to establish a relationship between select RT parameters and hypertrophic outcomes. Given the focus of this dissertation, a discussion of meta-analytical findings and other relevant findings provoking the design of the RT paradigm for this dissertation are particularly emphasized. For a more targeted discussion, three key training parameters will be highlighted in this section as they pertain to RT paradigms for eliciting

skeletal muscle hypertrophy: 1) training volume, 2) training intensity, and 3) training frequency. Given this, defining each of these concisely is warranted.

Although different definitions exist in regard to RT, a more technical definition of training volume pertains to the total work completed during a set of repetitions for an exercise, a training session, or another period of time. Invoking the term work denotes the importance of considering the magnitude of force produced and the displacement of the external load. Although this definition is robust, measuring displacement is technically demanding in the practical setting and is often disregarded with the assumption that a post-pubescent individual with given limb lengths will displace the external load a consistent magnitude when a certain exercise is performed, therefore largely standardizing this measurement over time. With this in mind and practically speaking, training volume is often defined as the total number of repetitions completed for an exercise multiplied by the weight used for the exercise relative to a specific time period. For example, this can be calculated as a function of a single set for a single exercise, the sum of this calculation for multiple sets of an exercise, expressed for an entire week of training where multiple exercise volumes are summed together, or even for a longer duration of time. This parameter is often calculated for an exercise completed during a single training session or over the course of a week so that an approximation of the training dose can be built upon in later weeks or successive training cycles. Training intensity can be defined in a variety of ways, but is commonly calculated as a percentage of 1RM for an exercise in context of RE. A more appropriate definition likely includes consideration of rest between sets (95), work completed relative to time (i.e., power), and other factors. However, given the technical nature of

these measurements and demands on practitioners, a percentage of 1RM is often employed as a proxy of intensity. For example, Marston et al. recently showed significantly stronger correlations between the blood lactate response and a novel intensity metric referred to as "Exercise Density" which considered work in joules divided by the summed interest recovery seconds of the session when compared to the traditional metrics of volume load (VL = sets x reps x weight) and intensity (VL / average % 1RM). Additionally, and in a more relative sense, training intensity can be expressed as a proximity to repetition failure (e.g., reps in reserve or reps left) or a perception of exertion magnitude (e.g., RPE). With that said, it is typical to employ either a percentage 1RM intensity parameter or relative intensity parameter (96) currently in the practical setting and in much of the RT literature. Training frequency can be defined as the rate of execution of a certain exercise or training session relative to a specific period of time. Further, training frequency can be denoted where a count is extrapolated from a number of sessions emphasizing a certain set of movements or muscles, or, from a combination of exercises emphasizing a certain muscle group relative to a specific duration of time (e.g., sets or sessions per muscle per week). Hence, training frequency is commonly characterized as the number of training sessions emphasizing a certain muscle or exercise per week.

Training volume, of the three parameters discussed so far, seems to be of primary importance regarding RT program design for hypertrophy, although not exclusive of the other factors mentioned above. That is, training volume inherently includes both intensity and frequency in its calculation. Importantly, intensity and frequency relate more to how volume is partitioned over the course of an RT program, rather than the total amount of work completed.

Commonly, given the practical nature of applying RT evidence, these concepts are expressed relative to a week's time (i.e., 7 days). For this reason, most of the research examples provided in this section relate to this time frame.

In 2007, Wernbom et al. published a systematic review suggesting that between 4-6 sets of RT per week resulted in slightly greater increases in CSA of the quadriceps than ≥10 sets per week (97). A similar pattern for the biceps was also noted, where 4-6 sets produced slightly greater hypertrophy than ≥9 sets per week. This seems to point to excessive tissue damage and thus MPB relative to MPS past these set per week values for these muscle groups. However, as noted by the authors, significant heterogeneity in the number of repetitions per set and per week, along with significant differences in training intensity confound the straightforward interpretation of a set per week upper limit for hypertrophy. For example, repetitions per session for studies examining the quadriceps ranged from ~20 - ~150. Indeed, the metabolic and adaptive responses to a single set of RE can be vastly different if a significant difference between reps per set, intensity, and rest between sets are involved (98). Stated differently, the term set should be carefully presented and interpreted since an individual set of repetitions can involve a host of different characteristics (e.g., load, repetition duration, etc.). Hence, caution should be exuded when interpreting set per week dosing effectiveness by considering the load employed during a set, the number of repetitions completed, the rest between sets, and the repetition tempos, for example. Of note, this has persuaded scientists to posit standardized metrics of RE dosing terminology and consideration of the specific parameters of a set of RE before judgement of a paradigm's effectiveness (95). In other words, standardizing nomenclature and the manner

by which certain RT parameters are defined and measured is critical to understanding specific dose-response relationships as we move forward into the 21st century.

Another important limitation in the data from Wernbom and colleagues is the severely low number of studies reviewed involving previously resistance trained subjects. This is relevant as the acute responses to RT and adaptive patterns to similar RT programs are expectedly different in trained subjects (99). Trained muscle tissue has been shown to exhibit different transcription, translation, and adaptive responses to similarly dosed RT, which will be discussed further in the following section. Furthermore, greater training volumes are likely required for a relatively similar quantitative response in trained subjects (100). As discussed prior, if the training dose is excessive too early in an RT program, MPB rates can outpace or match MPS rates mitigating the hypertrophic response to RT. This concept denotes that maximum effective doses for hypertrophy should take into account previous dosing and successive training strategies relative to the aim of the long-term training process, and that this optimal dose value is dynamic not static. Stated differently, it is very unlikely that a generalized maximal effective dose of RT for hypertrophy exists consistently across populations but rather an approximation of appropriate volumes relative to an individual's stage of training and physiological status warrant consideration before confident dose application. As an example of this disparity, in untrained muscle, based on the review from Wernbom and colleagues, it would seem the maximum effective dose expressed in terms of sets per week during sub-chronic RT is ~4-6 sets. However, our laboratory recently published data revealing ~15 % increases in vastus lateralis thickness measured by ultrasound in untrained subjects completing 15 sets per week for a period of 12

weeks (101). Indeed, others conducting meta-analyses and systematic reviews since this 2007 review have reported no clear upper limit in untrained and trained subjects over similar time frames. Some of the most statistically powerful examples are discussed below.

In 2010, Kreiger published a meta-analysis considering 8 separate studies and 19 treatment groups demonstrating greater hypertrophic responses to RT proportional to the number of sets per exercise completed per week where the following effect sizes were observed: a) 0.24 ± 0.03 for 1 set per week, b) 0.34 ± 0.03 for 2-3 sets per week, and c) 0.44 ± 0.09 for 4-6 sets per week, indicating 40 % more hypertrophy when multiple sets were completed compared to a single set (21). In 2016, Schoenfeld et al. published a systematic review and meta-analysis including evidence from 15 studies with strict inclusion criteria further expounding upon Kreiger's original work showing the greatest effect sizes for hypertrophy when >9 sets per muscle group per week were performed, compared to less than 5 sets or 5-9 sets per week. Specifically, <5 sets resulted in a 5.4 % percentage gain, 5-9 sets resulted in a 6.6 % gain, and >9 sets an 8.2 % gain (24). These data indicate that greater training volumes, to a point, result in greater hypertrophic outcomes. In discussion of their meta-analytical findings in a recent letter to the editor published in the Journal of Sport Sciences in 2017, Schoenfeld et al. state: "…we contend that a minimum of 10+ sets per muscle per week is necessary to maximize the hypertrophic response to RT (102). Again, this represents a minimum threshold as there were not enough studies that investigated higher volumes to carry out sub-analysis. What now needs to be determined is where the upper threshold for volume lies to promote the greatest increases in

muscular gains.”. This point served to facilitate the general design of the RT program for this dissertation, further described in Chapter 3.

Dosing RT intensity for hypertrophic outcomes, expressed as a percentage of 1RM, is likely the most controversial of the three parameters. Conveniently, loads are generally described in the RT literature as “heavy” or “high” vs “light” or “low” (103). Although no clear standards exist for this nomenclature, loads corresponding to ≤ 40 % 1RM are often termed light, while loads ≥ 60% 1RM are often termed heavy (91,98). Recently, evidence has suggested that RT involving regular employment of relatively light loads (≤40 % 1RM) can result in similar or even greater short-term hypertrophic responses than heavy loads (≥ 60% 1RM) (91). These findings challenge traditional thought that heavy loads are consistently superior to light loads for hypertrophic outcomes. However, certain considerations regarding this evidence and application in the practical setting are warranted.

Neuromuscular physiology research indicates that lifting heavy loads results in comparatively greater electromyography amplitudes, on average (98), when contractions are performed to momentary muscular failure. The observed higher EMG amplitudes during heavy lifting is generally thought to be due to the earlier and more frequent recruitment of higher threshold motor units during an RE set. Albeit, the validity of surface EMG to detect MU recruitment, and thereby surmise hypertrophic potential has been challenged due to the influence of peripheral factors like intracellular action potentials and muscle fiber propagation velocities during dynamic contractions, particularly in the presence of fatigue (104). Notwithstanding, higher threshold motor units tend to innervate larger, predominantly fast-twitch muscle fibers.

The size principle denotes that motor units are generally recruited based on their size and their recruitment depends on the force production requirements of a task. That is, high-force tasks of a sufficient duration generally involve the recruitment of both more total and greater sized motor units than low-force tasks. Additionally, motor units tend to be recruited in an orderly fashion, from small to large. Consequently, on a per rep basis, heavy loads are assumed to recruit both a greater total number of motor units and larger motor units in comparison to light loads. This implicates greater muscle fiber recruitment and suggests a greater hypertrophic effect per repetition during heavy RE. However, fatigue during dynamic contractions can affect the presupposed orderly recruitment of MUs based on their size. Fatigue, in this case, can be defined as the failure to maintain the required or expected force (105). For example, larger motor units with higher recruitment thresholds tend to exhibit a reduced recruitment threshold during fatigue. Given this, lifting light loads until presentation of fatigue can result in the recruitment of higher threshold motor units and/or MU cycling, where MUs are recruited in an acyclic manner to meet the demands of the loaded task. Logically, this indicates that with enough repetitions, seemingly near or at the point of momentary muscular failure, a similar or greater number of motor units could be recruited during RE with light loads and this could result in stimulation of MPS to a similar degree as RT involving heavy loads. Indeed, Burd et al. have shown greater MPS responses from light RE compared to heavy RE when both loading conditions involve subjects achieving momentary muscular failure (106). Additionally, Dr. Stuart Phillips' lab has conducted a series of investigations indicating similar or greater hypertrophic effects of light RE compared to heavy RE in both acute and sub-chronic models in humans (91,93). As stated prior, it is of

paramount importance for long-term hypertrophic outcomes to consider previous and successive RE bouts in way of better ensuring desired outcomes void of injury or overtraining. Our laboratory published evidence in 2017 suggesting that light RE to momentary muscular failure required a greater amount of time to recover force production capabilities while exhibiting the same anabolic responses as heavy RE in well-trained young men (98). Furthermore, ~75 more repetitions were required to achieve momentary muscular failure in the light RE condition compared to the heavy. Other investigations have shown similar trends(99,107). Hence, although light RE to momentary muscular failure can induce similar hypertrophic adaptations, heavy RE seems to be more efficient on a per repetition basis. Furthermore, our investigation was in trained subjects while Phillips et al. investigated untrained subjects. Thus, not only is training status a worthwhile consideration before practical application, but also the subsequent effects on training sessions to follow.

In addition to these points, Dr. Andrew Fry published a review article in 2004 suggesting greater hypertrophic effects for both type 1 and type 2 fibers from higher average intensities during a training studies available for review (19). That is, as the average % 1RM of exercise intensities in training studies increased beyond 40 % 1RM, greater increases in fCSA were observed in both type 1 and type 2 muscle fibers. Also, Dr. Jacques Poortmans published a review article in 2016 revealing higher acute MPS responses to loads above 50 % 1RM compared to loads below this value (108). Finally, a recent investigation reported significantly greater strength and hypertrophic adaptations to RT at 80% 1RM compared to 20 % 1RM indicating that loads less than ~30 % 1RM are likely below a hypothetical minimum intensity

threshold for stimulation of desired adaptations to RT (107). Lastly, training to momentary muscular failure is a questionable practice. A review article published by Peterson et al. in 2004 revealed RT where sets were not taken to failure resulted in greater improvements in strength than RT where sets were taken to failure (22). Other investigators have reported similar findings, calling into question the practice of training failure, particularly given the increased risk of injury in this training context (109). With this in mind, research investigating RT with light and heavy loads where sets are not taken to failure and adaptations are compared can help clarify practical implications of this RT practice. Since light RE seems to match or surpass the effects of heavy RE only when taken to failure, this practice deserves strategic inclusion in the long-term training process, if at all, and warrants careful attention to execution in exercises where injury risk is higher (e.g., multi-joint exercises).

Another proposed benefit of light RE for hypertrophic outcomes is the accumulation of metabolites that may be important for stimulating MPS maximally in response to RT. Although more detailed mechanisms are reserved for the following section, the concept warrants mention here. The American College of Sports Medicine and National Strength and Conditioning Association promote repetition per set values of between 6-12 to strike a balance between enunciating mechanical tension generated by muscle fibers and the magnitude of metabolic stress experienced by said fibers (110). Additionally, RT studies involving relatively short rest intervals (e.g.., 60-90 seconds) between sets have been shown to result in greater acute elevations in testosterone, growth hormone, and metabolic stress (110–113). For example, muscle lactate levels were significantly increased after 1 set of 12 repetitions at 80 % 1RM and further

increased after 3 sets to failure (114). This is thought to be in contrast to higher intensity protocols involving lower rep-per-set values where much of the energy provision occurs via the more immediate phosphagen system (115). The lower rep per set value is thought to result in comparatively lower metabolic stress due to reduced glycolytic activity and subsequent formation of lactate and other metabolites (116). Furthermore, temporary occlusion of blood flow due to compression of vascular structures during contraction can result in acute muscle hypoxia further augmenting metabolic stress (117). Thus, a larger number of contractions over a fixed amount of time (e.g., greater reps per set) would evidently result in greater metabolic stress, and potentially greater hypertrophic responses. In support of thesis, Goto et al. showed significant increases in fCSA after a period of training similar to the style discussed above (~10 reps per set with short rest intervals) while no significant increases in fCSA were observed after a period of higher intensity training with longer rest intervals in the same group of subjects (118). However, a recent systematic review by Grgic et al. suggested that longer rest intervals tended to be associated with greater hypertrophy than shorter rest intervals (9.2% vs 5.8%), although considerable heterogeneity in the six studies analyzed was noted (119).

Another provocative RT practice for hypertrophy is related to movement velocity. Particularly, since potentially more cross-bridge cycling would occur during intentionally slower movement velocities given greater time allowed for cross-bridging to occur, it has been speculated that slower movement velocities may provide a greater hypertrophic stimulus (120). Hypothetically, this practice would allow longer durations for fibers to produce tension and potentially more metabolic stress via increased cross-bridge cycling and metabolite formation

per set. However, only a limited number of studies have investigated movement velocity effects on hypertrophic outcomes which were recently reviewed by Hackett et al. (121). Briefly, the quadriceps muscle group seems to respond slightly better to movement velocities between 2-4 seconds while the biceps muscle group seems to respond better to faster movement velocities (e.g., < 2 seconds). Another meta-analysis by Schoenfeld et al. suggests that significant hypertrophy can occur from repetition durations of 0.5 to 8 seconds per repetition, with little observed difference in hypertrophy between this time range, while >10 seconds per repetition seems suboptimal (122). Albeit, both reviews highlight the need for more evidence to clarify an optimal repetition duration for hypertrophic purposes on a per-muscle/per-exercise basis.

Another important consideration in light of these data is that low-load blood flow restriction training has been shown to result in significant muscle hypertrophy (123). It has been posited this is potentially due to comparatively greater metabolic stress from the accumulation of metabolites (e.g., lactate, inorganic phosphate, hydrogen ions) formed in response to continued muscle contractions while venous occlusion is induced by a physical implement (e.g., tourniquet, cuff, etc.) local to the muscle. Occlusion of venous return consequently augments the pooling of metabolites and results in acute muscle hypoxia (123). The metabolites formed during blood flow restriction training are thought to potentially upregulate anabolic processes beyond the stimulus of muscle contraction alone (i.e., tension). For example, in vitro, lactate has been shown to induce myogenesis in C2C12 myoblasts, increase the phosphorylation of p70S6K (an important signaling protein involved in increased MPS), and increase myotube diameter via the activation of the MEK/ERK pathway (124,125). Hence, common recommendations for RT

programs aimed at hypertrophy are rep per set values from 6-12 with 60-90 second rest intervals. However, based on this review of the literature, no direct evidence exists from human RE studies elucidating a clear hypertrophic role of metabolites beyond muscle contraction-induced signaling alone (i.e., tension). Rather, as noted by Dankel et al., metabolite accumulation may augment muscle activation and thus tension generation in more fibers since inorganic phosphate and hydrogen ion accumulation has been shown to interfere with cross-bridge cycling (126). That is, significant metabolic stress in some fibers might simply result in tension generation in other fibers during RE thereby catalyzing more anabolic signaling in a greater total number of fibers compared to the number of fibers recruited in a less metabolically stressed state. Based on these data, it seems logical to design RT paradigms aimed at maximizing whole-body hypertrophic outcomes in such a way to induce the greatest total tension in the greatest number of fibers through a combination of sufficient intensities and metabolic stress. Practically, this intention is likely best accomplished through creative alteration in training parameters to prioritize the recruitment of a large number of muscle fibers through the complete range of motion of a joint, or multiple joints, for a sufficient amount of time to maximize tension and metabolic stress, to a point. Notwithstanding, tissue damage and time required for recovery-adaptation are vital to consider so that hypertrophic outcomes may be optimized over long periods of time. As such, the above data suggest regular employment of loads $\geq$ 60 %1RM where training volume loads are increased over time (e.g., number of sets near momentary muscular failure per week) and precede extended periods of recovery-adaptation to avoid overtraining. However, lighter loads can be employed for hypertrophic outcomes, so long as a sufficient number of repetitions are

performed to induce the recruitment of most of the fibers in a muscle (e.g., near momentary muscular failure). Further, the specific durations wherein training volumes can be increased, along with the magnitude of training volume optimal to elicit the maximal hypertrophic response seems to depend on individual characteristics (127). Importantly, considering preceding and succeeding training bouts is vital to maximize the frequency at which the aforementioned training stimulus can be applied on an individual basis. Sensibly, this is to provide the most regular hypertrophic stimulus possible relative to an individual's current physiological state. These points lead to a brief discussion of training frequency for hypertrophy.

A systematic review article published in 2016 by Schoenfeld et al. suggests that training muscle groups twice a week promotes superior hypertrophy compared to once per week (128). However, insufficient data beyond twice-per-week protocols were available for review, leaving the authors to conclude that the effectiveness of training frequencies for hypertrophy beyond twice per week remained to be determined. Interestingly, a recent survey of bodybuilders revealed that ~70% train a muscle group only once per week, and of the approximately 130 surveyed, no bodybuilders reported training a muscle more than twice per week (129). Dankel et al. proposed that higher frequencies would be hypothetically more effective for highly trained individuals given the less prolonged MPS response to RE (~24 hours) compared to untrained individuals (79). Dankel et al. argue a response similar to the "muscle-full" effect in response to amino acid infusion or ingestion (a refractory period of the stimulation MPS to amino acid availability) exists for RE-induced stimulation of MPS as well (130). Evidence from Hakkinen's lab showing numerically greater increases in muscle size in a group training twice per day

compared to once per day where volume was equated is cited by Dankel et al. to support this thesis. However, long-term training studies in humans wherein training a specific muscle group with frequencies beyond 2 days per week are warranted to clarify hypertrophic responses as insufficient data exist to confidently adopt this hypothesis. A recent investigation by Gomes et al. reported no significant differences in strength improvement or lean tissue mass between a group of well-trained men training specific muscle groups once per week compared to a group training specific muscle groups five times per week, although the group training five times per week realized greater numerical improvements in each dependent variable for strength and lean mass (131). Another more recent investigation reported similar findings in trained young men where strength and hypertrophic adaptations were statistically equal between a group of subjects training 3 x's/week vs a group training 6 x's/week (132). Considering this, the current state of the evidence indicates a training frequency of somewhere between 2 and 5 sessions per week per muscle group to elicit a maximal hypertrophic response to RE. However, insufficient data exist to confidently assert that higher training frequencies are suboptimal.

To summarize this section, the following points are provided: a) greater than 10 sets per muscle group per week likely result in maximal hypertrophic responses, while the upper limit of dosage is not clear, b) both low and high loads can instigate hypertrophy to an equal extent when contractions are performed to failure, however, loads ≥ 60 % 1RM likely do so more effectively on a per repetition basis and particularly so if contractions are not performed to failure, and c) training frequencies ≥ 2 times per muscle group per week are superior to training a muscle group once per week for hypertrophy. Although these points provide insight into RT program design

for hypertrophy, a discussion of mechanisms underlying hypertrophy malleable to RT can provide further insight into parameter dosing and bring attention to areas deserving future research in way of maximizing the hypertrophic response to RT. To this end, underlying mechanisms of RT-induced muscle hypertrophy are discussed in the next section followed by whey protein supplementation's potential role in maximizing the hypertrophic response to RT.

Since knowledge gaps remain in regard to dosing RT for maximizing hypertrophic outcomes, clarification of the underlying mechanisms of hypertrophy can elucidate potential training strategies worthy of further investigation in humans. RT-induced skeletal muscle hypertrophy is an integrative, multifactorial physiological response involving multiple physiological systems. Additionally, skeletal muscle is considered a postmitotic tissue indicating that, upon development, mature skeletal muscle cell cycles are arrested, in a sense, in the G_0 phase of the canonical cell cycle and do not undergo significant cell replacement throughout life. Stated differently, skeletal muscle cells are terminally differentiated and have apparently lost their ability to proliferate. In general, this means that skeletal muscle cells respond to various nutritional and training stimuli by increasing in mass (i.e., hypertrophy) rather than dividing into multiple cells (i.e., hyperplasia). Therefore, hypertrophy is distinct a distinct process from hyperplasia. Furthermore, muscle fiber hyperplasia has not been clearly demonstrated in humans. Thus, hypertrophy is considered the biological construct responsible for increases in muscle size. The central dogma of molecular biology denotes that genes housed within the cell nucleus composed of deoxyribonucleic acid (DNA) can be transcribed into messenger ribonucleic acid (mRNA) molecules, and these mRNA can be translated into proteins. Various stimuli signaling

skeletal muscle fibers to grow can eventually converge in nuclei of skeletal muscle cells where the process of gene transcription into mRNA molecules can begin. Further, ribosomal translation rates of mRNA transcripts can be affected by training and nutrition stimuli (127). Given the complexity of this hypertrophy-related signaling within muscle, roles of other physiological systems (e.g., central nervous system, endocrine system), and the influence of circulating factors in blood (e.g., hormones, nutrients) relevant to skeletal muscle fiber growth, categorizing signals into the following categories is helpful: a) extrinsic factors (originating outside of skeletal muscle), and b) intrinsic factors (originating inside of skeletal muscle). Stated differently, both extrinsic and intrinsic processes to muscle can eventually affect gene transcription and translation processes in muscle, and, consequently, the accrual of muscle protein. To ensure this discussion remains concise, RT-related mechanisms discovered in humans or cultured muscle cells are primarily discussed while analysis of supraphysiological pharmacological stimulation and exotic animal models of hypertrophy (e.g., synergist ablation) are avoided. First, well-established intrinsic mechanisms will be discussed. After this, an overview of various extrinsic factors particularly relevant to this dissertation will be provided.

As discussed prior, changes in muscle fiber size due to RT are primarily related to the difference between MPB and MPS. Significant changes in MPB or MPS are eventual outcomes of molecular signaling malleable to RE. In particular, two intrinsic signaling pathways particularly responsive to RE stimuli play key roles: 1) mammalian target of rapamycin (mTOR) pathway, and 2) Ubiquitin-Proteasomal (UPP) pathway. The mTOR protein signaling pathway has emerged as a primary signaling network in muscle tissue directly responsible for increases in

myofibrillar protein synthesis above basal levels in response to RE (133–135). Although the specific structural and electrochemical changes to the signaling molecules involved in this pathway in response to RE are still being unraveled, signaling events leading to increases in MPS after RE seem to adhere to the following sequence: 1.) upon PI3K phosphorylation via a myokine (e.g. MGF), interaction with an integrin molecule responsive to mechanical tension (e.g. FAK, ILK), or PI3K phosphorylating a lipid (phosphatidylinositol) sourced from the fiber sarcolemma, the PH domain of Akt (i.e. PKB) is phosphorylated (136), 2.) Akt "de-represses" mTOR from repression of the TSC1/TSC2 complex (137), and mTOR is phosphorylated (138,139), 4.) mTOR can phosphorylate both 4E-BP1 and p70s6k (138,139), 5.) p70s6k phosphorylates rpS6 eventually leading to enhanced translation of mRNAs coded for ribosomal protein expression and elongation factors, while 4E-BP1 hyperphosphorylation leads to translation initiation of mRNA and thereby increases in protein synthesis (133,135,140). Evidence indicates that the magnitude of increase in p70s6k phosphorylation in response to a single bout of RE is strongly correlated with significant increases in muscle mass, 1RM squat strength, and increases in type IIa muscle fiber CSA in both human and rodent models in response to 6 and 14 weeks of RT (133,140).

During and in response to RE, Schoenfeld proposed three primary mechanisms responsible for skeletal muscle hypertrophy: a) mechanical tension, b) muscle damage, and c) metabolic stress (64,141). The term mechanical denotes relation to physical forces or motion and invokes the interplay of parts of a whole, as in a machine. Tension, derived from "tendere" or "tensio" in Latin, denotes a state of being stretched tight or pulling forces at two ends of a rope

or string. Consequently, mechanical tension, in relation to skeletal muscle fibers, can be considered a pulling force generated at the level of the sarcomere and subsequent tensile forces experienced by the muscle fiber in response to the fiber's contraction or when the fiber is stretched. Typical RE involves both voluntary tension development, when the fibers are contracting, and passive tensile forces, when muscle fibers are stretched beyond resting levels during various resistance exercises. Skeletal muscle cells have been shown to be particularly sensitive to these tensile forces, in both culture and human models. For example, Hornberger et al. have shown mechanical tension alone can directly stimulate mTOR in vitro (142). Additionally, Miyazaki et al. reported significant activation of mTOR independent of canonical nutrient-sensitive signals in response to mechanical overload (143). Furthermore, both the magnitude and frequency of tension have been shown to affect the activation of p70s6k (144). The process of transmitting the mechanical signal of these tensile forces into molecular signals affecting MPB and MPS has been termed *mechanotransduction* (64). Mechanotransduction seems primarily accomplished through integrin proteins residing at the cell surface and interacting with both the extracellular matrix and intracellular milieu of skeletal muscle. For example, focal adhesion kinase, an integrin protein found in skeletal muscle cells, has been shown to be crucial to mediate the tension-induced increase in mTOR signaling in muscle cells (145). Mitogen-activated protein kinase (MAPK) signaling (e.g., ERK 1/2) and calcium-dependent signaling processes (e.g., calcineurin) have also been shown to be particularly responsive to RE and in some cases additive to the hypertrophic signaling of the mTOR pathway (134). MAPK signaling seems primarily responsive to increased metabolism and redox status

while calcium-dependent signaling, hence the name, is primarily responsive to intracellular changes in calcium concentrations. However, their roles seem more additive than necessary for significant increases in MPS, and conflicting data warrant question of their necessity. For example, phosphorylation magnitude of c-jun n-terminal kinase (JNK), a signaling protein in the MAPK pathway, has been shown to increase linearly with the magnitude of contractile force (146). However, other evidence suggests inhibiting JNK can enhance muscle protein accretion (147). Further, calcineurin does not seem to be required for significant muscle growth responses in context of mechanical overload (148). On the other hand, the mTOR pathway's importance for significant increases in MPS is quite clear. As proof, Drummond et al. have shown that blocking mTOR signaling through rapamycin administration in humans mitigates the post-RE increase in MPS (149). Hence, mechanical-tension induced increases in mTOR signaling underpin significant increases in MPS in humans. Therefore, both mechanical tension and the mTOR pathway have been deemed primary mechanisms underpinning RT-induced hypertrophy. Importantly, tension precedes the potentially additive hypertrophic mechanisms of metabolic stress and muscle damage in response to RE, which are further discussed below.

Metabolic stress, as discussed previously, denotes a physiological state in skeletal muscle where metabolites formed from increased metabolism (particularly glycolysis) increase in concentration outside of typically occurring (homeostatic) ranges. Conversely, a decrease in the concentration of muscle oxygen (i.e., hypoxia) can also be characterized as metabolic stress. Compared to that of mechanical tension, the role of metabolic stress in RT-induced hypertrophy outcomes is less clear. Hence, the potentially anabolic nature of metabolic stress is posited to be

associated with both muscle hypoxia and increased concentrations of H+ ions, inorganic phosphate, reactive oxygen species, and lactate during RE. However, these processes are consequences of, or secondary to, tension development and an increase in metabolic stress seems to augment the recruitment of more fibers during RE thereby resulting in greater gross amounts of tension in whole active muscle rather than serving a distinct, mechanistic hypertrophic role. This is to say that induction of metabolic stress may be more of a "means-to-an-end" for further tension development, rather than specific metabolites ramping up MPS signaling. For example, investigations using surface electromyography (sEMG) have suggested recruitment of higher threshold motor units when muscle is faced with steeped reductions in muscle glycogen and increased metabolic stress during blood-flow restricted training (150,151). Notwithstanding, select evidence in humans suggesting metabolic stress may play an additive role is available, although these data should be interpreted in light of the above points.

Hormonal changes and myokine production are other posited occurrences potentially affecting the hypertrophic response to RT-induced metabolic stress. Both autocrine and paracrine myokine signaling are characterized as intrinsic signals herein since their origin is inside muscle cells whereas hormones will be briefly discussed below in context of extrinsic signaling. In addition to changes in metabolite concentrations and cell hydration status, metabolic stress is also thought to play a hypertrophic role through the induction of significant alterations in myokine production and anabolic hormone concentrations in blood. Myokines are described as growth factors, proteins, or cytokines that are secreted by skeletal muscle cells able to interact with the secretory cell itself, an adjacent muscle cell, or act systemically on other tissues (152).

Myokines have been shown to be related to the hypertrophic response to RT. For example, Bamman et al. showed significant increases in the myokine mechano growth factor (MGF) in a cluster of extreme responders (+126% increase after 16 weeks) in VL biopsy samples and no significant increase in a cluster of non-responders to the training intervention (153). Evidence related to metabolic stress induced by RE and subsequent effects on myokine signaling are limited. However, Takarada et al. showed a gradual increase in interleukin 6 (IL-6) after multiple sets of knee extensions employing the BFR training technique compared to a volume-matched group without BFR (154). However, other evidence indicates little to no effect on specific myokines in context of RE with high metabolic stress, calling into question the relationship (123).

Manini et al. reported significant reductions in atrogin-1 and MuRF-1 (proteolytic signaling proteins) ~8 hours after BFR training compared to a group not completing BFR (155). Additionally, Laurentino et al. showed significant reductions in myostatin (MSTN) gene expression after an 8-week period of BFR compared to a group of subjects not performing BFR (156). Increases in intracellular hydration (i.e., cell swelling) are thought to occur during metabolic stress in muscle cells and changes thereof are thought to affect muscle protein turnover (157). Interestingly, primarily fast-twitch muscle fibers have been shown to respond more robustly to RE than primarily slow-twitch fibers (19,158) and fast-twitch fibers tend to contain a high concentration of aquaporin-4 protein (water transport channel) (159). Schoenfeld posited this may be at least one reason for this observation, and this phenomenon may be partially explained by metabolic stress-induced cell swelling effects of RE (160). In summary, the

importance of metabolic stress for maximizing hypertrophy is questionable, but seemingly additive. Both mechanical tension and metabolic stress can result in significant increases in muscle damage, the third intrinsic signal posited as important for skeletal muscle hypertrophy.

Muscle damage refers to the disruption of muscle fiber structure that can result in an impairment in normal fiber function. Importantly, damage of muscle can occur to different extents and at different levels of organization of muscle tissue. For example, damage to the sarcolemma compared to damage of the sarcomere. To be concise, damage herein refers to both occurrences and in the more general sense of RT-induced damage to muscle fiber structures. Damage has been traditionally visualized through z-disc streaming of the sarcomere as a hallmark of muscle damage due to unaccustomed exercise (161). Further, evidence suggesting somewhat of a dose-response relationship between training volume and increases in muscle damage exists(162). Significant damage precedes decreases in force production, swelling, and local inflammation (162). With training, an individual is generally less susceptible to significant damage from similar workloads (163) (i.e., repeated bout effect). However, significant hypertrophy can still occur in trained populations. This would seem to combat the relative importance of damage to the hypertrophic response. Gibala et al. showed significant damage after eccentric muscle actions of the biceps against 80% 1RM resistance for 8 sets of 8 repetitions in resistance-trained young men, but not when only concentric muscle actions were performed (164). This suggests that trained individuals can still experience significant muscle damage so long as RE involves sufficient load and eccentric muscle actions. As mentioned prior, MPB rates influence changes in fiber size, and MPB is clearly important for remodeling

damaged tissue (e.g., clearing damaged proteins or recycling amino acids of damaged proteins for synthetic processes) as shown in the photo above. MPB rates, although increased in response to RE, have been suggested to be ~3 fold lower than MPS rates (86). Hence, MPS is primarily studied being the more dynamic of the two. However, since changes in cell size result from both processes, and MPB seems particularly important for remodeling after damaging RE, maximizing each response in the appropriate timeframe seems the logical pursuit of the training process for hypertrophy. MPB is primarily achieved through the coordinated activity of the UPP, autophagy, and calpain calcium-dependent cysteine proteases. The process of MPB in response to RE has been extensively reviewed recently (86), and is only briefly discussed below; particularly to connect this section to the following section on whey protein and muscle hypertrophy.

The UPP degrades target proteins that have been poly-ubiquinated with multiple ubiquitin monomers through the coordinated process of three enzymes ("E1", "E2", and "E3"), (165). Atrogin-1 and MuRF-1 are two, muscle-specific E3 ligases catalyzing the conjugation of ubiquitin to a substrate protein and have been shown to be specific to contractile proteins. The 20S core protein is the "catalytic" portion of the 26S proteasome and is involved in the degradation of contractile proteins (86). Importantly, the UPP doesn't seem to act in isolation to autophagy and calcium-related proteolytic signaling. Rather, the process of autophagy and calpain activity seem to be involved with the first steps of RT-induced MPB increases by facilitating the formation of the phagophore (a nascent membrane structure) around damaged proteins or, in the case of calpains and caspases, processing damaged proteins for breakdown

(166). Upon protein degradation in the lysosome or autophagophore, coordination with the UPP can allow for specific breakdown processes to proceed where specific proteins or degraded segments thereof can be tagged for further breakdown and liberated amino acids can be utilized for synthetic processes. Indeed, multiple studies have shown increased proteolytic mRNA expression after RE (167,168). Surprisingly, as noted by Tipton et al., breakdown rates of myofibrillar proteins specifically in response to RE are unclear although fractional breakdown rates of mixed muscle proteins have been made (86). Although validity of the technique has been called into question, MPB rates are generally considered to increase after RE via the pathways discussed above, and for the purposes of remodeling muscle and supplying amino acids for synthetic processes. Recently, Damas et al. argued that significant increases in fCSA and muscle size occur only after significant damage from training and MPB is attenuated, while significant increases in MPS continue with training (89). Additionally, Flann et al. recently showed equal hypertrophic responses to high-force eccentric-cycle ergometry between a group naïve subjects and a group of subjects that were trained for three weeks leading up to the study to mitigate the magnitude of damage to muscle (169). Indeed, the naïve group showed significantly greater signs of muscle damage than the pre-trained group, while both groups hypertrophied from the intervention. Notwithstanding, based on the state of the current evidence, it seems logical that MPB is important for tissue remodeling and, particularly, to "clean up" damaged fibers to make way for sarcomere construction and subsequent increases in fiber size, at least in the early stages of an RT program. However, the extent to which this process is necessary likely exists on a continuum resembling a U-Shaped curve, where some damage is beneficial, but too much can

result in maladaptation (160). Increases in inflammation can occur in response to significant muscle tissue damage from RE, where neutrophils and other immune cells can infiltrate the damaged tissue and aid in protein breakdown and tissue repair. Interestingly, non-steriodal anti-inflammatory drugs have been shown to blunt significant increases in MPS after RE in humans (170). Hence, at least a moderate relationship between muscle damage from RE and hypertrophic outcomes seems plausible. Furthermore, the role of protein supplementation and resultant increase in amino acid availability to muscle cells seem to affect both MPB and MPS responses. Amino acid availability and hormonal effects subsequent to RE can be thought of as extrinsic signals that can affect MPS and MPB. Interestingly, Areta et al. showed increased expression of MuRF1 mRNA following RE when 10 or 20g of protein were ingested, however, 40g seemed to prevent the increase in MuRF1 mRNA (171). This seems to indicate that consumption of a relatively high amount of protein post exercise may reduce the need for MPB to supply amino acids for synthetic processes. Indeed, Tipton et al. recently concluded in a detailed review of the literature on MPB that nutritional strategies (e.g., whey protein supplementation) can suppress MPB in response to RE, and this may translate to higher NBAL. However, the authors are careful to highlight that little is known regarding which specific proteins are being broken down in response to RT and that significant suppression of MPB might actually result in maladaptation until further research reveals more explicit relationships. This concept will be discussed in the following section. Changes in hormone concentrations at rest and in response to RE were traditionally thought to play critical roles in the hypertrophic response to RT (172). However, recently Dr. Stuart Phillips' lab completed a series of studies in both trained and untrained

individuals demonstrating little to no effect on hypertrophic outcomes from RT when hormones change within normal ranges (173,174). Of course, pharmacological intervention where supraphysiological doses of testosterone are provided result in significant hypertrophy, however, the state of the current evidence indicates little influential role of acute changes in hormonal status on hypertrophic outcomes to RT.

Each of these underlying processes discussed above are thought to play an important role in the hypertrophic response to RT and correspond to both extrinsic and intrinsic signals potentially resulting in fiber hypertrophy. Since the primary focus of this dissertation is related to the observed hypertrophic effects of the RT paradigm described in Chapter 3, and secondarily related to any potential differential effects of whey protein supplementation on these responses, this section is meant to provide a general overview of mechanisms potentially related to differential responses between groups, and to provide direction to potential follow-up analyses on collected biological samples. To summarize, intrinsic molecular signaling of mTOR pathway molecules and proteolytic pathway intermediates (e.g., UPP, autophagy, calpains) are particularly responsive to mechanical tension, metabolic stress, and muscle damage occurring during and in response to RT. These intrinsic processes affect eventual changes in MPS and MPB, and, consequently, changes in fiber size. Extrinsic signals like hormones and amino acid availability can also affect alterations in MPS and MPB. Of the two, amino acid availability likely plays a more prominent role in hypertrophic outcomes to RT, so long as hormonal concentrations are not pharmacologically altered. Of note, satellite cells, immune cells, and other factors fairly characterized as extrinsic and related to RT-induced hypertrophy were not

thoroughly discussed given the primary aim of the investigation for this dissertation described in Chapter 3, and given the fact that the importance of their roles in significant hypertrophic outcomes in humans are not yet entirely clear. For example, McCarthy et al. have shown significant hypertrophy in rodent models when satellite cells were ablated, suggesting that satellite cell donation of myonuclei are not necessary for significant increases in muscle size (175). However, Bamman et al. have shown strong relationships between the number of satellite cells present in VL biopsy samples at baseline and the hypertrophic response to RT (176). Further, how satellite cells are measured in human biopsy samples and at which time points after RE has clouded specific satellite cell physiology relationships to the hypertrophic response to exercise, with many investigators choosing immunohistochemical methods using a very small amount of tissue sample (177). Additionally, related to the discussion above on muscle damage, immune cell roles and necessity in the hypertrophic response to RE are not as explicit as the factors described above, and neither immune cell content nor satellite cell content were directly investigated in this arm of the study. However, we intend to investigate these factors in follow-up experiments characterizing underlying mechanisms related to the heterogeneity in responses of the training protocol described herein. To be clear, the primary aim of this dissertation was to observe the hypertrophic effects of the extreme training volumes not yet investigated in humans to help clarify an upper limit of adaptability. Second, this research sought to clarify differential hypertrophic responses to supplementing with either a graded dose of whey protein, or a standard, single serving each day in context of the extreme training volumes. Hence, the research questions and focus of this project were exploratory first and foremost, and follow-up

experiments are planned to address more explanatory mechanisms. Since the next section will focus on whey protein and underlying factors suggestive of its potentially additive role in RT-induced hypertrophy, the primary aim of this section was to set the stage for the following section. Below, evidence suggesting whey protein's role in maximizing hypertrophic outcomes will be provided along with a terse description of underlying mechanisms prior to presentation of the original research for this dissertation in Chapter 3.

Whey Protein Supplementation

A brief history of whey protein supplementation was provided previously (see *Brief History of Whey Protein Supplementation*), along with select findings pertinent to RT adaptations. As noted prior, the last three decades of research in this area have afforded enough data for relatively comprehensive analyses on the effects of protein supplementation combined with RT. Particularly, in the past decade, original research, multiple review articles, and meta-analytical findings have suggested an additive hypertrophic response to RT when combined with whey protein supplementation (37). Hence, this section will focus briefly on gaps in our knowledge pertaining to whey protein supplementation and consider additional supplementation strategies that may further potentiate the positive effects of whey protein on RT-induced hypertrophic outcomes.

Whey protein is rich in the amino acid leucine (178). At the molecular level in skeletal muscle cells, increases in leucine concentrations result in the activation or increased activity of

mTORc1 whereas other amino acids do not seem to exert this effect. As stated prior, mTORc1 activation seems to be required for the hypertrophic response to RE in humans (149). Leucine seems to enhance the activation of mTORc1 by first binding to the leucyl-transfer RNA synthetase (LRS). LRS facilitates the hydrolysis of a molecule called guanosine triphosphate (GTP) bound to a small G-protein (RagD), allowing the interaction of various other Rag proteins which eventually direct mTORc1 to an organelle in the cell called a lysosome. A molecule located at the lysosome called Rheb (Rag homolog enriched in brain) is a critical activator of mTOR and, therefore, the ultimate effect of leucine on muscle protein synthesis seems to result from the direction of mTOR to interact with Rheb at the lysosome. Upon mTOR-Rheb interaction, downstream targets of mTORc1 involved in the translation of mRNA into proteins (e.g., contractile proteins) are activated thereby increasing the rates of muscle protein synthesis (179).

Morton et al. recently reported meta-analytical findings considering 49 studies which revealed significant, positive effects on both increases in fat-free mass and strength (37). Hence, at this point, it seems quite clear that protein supplementation is a reasonable adjuvant to RT. Interestingly, the statistical analysis of Morton et al. revealed greater effects of protein supplementation in previously trained young men. It logically follows that maximizing the hypertrophic response to RT in young men should include protein supplementation. Additionally, a number of studies suggest that whey protein results in higher FSR responses, per equivalent dose, than other fractions of protein (38). For example, Tang et al. reported whey protein consumption resulted in higher MPS responses than casein or soy in young men both at

rest and after RE (38). Further, Hartman et al. reported significantly greater lean mass accretion in male weightlifters after 12 weeks of training and supplementation with fat-free milk, containing whey protein, compared to soy or carbohydrate (180). After a 9-month intervention of both supervised RT and supplementation of either whey, soy, or carbohydrates, Volek et al. reported significantly greater increases in lean mass in young men supplementing with whey (+3.3 ± 1.5 kg) compared to soy (+1.8 ± 1.6 kg) or carbohydrate (+2.3 ± 1.7 kg) (181). A host of other studies have reported similar findings (178). However, the amount and timing (i.e., serving dose and frequency) of whey protein supplementation are less well characterized.

In 2002, Borsheim et al. suggested a dose-dependent effect of essential amino acid ingestion on muscle protein synthesis (182). This suggestion was based on the finding that two doses of a mixture of 3 g of EAA + 3 g of NEAA resulted in double the NBAL response. More recently, Macnaughton et al. reported significantly greater FSR responses after RT when a 40g dose of whey protein was ingested post-training, compared to a 20g dose (183). Yang et al. have reported significantly greater FSR responses from 40g of whey protein supplementation compared to 0, 10, and 20g in older men (184). Similarly, Pennings et al. have reported similar findings wherein significantly higher myofibrillar FSR responses occurred in older men who ingested 35g compared to 10 or 20g (185). Witard et al. compared myofibrillar MPS responses from ingestion of 40g of whey protein to 0, 10, and 20g in young, resistance-trained young men revealing numerically larger, but not significantly different FSR responses from ingestion of 40g vs 20g, while 0, and 10g resulted in significantly lower responses (186). Interestingly, Witard et al. reported significantly greater urea production and phenylalanine oxidation rates in the 40g

condition, suggesting increased whole body amino acid catabolism. Although using egg protein supplementation, Moore et al. reported similar acute findings in young men after exercise and ingestion of either 0, 5, 10, 20, or 40g where 20 and 40g resulted in significantly greater FSR responses but no significant difference was observed between 20 and 40g doses, albeit the mean FSR response was numerically higher in the 40g condition but did not reach significance ($p=0.29$). Similar to Witard et al., Moore et al. reported greater leucine oxidation rates in the 40g condition. Furthermore, Moore et al. suggested that ~20g of protein (~8.6 of EAA) likely represents a maximal effective dose for increasing FSR, noting significant increases in oxidative losses of consumed amino acids beyond this point. Moore et al. also suggest that regular consumption beyond this value could dampen the FSR response to protein supplementation after RT since amino acid oxidative capacity can adapt to the diet, and thereby regulate protein stores (187). Although involving the consumption of beef, another study from the Phillips Laboratory revealed higher MPS rates after consumption of 36g of protein compared to 12 or 24g (188). Scientists discussed above attribute the saturation of the MPS response to protein consumption to the posited "muscle full" hypothesis (189). The muscle full hypothesis denotes a refractory period of the sensitivity of muscle to amino acids wherein FSR cannot be further enhanced by increased amino acid availability through either infusion of amino acids or feeding. This hypothesis is based upon the observation that infusion of amino acids resulting in hyperaminoacidemia combined with ingestion of protein failed to result in further increases in FSR after a period of ~4 hours in humans (189). However, this concept is largely based on acute studies and the muscle full effect has not been clearly demonstrated in humans undergoing

chronic RT combined with ingestion of protein doses ≥ 40g. In fact, upon review of the literature, it seems that neither sub-chronic (e.g., 2-6 weeks) nor chronic (e.g., > 6 weeks) supplementation of whey protein dosed at ≥ 40g per dose combined with supervised RT have been investigated. Given the above evidence, and since high-protein diets have been shown to result in more favorable body composition changes and quantitatively larger changes in FFM in resistance-trained young men (190,191), it stands to reason that doses ≥ 40g multiple times a day for a period of weeks may result in larger changes in FFM, particularly if combined with high training volumes. Furthermore, since increases in training volumes result in proportionally greater increases in hypertrophy over time, to a point yet unclear, it seems logical that concurrently increasing dosages of whey protein with short-term increases in training volume could result in greater increases in FFM (24). This hypothesis is also underpinned by evidence indicating higher protein turnover rates in response to higher training volumes (192,193). Furthermore, a recent meta-analysis considering 13 randomized controlled trials by Davies et al. reported positive effects of whey protein supplementation on recovery of force production after RT (194). These observations suggest a heightened ergogenic potential for whey protein supplementation if consumed proportional to increases in training volume. For these reasons, we sought to investigate the effects of either a standard fixed-dose of whey protein (WP), a graded dose of whey protein (GWP), or a maltodextrin-based carbohydrate supplement (MALTO) on body composition during and after a period of high volume RT.

CHAPTER 3: JOURNAL MANUSCRIPT

Effects of graded whey protein supplementation during extreme-volume resistance training

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Short title: Extreme-volume resistance training and graded whey protein supplementation

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ABSTRACT

Purpose: We examined hypertrophic outcomes of weekly graded whey protein dosing (GWP) versus whey protein (WP) or maltodextrin (MALTO) dosed once daily during 6 weeks of resistance training (RT). Methods: College-aged resistance-trained males (training age=5±1 yrs; mean±SE) were assigned to WP (25g/d; n=10), MALTO (30g/d; n=10), or GWP (25-150 g/d from weeks 1-6; n=11). RT occurred 3d/wk (2 upper- and 2 lower-body exercises/d, 10 repetitions/set), and RT volume increased from 10 sets/exercise (week 1) to 32 sets/exercise (week 6). The 6-week RT program implemented was designed to involve higher RT volumes than ever investigated in this timeframe. Tests performed prior to training (PRE) and after weeks 3 (MID) and 6 (POST) included dual-energy x-ray absorptiometry (DXA), vastus lateralis (VL) and biceps brachii ultrasounds, and bioelectrical impedance spectroscopy (BIS). VL biopsies were also collected for immunohistochemical staining. Repeated-measures ANCOVAs were performed, although emphasis was also placed on effect size calculations. Results: The GWP group experienced the greatest PRE to POST reduction in DXA fat mass (FM) (-1.00 kg, d=-0.24, $p<0.05$) and increase in DXA lean body mass (LBM) (+2.93 kg, d=0.33, $p<0.05$). DXA LBM increases (ΔLBM) occurred from PRE to MID (+1.34 kg, $p<0.001$) and MID to POST (+0.85 kg, $p<0.001$) across all groups. However, when adjusting ΔLBM for extracellular water changes, a significant increase occurred from PRE to MID (+1.18 kg, $p<0.001$), but not MID to POST (+0.25 kg; $p=0.131$). Conclusions: Larger effects on FM and LBM in GWP subjects indicates a need for longer-term investigations with greater sample sizes examining graded WP

intakes and RT. Additionally, ECW-corrected LBM gains were largely dampened, but still positive, in resistance-trained subjects when RT exceeded ~20 sets/exercise/wk.

Keywords: muscle hypertrophy, resistance training, recovery, adaptation, graded whey protein

INTRODUCTION

Resistance training (RT) and increased consumption of dietary protein have been well documented to enhance indices of skeletal muscle hypertrophy in humans. Regarding the former, current scientific evidence suggests a positive relationship between RT volume (e.g., sets per muscle per week) and hypertrophy (39). However, the upper limit of RT volume to elicit maximal hypertrophic responses while avoiding maladaptation is unclear (38). Considering this, RT studies investigating higher doses than previously studied are warranted to better understand dose-response relationships in various populations. To this end, Schoenfeld et al. (38) recently argued: "What now needs to be determined is where the upper threshold for volume lies to promote the greatest increases in muscular gains". The RT program designed for this investigation was intended to involve the highest RT volumes formally investigated in humans to date in a 6-week timeframe.

Given that ~90% of skeletal muscle dry weight is comprised of protein (27), significant changes in skeletal muscle size are likely associated with alterations in muscle protein breakdown (MPB) and muscle protein synthesis (MPS) (44). RT volume and muscle protein

turnover seem to exhibit a dose-response relationship, where increases in RT volume are associated with increased muscle protein turnover (8). This phenomenon suggests a potential ergogenic role of protein supplementation during high-volume RT programs. Indeed, numerous studies indicate protein ingestion acutely stimulates significant increases in MPS following a resistance exercise bout [reviewed in (4)]. Individuals self-reporting chronic high protein intakes (> 2.0 g/kg/day) while undergoing chronic RT have exhibited greater reductions in body fat and greater increases in FFM (2, 3, 20). Significantly greater acute MPS responses to ≥ 35 g of whey protein compared to lower doses (e.g., ≤ 20 g) have also been reported (29, 43). However, studies examining chronic (i.e., > 6 weeks) supplementation of whey protein dosed at ≥ 40 g per day combined with supervised RT in humans are sparse, particularly in young men with prior training experience. In this regard, and to our knowledge, only a handful of studies have examined effects of whey protein doses ≥ 40 g per day on body composition in resistance-trained young men undergoing supervised, chronic RT (11, 21, 22, 24, 26). While four of these studies reported high-dose whey protein supplementation significantly increased FFM following 8-12 weeks of RT (11, 21, 22, 24), Lockwood et al. (26) reported 60 g/d of whey protein concentrate or hydrolyzed whey protein did not further promote increases in FFM beyond those observed in subjects supplementing with maltodextrin. Given this underwhelming amount of evidence overall, investigations of these dosages in previously trained young men deserves further inquiry. Additionally, studies implementing high whey protein supplementation doses are lacking in the context of extremely high training volumes. Critically, it seems that no studies have investigated dosing whey protein in a practical and proportional manner to RT volume where doses are

increased concurrently during an RT program to elicit a greater hypertrophic response (i.e., a "proportional supplemental protein hypothesis"). Given the graded structure of RT volume in this design, where RT volume was significantly increased each week, we also sought to explore the effects of a graded dose of whey protein concurrent to the increase in RT volume.

As such, we first intended to examine effects of RT volumes higher than previously investigated in a 6-week timeframe. Secondarily, we sought to observe any differential effects between a group of subjects consuming a single 25 g supplemental dose of whey protein per day (WP), a group consuming a graded dose of protein throughout the study where the dose per day was increased by 25 g each week (GWP [25 g – 150 g from week 1 to week 6]), and a group consuming a single 30 g supplemental dose of a maltodextrin-based carbohydrate supplement per day (MALTO). Given the exploratory nature of this work, we posited the null hypothesis as true for all independent and dependent variable relationships.

METHODS

Ethical approval and subject screening

Prior to engaging in data collection, this study was approved by the Institutional Review Board at Auburn University and conformed to the standards set by the latest revision of the Declaration of Helsinki (IRB approval #: 17-425 MR 1710). Resistance-trained young men from the local community were recruited to participate in this study. Subjects provided both verbal and written consent, and completed a medical history form prior to screening. Two primary

criteria were used to establish subject training status: a) self-reported > 1.5 years of RT, and b) back squat 1RM ≥ 1.5 × body mass (estimated from a three-repetition maximum [3RM] test conducted for each subject with strict criteria [e.g., crease of the hip below the top of the knee joint at the bottom of the squat]) (7). After screening, 34 subjects were counterbalanced between groups to ensure no significant differences existed between groups in lean body mass (LBM) and 3RM squat at baseline. Due to illnesses, three subjects withdrew from the study; specifically, one subject during week 1, a second subject during week 3, and a third subject after week 4. Hence, 31 subjects completed the study and were partitioned to one of three groups: 1) daily single dose of whey protein (WP, 25g/d; n = 10), 2) daily single dose of maltodextrin (MALTO, 30g/d; n = 10), or 3) graded dose of WP (GWP, 25-150 g/d from weeks 1-6; n = 11). Descriptive characteristics are provided in supplementary table 1 (haun_supplementary_tables.pdf).

Study design

Figure 1 provides a visual representation of the study design. Briefly, a battery of tests were performed prior to week 1 (PRE), after week 3 (MID), and after week 6 (POST). These tests will be further described below following an explanation of the resistance training program, supplementation paradigm, and nutritional recommendations.

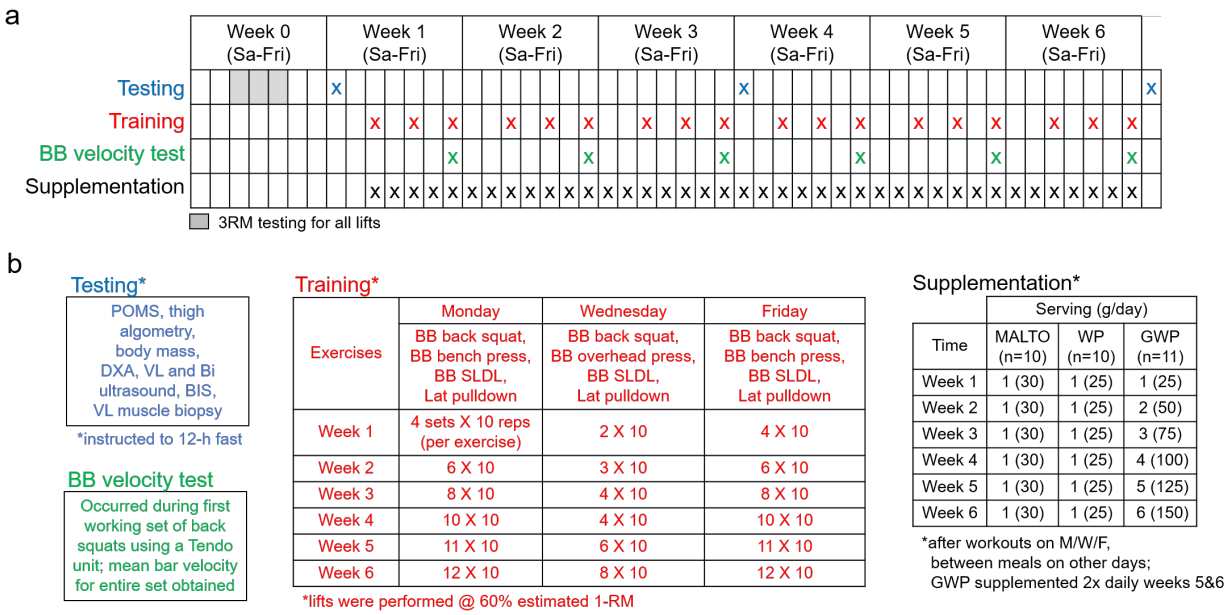


Figure 1. Study design

Legend: Panel a outlines testing, training and supplementation days. Panel b (upper left inset) describes the testing battery which included (in order) a profile and mood state questionnaire (POMS), outer thigh pain assessment using algometry, body mass assessment, and whole-body dual x-ray absorptiometry (DXA) scan, a vastus lateralis (VL) and biceps (Bi) ultrasound, total body water assessment using bioelectrical impedance spectroscopy (BIS), and a VL muscle biopsy. Panel b (lower left inset) describes the BB squat velocity test that occurred during the first set of barbell squats every Friday from weeks 1-6 of training. Panel b (middle inset) outlines the supervised training regimen described in greater detail in the methods. Panel b (right inset) outlines the supplementation regimen described in greater detail in the methods.

Resistance training

Subjects were familiarized with the design of training and technical parameters during testing of 3RMs which occurred 3-7 days prior to PRE testing and training initiation. Strict technical parameters were employed for testing to ensure accurate reflections of strength under direct supervision of research staff holding the Certified Strength and Conditioning Specialist Certification from the National Strength and Conditioning Association.

Following the PRE testing battery and 3RM testing, RT occurred 3 days per week and was progressed according to Figure 1b. Loads corresponding to 60% 1RM, based on 3RM testing, were programmed for each set of each exercise. Sets of 10 repetitions were programmed for each set of each exercise throughout the study. Prior to beginning each training session, subjects were instructed to perform a general warm-up involving 25 jumping jacks, 10 bodyweight squats, 10 push-ups, and 10 bodyweight standing reaches mimicking the kinematics of the stiff-legged deadlift (SLDL) for 2 rounds. Next, subjects were instructed to perform the following specific warm-up for each exercise: 50% of working set weight for 10 repetitions, 75% for 3 repetitions, and 95-100% for 1 repetition. Exercises were completed one set at a time, in the following order during each training session: Days 1 and 3 – barbell (BB) back squat, BB bench press, BB SLDL, and an underhand grip cable machine pulldown exercise designed to target the elbow flexors and latissimus dorsi muscles (Lat Pulldown); Day 2 –BB back squat, BB overhead (OH) press, BB SLDL, and Lat Pulldown. A single set of one exercise was completed, followed by a set of each of the succeeding exercises before starting back at the first exercise of the session (e.g., compound sets or rounds). Subjects were recommended to take 2 minutes of rest between

each exercise of the compound set. Additionally, subjects were recommended to take 2 minutes of rest between each compound set. However, if subjects felt prepared to execute exercises with appropriate technique under investigator supervision they were allowed to proceed to the next exercise without 2 minutes of rest. Additionally, if subjects desired slightly longer than 2 minutes of rest, this was allowed with intention for the subject to execute the programmed training volume in less than 2 hours each training session. This design was based on evidence indicating that total volume load (sum of the total repetitions x weight for each individual exercise) for a week of training is primarily related to hypertrophic outcomes, with specific rest intervals between sets being less important (16, 39). In that we sought for the nature of this design to be ecologically valid, we elected a more self-regulated pace of the training session where subjects could be somewhat autonomous while under direct supervision of research staff ensuring technical execution of exercises. Both the extremely high training volumes planned for this investigation, having never been investigated in humans, and pilot testing of this design by our research staff persuaded the implementation of this rest scheme paradigm.

During training sessions, subjects provided a repetition in reserve (RIR) rating after each set of each exercise to a researcher, having been instructed to provide a number of repetitions the subject felt they could have completed with good technique beyond the 10 repetitions completed for the set (19). If the execution of repetitions during a working set were deemed unsafe by research staff, or the subject felt unsafe or too fatigued to continue the set or the session, the set or session was terminated. This occurred on only a few occasions, and if repetitions were missed, attempts were made to make these up within the same week of training. The number of

repetitions completed for each exercise and the load used for each exercise each week were recorded in Google Sheets (Mountain View, CA, USA) by research staff, along with the RIR rating provided by the subject for each individual set. RT volume and RIR data are available in the supplementary .csv file (haun_supplementary_data.csv). A priori, based on pilot testing of the training, we elected a systematic approach to load manipulation within each training session where the load was decreased by 5% for each repetition below 10 (e.g., 9 repetitions = -5%, 8 repetitions = -10%, 7 repetitions = -15%, etc.). However, this was only necessary on a few occasions, and the majority of the training was executed according to the planned study design. BB velocity was also measured using a Tendo unit (TENDO Sports Machines, Trencin, Slovak Republic) on Friday of each week as a proxy of fatigue status and recovery on the first set of BB back squats similar to the methods of Zourdos et al. (46). However, due to logistical constraints, BB velocity was only obtained from a subset of subjects at all time points (n = 6-7 per group). Finally, subjects were allowed to train from either 0700-0900 or 1530-1830 on Monday, Wednesday, and Friday each week, and were instructed to perform no other vigorous exercise outside of the study.

Supplementation

As illustrated in Figure 1, subjects were assigned to either MALTO, WP, or GWP groups. All supplements were graciously provided by Dymatize Nutrition® (Dallas, TX, USA). Packaging and delivery to subjects was designed to blind subjects to the supplement condition; however, investigators of the study were not blinded. The WP utilized herein (Elite 100%

Whey) was comprised of the following nutrition profile per scoop: calories – 140, total fat – 2 g, cholesterol – 70 mg, sodium – 70 mg, potassium – 150 mg, total carbohydrate – 3 g, protein – 25 g. Additionally, WP contained 5.5 g branched chain amino acids (2.7 g L-leucine, 1.4 g L-isoleucine, 1.4 g L-valine), 3.5 g of other essential amino acids, 4.4 g of L-glutamine, 2.4 g of conditionally essential amino acids, and 6.5 g of non-essential amino acids. The MALTO supplement contained 120 calories from 30 g of maltodextrin powder (~30g of carbohydrates) with <1g of vanilla flavoring.

Drinks were formulated by research staff for each subject by combining the appropriate serving size with ~500 ml of tap water, and subjects consumed drinks after each training session under investigator supervision. MALTO and WP consumed a single scoop each day for the duration of the study; specifically, 1 after training sessions on training days and 1 between meals on non-training days which subjects prepared themselves. GWP consumed the protein supplement according to the following dosage and timing breakdown:

Week 1: 1 scoop with 500 ml of water post-training on training days, 1 scoop with 500 ml of water between meals on non-training days (1 total scoop each day)

Week 2: 2 scoops with 500 ml of water post-training on training days, 2 scoops with 500 ml of water between meals on non-training days (2 total scoops each day)

Week 3: 3 scoops with 500 ml of water post-training on training days, 3 scoops with 500 ml of water between meals on non-training days (3 total scoops each day)

Week 4: 4 scoops with 500 ml of water post-training on training days, 4 scoops with 500 ml of water between meals on non-training days (4 total scoops each day)

Week 5: 4 scoops with 500 ml of water post-training on training days, 4 scoops with 500 ml of water between meals on non-training days, 1 scoop prior to bed each day (5 total scoops each day)

Week 6: 4 scoops with 500 ml of water post-training on training days, 4 scoops with 500 ml of water between meals on non-training days, 2 scoops prior to bed each day (6 total scoops each day)

Beyond post-exercise supplementation which was supervised, subjects from all groups verbally reported compliance to the supplementation paradigm on a weekly basis to research staff. Additionally, subjects were asked to refrain from the use of other protein supplements or protein bars throughout the duration of the study.

Nutritional recommendations and monitoring throughout the protocol

In collaboration with a Registered Dietitian (A.K., PhD, RD), subjects were provided with calorie and macronutrient recommendations along with lists of potential food choices to help meet recommendations for each day during the study. Specifically, recommended values and calculations can be found in the supplementary .csv file (haun_supplementary_diet.csv). Briefly, these recommendations were based on the following: 1) resting metabolic rate estimates from the Harris-Benedict equation, 2) an estimated non-exercise activity expenditure in this age cohort, 3) an estimated energy expenditure from training each week, and 4) the desire for subjects to be in a modest calorie surplus (~500 calories above the estimated total daily energy expenditure [TDEE]) throughout the study. These calculations and supplementary formulae can

be found in the supplementary .csv file (haun_supplementary_diet.csv). These recommendations were provided directly to subjects through Google Sheets. Subjects were asked to enter dietary intakes each day throughout the study, and include the consumption of their supplement in their daily tracking using a mobile application (MyFitnessPal, Inc.; Baltimore, MD, USA). Notably, this mobile application has been validated against paper-based food records (41). Data were exported on a weekly basis by research staff for analysis. A de-identified generic food item was created in the application's database for WP, and subjects were instructed to log this food item each time a single scoop of their respective supplement was consumed. Entries for subjects in the MALTO group were corrected by research staff following the study to account for macronutrient differences between the WP and MALTO supplements.

Subjects in the WP and GWP groups were recommended to consume the same daily amount of dietary protein where, during week 1, subjects were recommended to consume 1.6 g/kg/day assuming the consumed supplement contributed 25 g/scoop to this total. Subjects in the MALTO group were recommended to consume 1.6 g/kg/day protein for the entire duration of the study. This recommendation was based on the findings of Morton et al. (35) suggesting a maximum effective dose of daily protein around this value in young, resistance-trained men. Suggested protein intakes for WP and GWP were increased in proportion to one another throughout the study. However, GWP increased their dosage through supplemental whey protein, as described above, and were informed to log the specific number of servings of whey protein each day so that this contributed to the recommended total. WP was instructed to consume 1 scoop each day for the entire study, and attempt to meet the recommended protein intake through

dietary sources beyond the single scoop allowed from the supplement. Hence, there was no difference in recommended protein intakes between GWP and WP groups, but GWP was provided supplemental protein in proportion to the doses and guidelines described above while WP was not. Subjects were instructed to consume ~3 g/kg/day of dietary carbohydrate starting on week 1 of the study. A modest amount of carbohydrates (~30 g) were added to this value on training days each week to address expected reductions in muscle glycogen from increases in training volume specific to the design herein based on the recommendations from Scott et al. (40). Fat recommendations were based on remaining calorie values upon setting targeted protein and carbohydrate values. Subjects were instructed to attempt to meet the dietary fat recommendation through primarily monounsaturated and polyunsaturated fatty acid sources, while confining saturated fat intakes to no more than 10% of total calorie intake. Logged nutrition data were stored in Google Drive and are provided in a supplementary file in .csv format (haun_supplementary_data.csv).

Testing battery procedures

As outlined in Figure 1, the following tests were performed prior to (PRE), during (MID) and following the 6-week protocol (POST). Notably, subjects were encouraged to arrive to these testing sessions in an overnight fasted condition, and the following tests were performed:

Hydration Status and Profile of Mood State. Subjects were instructed to submit a urine sample (~5 mL) to assess normal hydration specific gravity levels (1.005-1.020 ppm) using a handheld refractometer (ATAGO; Bellevue, WA, USA). Subjects with a urine specific gravity >1.020

were asked to consume 400 ml tap water and were re-tested ~10 minutes later. Following urinalysis, profiles of mood state (POMS) were collected on Google Forms using the questionnaire published by Grove and Prappavessis (17). From this, total mood disturbances (TMD) could be inferred by summing negative emotion scores and subtracting positive emotion scores from this summed value according to a subject's specific responses to approximately 50 questions where the subject's baseline score served as its own control.

Algometry. Following POMS, pressure-to-pain threshold (PPT) of the outer aspect of the right upper thigh was measured using a handheld algometer (Force Ten FDX, Wagner Instruments, Greenwich, CT, USA) according to methods described previously from our laboratory (18). Briefly, focal pressure was applied by the algometer to proximal, medial, and distal portions of the right vastus lateralis (VL) which were marked for accurate application of force. Algometry pressure was applied at a rate of approximately 5 Newtons (N) per second at each site until the subject audibly indicated the specific moment at which the applied pressure became painful. At this point, the PPT value in N was recorded. The digital display of the algometer indicating the force value was blinded to subjects. The PPT was measured sequentially from proximal, medial, and distal sites, respectively, three times for triplicate measures with ~30 s between cycles of measurement. The average of the triplicate measures at each site was calculated as the respective PPT of the site, and these values were averaged for a total PPT.

Body composition assessment. Following algometry, height and body mass were assessed using a digital column scale (Seca 769; Hanover, MD, USA) with weights and heights being collected to the nearest 0.1 kg and 0.5 cm, respectively. After this, subjects were subjected to a full body

dual x-ray absorptiometry (DXA) scan (Lunar Prodigy; GE Corporation, Fairfield, CT, USA). All DXA scans were completed by the same investigator (M.A.R.). According to previous data published by our laboratory (23), the same-day reliability of the DXA during a test-calibrate-retest on 10 subjects produced an intra-class correlation coefficient (ICC) of 0.998 for total body lean mass.

Ultrasound muscle thickness measurements. Subjects also underwent duplicate ultrasound assessments per testing session to determine average right leg VL muscle and right bicep brachii thicknesses with a 3 to 12 MHz multi-frequency linear phase array transducer (Logiq S7 R2 Expert; General Electric, Fairfield, CT, USA). VL measurements were taken from the midway point between the iliac crest and patella of the right femur whereby subjects were in a standing position and all weight was placed on the left leg. Similarly, bicep brachii thickness measurements were taken ~60% distal from the acromial process of the scapula to the lateral epicondyle of the humerus. All ultrasound assessments were completed by the same investigator (P.W.M.). Reliability for duplicate ultrasound muscle thickness measurements on 33 subjects at PRE produced an ICC of 0.994.

Total body water assessment. Total body water (TBW), extracellular water (ECW), and intracellular water (ICW) were measured by bioimpedance spectroscopy using the SFB7 device (ImpediMed Limited, Queensland, AU) according to the methods described by Moon et al. (34). The SFB7 device measures whole-body bioelectrical impedance with over 200 frequencies, and uses complex Cole models to estimate TBW, ICW and ECW. Moreover, the SFB7 device: a) has excellent agreement with TBW assessed via deuterium oxide (34), b) has excellent

agreement with ECW assessed via sodium bromide dilution (6), and c) has been posited to be the best non-invasive methodology for the determination of fluid compartmentalization (33). This test involved subjects resting in a supine position for 5 to 10 minutes, and TBW estimates were collected thereafter while the subjects laid supine on a table with their arms ≥ 30 degrees away from their torso with their legs separated. The average of two readings was used to represent the subjects' TBW. All TBW assessments were performed by the same investigator (K.C.Y.). Reliability for duplicate TBW measurements on 24 subjects at PRE produced an ICC of 0.999.

Muscle Biopsies and Tissue Processing. After body composition and ultrasound measurements, VL muscle biopsies from the right leg were collected using a 5-gauge needle under local anesthesia as previously described (32). Immediately following tissue procurement, the obtained tissue was teased of blood and connective tissue, and ~20-40 mg of tissue was embedded in cryomolds containing optimal cutting temperature (OCT) media (Tissue-Tek®, Sakura Finetek Inc; Torrence, CA, USA). Embedding was performed whereby tissue was laid in cryomolds for perpendicular slicing in a non-stretched state prior to rapid freezing. Cryomolds were then frozen using liquid nitrogen-cooled isopentane and subsequently stored at -80ºC until immunofluorescent staining for determination of fiber cross sectional area (fCSA). The remaining tissue was wrapped in pre-labelled foils, flash frozen in liquid nitrogen, and subsequently stored at -80ºC. All biopsies were obtained by the same investigators (M.D.R. and C.T.H.), and biopsies were obtained ~2 cm apart at the same approximate depth each testing session.

Immunohistochemistry for fiber cross sectional area assessment

Similar methods for immunohistochemistry have been employed previously in our laboratory (32). Sections from OCT-preserved samples were cut at a thickness of 8 μm using a cryotome (Leica Biosystems; Buffalo Grove, IL, USA) and were adhered to positively-charged histology slides. Once all samples were sectioned, batch processing occurred for immunohistochemistry. During batch processing sections were air-dried at room temperature for 10 minutes, permeabilized in a phosphate-buffered saline (PBS) solution containing 0.5% Triton X-100 for 10 minutes, and blocked with 100% Pierce Super Blocker (Thermo Fisher Scientific) for 10 min. For fiber type staining, sections were subsequently washed for 2 minutes in PBS. Sections were then incubated for 10 minutes with a pre-diluted commercially-available rabbit anti-dystrophin IgG antibody solution (catalog #: GTX15277; Genetex Inc.; Irvine, CA, USA) and spiked in mouse anti-myosin I IgG (catalog #: A4.951 supernatant; Hybridoma Bank, Iowa City, IA, USA; 40 μL added per 1 mL of dystrophin antibody solution). Sections were then washed for 2 minutes in PBS and incubated in the dark for 15 minutes with a secondary antibody solution containing Texas Red-conjugated anti-rabbit IgG (catalog #: TI-1000; Vector Laboratories, Burlingame, CA, USA), and Alexa Fluor 488-conjugated anti-mouse IgG (catalog #: A-11001; Thermo Fisher Scientific) (~6.6 μL of all secondary antibodies per 1 mL of blocking solution). Sections were washed for 2 minutes in PBS, air-dried and mounted with fluorescent media containing 4,6-diamidino- 2-phenylindole (DAPI; catalog #: GTX16206; Genetex Inc.). Following mounting, slides were stored in the dark at 4ºC until immunofluorescent images were obtained. After staining was performed on all sections, digital 10x objective images were

captured using a fluorescence microscope (Nikon Instruments, Melville, NY, USA). All images were captured by a laboratory technician whom was blinded to the group assignment of each subject with intent to prevent any bias. Approximate exposure times were 400 ms for TRITC and FITC imaging. Our staining method allowed the identification of cell membranes (detected by the Texas Red filter), type I fiber green cell bodies (detected by the FITC filter), type II fiber black cell bodies (unlabeled), and myonuclei (detected by the DAPI filter). Measurements of type I and II fCSAs were performed using custom-written pipelines in the open-sourced software CellProfilerTM (9) per modified methods previously described whereby the number of pixels counted within the border of each muscle fiber were converted to a total area (μm2) (30). Notably, a calibrator slide containing a 250,000 μm2 square image was also captured, and pixels per fiber from imaged sections were converted to area using this calibrator image. Per the recommendations of Mackey et al. (28), at least 50 fibers per specimen were quantified in order to obtain accurate fCSA values. On average, 113 ± 26 fibers per cross-section were identified for analysis at each time point. A post hoc experiment performed in our laboratory to examine potential differences in fCSA measurements between sections on the same slide (n = 23 slides) revealed strong reliability using this method (ICC = 0.929).

Statistical Analysis

Statistical tests were performed in RStudio (Version 1.0.143; R Foundation for Statistical Computing, Vienna, AT), SPSS (Version 23; IBM SPSS Statistics Software, Chicago, IL, USA), and Google Sheets. Group (3 levels [WP, GWP, MALTO]) and time (3 levels [PRE, MID,

POST], or 6 levels [Week 1-6] for weekly measures) served as independent variables. A mean-centered covariate for each baseline measurement was added as a parameter to models to examine the explained variance in dependent variables relative to values at PRE. Since nutrition-related data was not available at PRE, and only after collection of data during week 1, no covariate was utilized in this model and a repeated-measures ANOVA was performed after assumptions testing. Statistical assumptions tests were completed prior to analysis consisting of: 1) Shapiro-Wilks tests of residual distributions for normality, 2) Levene's test of homogeneity of variance, and 3) Mauchly's test for Sphericity, given that a repeated-measures analysis of covariance (ANCOVA) was performed for the provision of p-values. Violation of these assumptions and appropriate data transformations (i.e., square root or log10 transformations) when residuals were not normally distributed were completed prior to ANCOVA for the avoidance of type 1 or type 2 errors. Data transformation and data removal were avoided with intention to analyze all raw data. For this reason, if the majority of levels of group (2 of 3 groups) at each level of time were normally distributed, ANCOVA proceeded without data transformation. If the assumptions of homogeneity of variance or sphericity were violated, Greenhouse-Geisser corrections to degrees of freedom were made. The alpha level of significance was set a priori to $p < 0.050$. For significant main effects of time and group×time interactions, LSD post hoc tests were performed at each level of time to elucidate between time point differences. A priori power analysis in RStudio using general linear model parameters in the "pwr" package (Version 1.2-1) revealed 84.5% power (power = $1 - \beta$) for the discovery of a large effect size when 2 predictors and 31 observations were employed (e.g., k=2 [time, y-

intercept], n = 31 [31 subject observations], f2 = 0.35 [large effect], p = 0.05 [a-priori level of significance]). However, a power analysis to detect a significantly large difference of an effect between groups when 3 groups (k = 3) included 10 subjects each (n = 10) revealed 44 % power. Therefore, Cohen's d effect sizes and 95 % confidence intervals were also calculated for each dependent variable, aside from nutrition data, to examine mean differences between groups from PRE to POST considering the pooled standard deviation of a dependent variable at baseline since population-based inferences were underpowered. These statistics are reported below as: d = x.xx , 95 % CI: xxx (lower-bound) to xxx (upper-bound). Supplementary Tables 2-11 provide descriptive statistics, effect sizes, and 95% confidence intervals for each dependent variable. Additionally, raw data are provided in .csv files (haun_supplementary_data.csv). For these reasons, only statistically significant findings are reported below along with Cohen's d and 95% confidence intervals (CI) values where relevant.

RESULTS

Self-Reported Nutrition

Nutritional analyses were performed on subjects who logged > 90% of days throughout the study where differences between calorie and macronutrient intakes could be accurately compared each week. Specifically, 12 subjects irregularly reported or did not report nutritional intakes each week resulting in 19 subjects' data inclusion in the nutritional reporting analyses. Hence, Table 1 contains self-reported dietary intakes from these 19 subjects. When considering data from these 19 subjects, no significant main effect of group, time, or group×time interaction was observed

for self-reported absolute or relative energy, protein, or carbohydrate intakes ($p > 0.05$). A significant main effect of time and group, but no interaction, was observed on reported fat intake, where reported intake decreased over time ($p = 0.006$), and WP averaged higher reported intakes than GWP and MALTO ($p = 0.017$). In reference to subject adherence to nutrition recommendations provided by the R.D., within-group LSD post hoc comparisons at each level of time between recommendations and reported consumption were similar for calories, protein, and carbohydrates. However, during weeks 1 and 6, GWP reported less protein consumption than recommended ($p < 0.05$), and the reported consumption of dietary fat relative to that recommended was significantly different during weeks 1-6 in MALTO, weeks 1-3 in GWP, and weeks 4-6 in WP ($p < 0.05$).

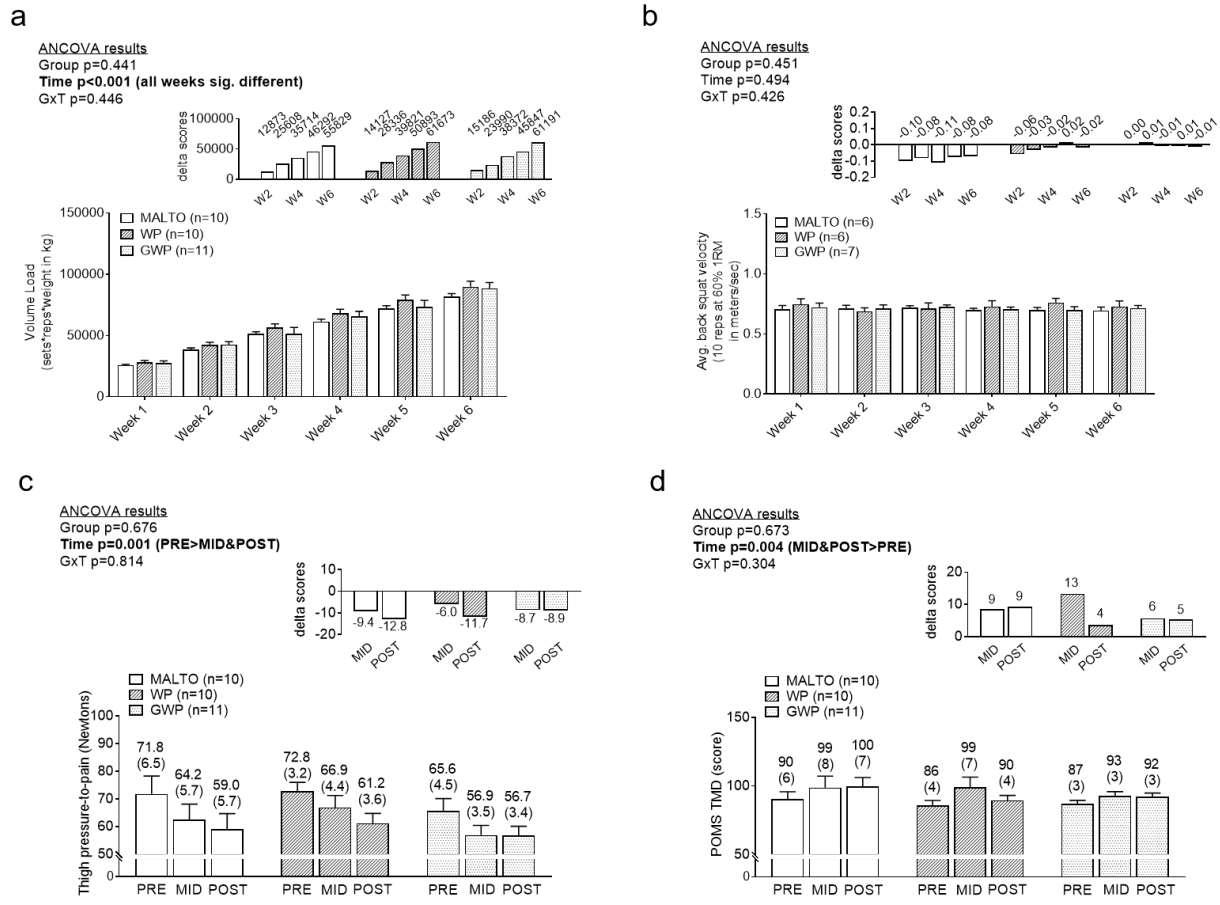
Table 1. Self-reported dietary data

		MALTO (n = 6)				WP (n = 6)				GWP (n = 7)				TOTAL (n = 19)			
	week	Abs	SE	Rel	SE	Abs	SE	Rel	SE	Abs	SE	Rel	SE	Abs	SE	Rel	SE
Energy (kcal/d) or (kcal/kg/d)	1	2869.7	188.4	35.2	2.2	2994.1	188.4	35.2	2.2	2625.1	319.3	32.2	3.9	2818.9	136.4	34.1	1.6
	2	2732.8	238.0	36.0	2.8	3064.9	238.0	36.0	2.8	2831.5	220.1	34.7	2.7	2874.0	119.2	34.7	1.4
	3	2736.5	250.6	34.9	3.0	2959.4	250.6	34.9	3.0	2677.2	194.6	32.6	2.4	2785.1	109.8	33.5	1.3
	4	2826.8	86.1	38.6	1.0	3288.1	86.1	38.6	1.0	2744.1	216.5	33.3	2.6	2942.0	101.4	35.2	1.2
	5	2912.2	114.8	37.5	1.3	3199.1	114.8	37.5	1.3	2557.8	312.5	30.9	3.8	2872.2	134.4	34.3	1.6
	6	2209.0	142.8	35.8	1.7	3046.5	142.8	35.8	1.7	2318.9	341.2	28.0	4.1	2513.9	189.9	30.1	2.3
PRO (g/d) or (g/kg/d)	1	148.1	15.5	2.1	0.2	177.9	15.5	2.1	0.2	185.6	23.1	2.3	0.3	171.3	10.4	2.1	0.1
	2	151.3	10.9	2.1	0.1	181.1	10.9	2.1	0.1	183.9	18.3	2.3	0.2	172.7	8.2	2.1	0.1
	3	166.2	21.4	2.3	0.3	191.3	21.4	2.3	0.3	170.1	20.0	2.1	0.2	175.6	10.5	2.1	0.1
	4	182.9	15.3	2.4	0.2	204.7	15.3	2.4	0.2	186.9	21.3	2.3	0.3	191.3	10.0	2.3	0.1
	5	189.6	23.1	2.5	0.3	213.5	23.1	2.5	0.3	183.9	30.8	2.2	0.4	195.0	14.9	2.3	0.2
	6	171.8	22.3	2.4	0.3	204.4	22.3	2.4	0.3	167.7	32.9	2.0	0.4	180.6	18.2	2.2	0.2
CHO (g/d) or (g/kg/d)	1	278.3	37.1	3.1	0.4	260.8	37.1	3.1	0.4	251.0	32.9	3.1	0.4	262.7	17.8	3.2	0.2
	2	260.0	21.5	3.1	0.3	262.1	21.5	3.1	0.3	279.4	20.7	3.4	0.3	267.8	11.5	3.2	0.1
	3	253.8	18.2	3.1	0.2	267.4	18.2	3.1	0.2	267.0	17.9	3.3	0.2	262.9	10.7	3.2	0.1
	4	271.1	16.8	3.4	0.2	285.7	16.8	3.4	0.2	262.9	28.8	3.2	0.3	272.7	12.2	3.3	0.1
	5	288.2	11.6	3.5	0.1	297.6	11.6	3.5	0.1	250.6	30.0	3.0	0.4	277.3	15.3	3.3	0.2
	6	206.5	14.4	3.4	0.2	290.1	14.4	3.4	0.2	210.7	34.3	2.5	0.4	234.4	18.8	2.8	0.2
FAT (g/d) or (g/kg/d)	1	122.7	8.7	1.7	0.1	141.1	8.7	1.7	0.1	102.0	14.6	1.3	0.2	120.9	7.5	1.5	0.1
	2	118.1	10.9	1.6	0.1	137.4	10.9	1.6	0.1	110.0	10.7	1.3	0.1	121.2	6.2	1.5	0.1
	3	111.3	13.5	1.5	0.2	129.8	13.5	1.5	0.2	101.0	9.7	1.2	0.1	113.3	6.2	1.4	0.1
	4	112.8	4.9	1.7	0.1	145.6	4.9	1.7	0.1	106.6	10.9	1.3	0.1	120.8	6.1	1.4	0.1
	5	107.8	5.9	1.5	0.1	126.7	5.9	1.5	0.1	91.0	12.2	1.1	0.1	107.6	5.8	1.3	0.1
	6	82.0	8.3	1.5	0.1	129.7	8.3	1.5	0.1	90.4	11.6	1.1	0.1	100.2	7.6	1.2	0.1

Legend: all data absolute (Abs) or relative (Rel) self-reported dietary intake data are presented as means ± standard error (SE) values. Only 19 subjects' data were included in the nutritional analyses given that 12 subjects irregularly reported (or did not report) nutritional intakes. No significant main effect of group, time, or group×time interaction was observed for reported absolute or relative calories, protein intake, or carbohydrate intake ($p > 0.05$); thus no significance is indicated.

Training Volume, Soreness, BB Velocity, and Total Mood Disturbance

Figure 2. Differences in training volume, back squat lifting velocity, thigh soreness, and total mood disturbance between supplementation groups



Legend: Only a significant time effect was observed for training volume whereby values were significantly greater from week to week (panel a). No main effects or group×interaction was observed for back squat lifting velocity (panel b). Only a significant time effect was observed for thigh pressure-to-pain values (lower values indicates greater soreness) (panel c). Only a significant time effect was observed for profile of mood state (POMS) total mood disturbance (TMD) (greater values indicates more mood disturbance) (panel d). All data are presented as means ± standard error values, and values in panel c and d are indicated above each bar; values

for panels a and b are not indicated due to space constraints but are provided in the raw data file. Additionally, each data panel has delta values from PRE included as inset data. Abbreviations: MALTO, maltodextrin group, WP; standardized whey protein group; GWP, graded whey protein group.

Training volume significantly increased over time where each week of training resulted in more volume relative to the previous week ($p < 0.001$), but no significant group or group×time interaction was observed (Figure 2a). No significant main effects or group×time interaction was observed for BB velocity assessed during set 1 of the back squat exercise at the beginning of each Friday training session (Figure 2b). Algometry PPT measures significantly decreased over time ($p < 0.001$), but no significant group or group×time interaction was observed (Figure 2c). PPT was significantly lower at MID compared to PRE ($p = 0.002$), and POST compared to PRE ($p < 0.001$), but not at POST compared to MID ($p = 0.122$). The largest effect occurred in MALTO from PRE to POST ($d = -0.84$, 95% CI = -22.20 to -3.36 N). POMS TMD significantly increased over time ($p = 0.002$) but no significant effect of group or group×time interaction was observed (Figure 2d). TMD was significantly higher at MID compared to PRE ($p = 0.002$), and at POST compared to PRE ($p < 0.001$), but not at POST compared to MID ($p = 0.254$). The largest effect occurred in MALTO from PRE to POST ($d = 2.16$, 95% CI = 7.60 to 14.8 units).

Body Composition Data

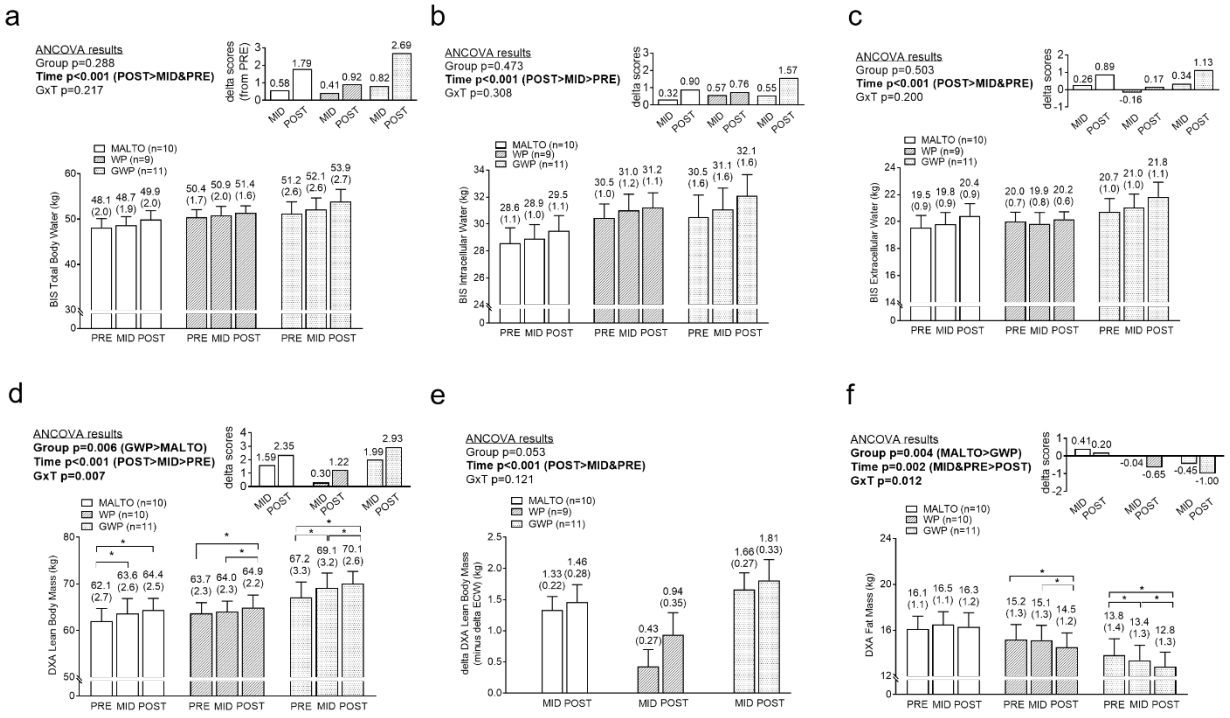
TBW significantly increased over time ($p < 0.001$), but no significant group or group×time interaction was observed (Figure 3a). The largest effect was observed in GWP ($d = 0.40$, 95% CI

= -1.32 to 6.70 kg). Both ICW (Figure 3b) and ECW (Figure 3c) significantly increased over time, but no significant group or group×time interactions were observed for these metrics. Again, the largest effects were observed in GWP (ICW: d = 0.38, 95% CI = -0.86 to 4.00 kg; ECW: d = 0.40, 95% CI = -0.53 to 2.77 kg).

DXA lean body mass (LBM) significantly increased over time ($p < 0.001$; Figure 3d). A significant group×time interaction ($p = 0.007$) was observed for LBM, although LSD post hoc tests revealed no significant differences between groups at any time point. However, the largest effect was observed in GWP from PRE to POST (d = 0.33, 95% CI = -2.32 to 8.19 kg). When corrected for changes in ECW, a significant increase in DXA LBM was observed from PRE to POST ($p < 0.001$). No significant group or group×time interaction was observed (Figure 3e).

DXA fat mass significantly decreased over time ($p = 0.004$; Figure 3f). A significant group×time interaction ($p = 0.012$) was observed and, while LSD post hoc tests revealed no significant differences between groups at any time point, the difference between GWP and MALTO at MID and POST approached significance ($p = 0.088$ and $p = 0.064$, respectively). The largest effect was observed in GWP from PRE to POST (d = -0.24, 95% CI = -3.46 to 1.46 kg).

Figure 3. Body composition differences between supplementation groups



Legend: Only significant time effects were observed for total body water content (panel a) assessed via bioelectrical impedance spectroscopy (BIS), BIS intracellular water content (panel b), and BIS extracellular water content (panel c). For all of these metrics, POST values were significantly greater than PRE and MID values. Significant main group and time effects as well as a group×time interaction was observed for lean body mass (panel d) assessed via dual x-ray absorptiometry (DXA). Post hoc tests indicated lean body mass increased within groups from PRE to MID (MALTO & GWP; *, $p < 0.05$), MID to POST (WP & GWP; *, $p < 0.05$), and PRE to POST (all groups; *, $p < 0.05$). However, no significant between-group differences existed at each level of time. A significant main time effect as well as a group×time interaction was observed for change scores DXA lean body mass corrected for change scores in ECW (panel e). Post hoc tests indicated this metric increased within groups from PRE to MID (MALTO &

GWP; *, p<0.05), and PRE to POST (all groups; *, p < 0.05). Additionally, MID WP was significantly lower than MID GWP (#, p = 0.004). Significant main group and time effects as well as a group×time interaction was observed for fat mass (panel f) assessed via DXA. Post hoc tests indicated fat mass decreased within groups from PRE to MID (GWP; *, p < 0.05), MID to POST (WP & GWP; *, p < 0.05), and PRE to POST (WP & GWP; *, p < 0.05). However, no significant between-group differences existed at each level of time. All data are presented as means ± standard error values, and values are indicated above each bar. Additionally, each data panel (except e) has delta values from PRE included as inset data. Abbreviations: MALTO, maltodextrin group, WP; standardized whey protein group; GWP, graded whey protein group

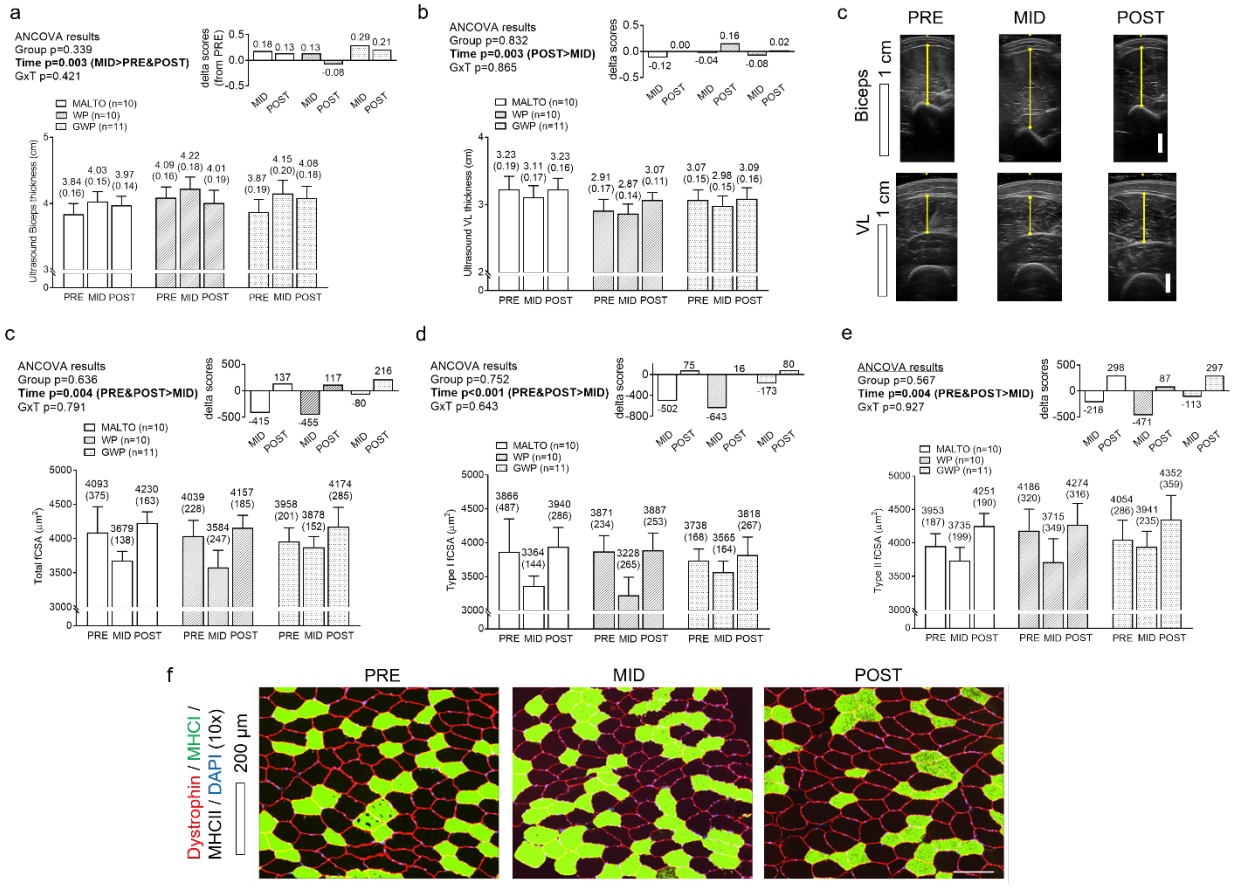
Muscle Thicknesses and fCSA

A significant effect of time was observed for bicep thickness where post hoc tests revealed a greater thickness at MID compared to PRE (p = 0.001) and POST (p = 0.040), but no significant group×time interaction was observed (Figure 4a). The largest effect occurred in GWP from PRE to POST (d = 0.39, -0.11 to 0.53 cm). A significant effect of time was also observed for VL thickness (p = 0.003) where post hoc tests revealed lower values at MID compared to POST (p < 0.001), and lower values at MID compared to PRE approached significance (p = 0.053; Figure 4b). However, a significant group×time interaction was not observed. The largest effect occurred in WP from PRE to POST (d = 0.30, -0.19 to 0.51 cm).

Significant reductions in VL total fCSA, type I fCSA, and type II fCSA were observed from PRE to MID (p = 0.045, p = 0.009, and p = 0.0410, respectively), followed by a significant increase from MID to POST (p = 0.004, p = 0.004, and p = 0.001, respectively) (Figure 4d-f). However, values in these metrics at POST were not significantly different from values at PRE, and no

significant group or group×time interactions were observed. The largest effect in total fCSA from PRE to POST occurred in GWP (d = 0.25, -286 to 719 µm2). Numerically equal effect sizes in Type II MHC fCSAs from PRE to POST were observed in WP and GWP, which exceeded the effects in MALTO (WP: d = 0.35, -442 to 617 µm2; GWP: d = 0.35, -207 to 803 µm2). The largest effects in Type I MHC fCSAs were observed in GWP (d = 0.09, -470 to 630 µm2).

Figure 4. Muscle thickness and VL fiber size differences between supplementation groups



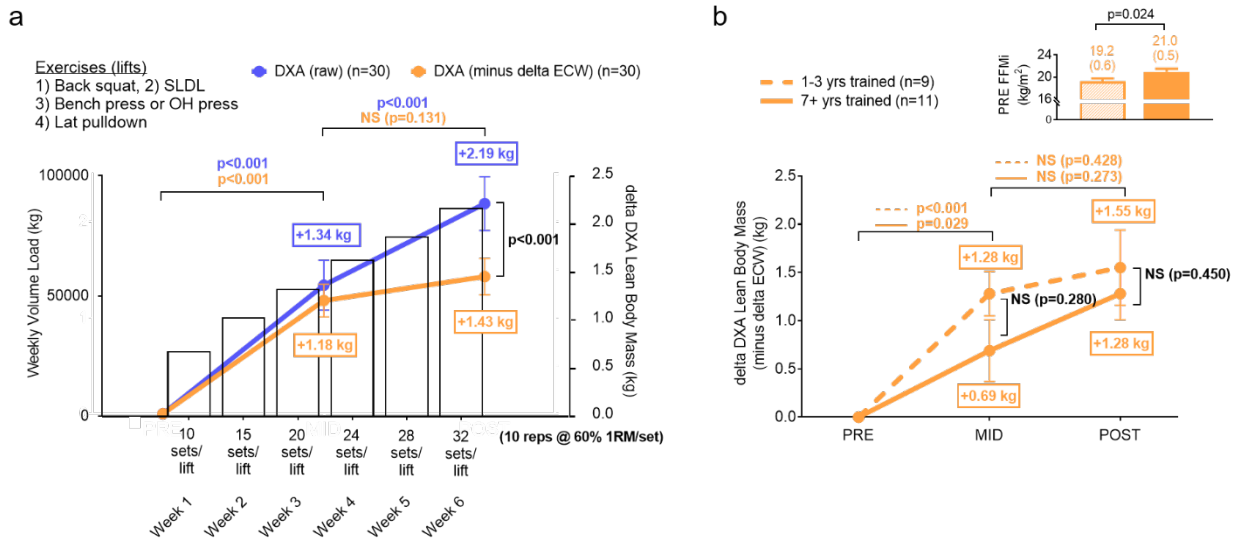
Legend: Only a significant time effect was observed for biceps thickness (panel a) assessed via ultrasound where MID values were greater than PRE and POST values. Only a significant time effect was observed for biceps thickness (panel a) assessed via ultrasound where MID values were less than POST values. Panel c provides representative images of ultrasound scans from the same subjects. Only significant time effects were observed for total fiber cross sectional area (fCSA) (panel d), type I fCSA (panel e), and type II fCSA (panel f) assessed via histology where MID values were less than PRE and POST values. Panel g provides representative 10x objective histology images from VL biopsies of the same subject. All data are presented as means ± standard error values, and values are indicated above each bar. Additionally, each data panel has delta values from PRE included as inset data. Abbreviations: MALTO, maltodextrin group, WP; standardized whey protein group; GWP, graded whey protein group

Training Volume versus change in DXA Lean Body Mass

As stated prior, we sought to examine the overall hypertrophic response independent of group given this is the highest RT volume investigated to date in 6 weeks. Interestingly, the significant increase in LBM from PRE to MID ($p < 0.001$) and MID to POST ($p < 0.001$) was proportional to the significant increase in training volume over time (Figure 5a). When correcting changes in LBM by subtracting changes in ECW (i.e., ECW-corrected LBM), we observed a similar increase across groups from PRE to MID ($p < 0.001$), but a non-significant increase from MID to POST ($p = 0.131$). Additionally, we decomposed these data into subjects reporting lower training ages (up to 3 years; $n = 9$) versus subjects reporting higher training ages (7+ years; $n = 11$) with the rationale being that training status may affect changes in ECW-corrected LBM (Figure 5b). Similar trends were observed in both cohorts whereby significant

increases in ECW-corrected LBM occurred from PRE to MID ($p < 0.05$), but not from MID to POST ($p > 0.05$).

Figure 5. Change in DXA lean body mass plotted against increases in training volume for all subjects



Legend: Data in panel a include DXA lean body mass changes (blue line graph), changes in LBM by subtracting changes in extracellular water (i.e., ECW-corrected LBM), and training volume (bar data) from all 30 subjects that underwent DXA and BIS testing. A significant increase in LBM from PRE to MID ($p < 0.001$) and MID to POST ($p < 0.001$) was observed in DXA LBM and this was proportional to the increase in training volume over time. When considering ECW-corrected LBM changes, a similar increase occurred across groups from PRE to MID ($p < 0.001$), but a non-significant increase from MID to POST ($p = 0.131$). Additionally, post DXA LBM was significantly higher than POST ECW-corrected LBM suggesting that raw DXA LBM scores may have been obscured edema or inflammation when > 20 sets per exercise per week were executed. In panel b, the ECW-corrected LBM data were decomposed into subjects reporting lower training ages (< 3 years; n = 9) versus subjects reporting higher training ages (7+ years; n = 11), and subjects reporting higher training ages possessed a greater fat-free

mass index at PRE (FFMi expressed as means ± SE; DXA LBM/height^2) which strengthens the self-reported training data. Similar trends were observed in both cohorts whereby significant increases in ECW-corrected LBM occurred from PRE to MID, but not from MID to POST.

DISCUSSION

Perhaps the most interesting finding of this investigation is the apparent dose-response relationship observed between RT volume and LBM changes corrected for alterations in ECW (Figure 5). It has been suggested a positive relationship between RT volume and skeletal muscle hypertrophy exists up to a certain point (42). A recent meta-analysis from Schoenfeld et al. (39) demonstrated significantly greater hypertrophic responses from completion of 10 sets per week of a resistance exercise emphasizing specific musculature compared to <5 sets per week. However, others have challenged this contention noting a plateau in the hypertrophic response beyond select RT doses but with comparatively less supporting evidence (25). Given these divergent viewpoints, we aimed to clarify hypertrophic responses to extreme RT volumes beyond those previously investigated in younger resistance-trained men. Our data indicate no clear plateau in RT-induced muscle mass increases when RT volumes are increased from 10 sets of 10 repetitions at 60 % 1RM per exercise per week up to 32 sets per week, and this interpretation stems from the significant increases observed in DXA LBM from weeks 1-3 and 3-6. However, when changes in DXA LBM were corrected for changes in ECW, a slightly different interpretation arises. Notably, subtraction of ECW changes from LBM changes were completed to better clarify changes in LBM unrelated to extracellular fluid retention possible

from tissue damage in context of these extreme RT volumes. In this regard, Yamada et al. (45) suggest expansions of ECW may be representative of edema or inflammation and can mask true alterations in functional skeletal muscle mass. Further, these authors suggest the measurements of fluid compartmentalization (e.g., ICW, ECW), which are not measured by DXA, are needed if accurate representation of functional changes in LBM are to be inferred. When considering the ECW-corrected LBM changes noted herein, week 1-3 increases were similar in magnitude to raw DXA LBM changes (+1.18 kg versus +1.34 kg, respectively). However, ECW-corrected LBM changes from weeks 3-6 were significantly lower than raw DXA LBM changes (+0.85 kg versus +0.25 kg, respectively), and we speculate this observation could be related to local inflammation or edema induced by increasing RT volume above 20 sets per exercise per week. Additionally, this phenomenon seemingly occurred regardless of training age. Consequently, it seems logical subjects were approaching a maximal adaptable volume beyond 20 sets per exercise per week. We are careful to generalize these findings across populations to avoid promotion of an assumed RT volume ceiling for eliciting hypertrophy since there is likely no "one size fits all" RT dose for eliciting a maximal hypertrophic response (5, 31). Rather, optimally dosing RT for hypertrophic outcomes should depend on the physiological status of an individual and particularly as it pertains to recent historical training (13). We feel moving toward a more dynamic model of RT dosing for eliciting maximal hypertrophic responses will require keen effort to characterize various biomarkers and monitoring methodology to help facilitate more objective, individualized dosing of RT. Therefore, characterizing individual responses and optimal dosing strategies of RT are particularly important at doses approaching and surpassing

these RT volumes since maladaptation and injury are potential consequences. These training considerations aside, we also agree with the conceptual basis suggesting the assessment of ECW changes during custom RT programs may better delineate changes in functional skeletal muscle mass.

Other interesting effects related to training (i.e., significant time effects) emerged from the current study. First, divergent adaptive responses in the bicep brachii and VL muscles assessed via ultrasound were observed, where increases in biceps thickness and decreases in VL thickness occurred from PRE to MID and the inverse effects occurred from MID to POST. While fiber type data in human biceps brachii muscle is lacking, Dahmane et al. (12) reported ~60% of fibers in the biceps brachii were type II, while ~40 % were type I. Herein, we observed the VL consisted of ~50% type II fibers, on average. Given that type II fibers typically hypertrophy to a greater extent in response to RT relative to type I fibers (14), the observed divergent responses in the biceps and VL muscle thickness measurements may be related to fiber-type distributions of these muscles. However, this hypothesis is speculative at best and more work is needed in determining how different muscle groups mechanistically adapt to high volume RT. Another striking observation was the PRE to MID decrease in VL thickness and fCSA values followed by the MID to POST increase in these metrics. Damas et al. (13) recently reported significant increases in muscle damage after a single bout of RT, followed by an attenuation of damage measured from a similar bout 3 and 10 weeks later. Additionally, while significant elevations in MPS were observed after each bout, significant increases in fCSA were only observed after 10 weeks. These findings led the authors to posit significant increases in muscle damage and MPB

from weeks 1-3 outpaced increases in MPS resulting in no significant increase in fCSA until the RT-induced damage response subsided from weeks 3-10. Relating these findings to our data, the initial atrophic VL muscle response during the first 3 weeks of training may have been due to high levels of muscle damage/MPB counteracting increases in MPS. However, during weeks 3-6, MPS levels may have outpaced muscle damage/MPB leading to increases in muscle thickness and fCSA. These findings are speculative given that we did not assess markers of muscle protein turnover, although it is interesting that our data agree with the model hypothesized by Damas et al.

Regarding the effects of GWP supplementation on body composition and fCSAs, we interpret the following as interesting and novel findings based on effect sizes: 1) the largest increases in DXA LBM and largest reductions in DXA fat mass were observed in GWP subjects, 2) the largest increases in bicep thickness were observed in GWP subjects, and 3) the largest fCSA increases from weeks 1-3 were observed in GWP subjects. Our observations related to muscle hypertrophy conceptually agree with prior literature examining the effects of single dose ingestion or longer-term supplementation with higher whey protein doses. For example, Macnaughton et al. (29) recently reported significantly greater MPS responses to a resistance exercise bout and whey protein ingestion when 40 g were consumed post-exercise compared to 20 g. Witard et al. (43) compared myofibrillar protein synthesis responses from ingestion of 40 g of whey protein to 0 g, 10 g, and 20 g in younger resistance-trained males and noted numerically larger, but not significantly different, responses from ingestion of 40 g versus 20 g, while 0 g and 10 g resulted in significantly lower responses. Regarding longer-term data, Cribb et al. (11)

reported previously-trained males consuming high doses of whey protein (i.e., 120 g/d for an 80-kg subject) experienced robust and significant increases in DXA LBM (+5.0 kg) compared to subjects consuming high doses of casein during 10 weeks of RT. Antonio et al. (3) reported ~2 kg increases in LBM (assessed via air displacement plethysmography) in a group of 20 subjects consuming ~4.4 g/kg/day of dietary protein over an 8-week period, much of which was supplemented via whey protein in the diet, compared to ~1.3 kg increases in LBM in another group of subjects consuming ~1.8 g/kg/day (11). Antonio et al. (2) conducted a follow-up investigation wherein a total of 31 subjects consumed ≥ 3 g/kg/d, and 17 subjects consumed their normal amount of dietary protein (1.8-2.3 g/kg/d) for 8 weeks while undergoing 5 days of RT per week. These authors reported both groups gained statistically equivalent amounts of LBM (+1.5 kg), however, the three highest hypertrophic responders in the study consumed ≥ 3 g/kg/d. Notably, some of these same studies have reported high-dose whey protein supplementation also promotes significant reductions in fat mass, and this seemingly agrees with our data supporting GWP for fat loss promotion. For instance, the abovementioned study by Cribb et al. (11) reported subjects supplementing with whey protein lost a significant amount of fat mass compared to casein-supplemented subjects (-1.4 kg versus +0.1 kg). Additionally, Antonio et al. (2) reported subjects consuming high amounts of protein lost significantly more fat mass relative to a lower protein intake group (-1.6 kg versus -0.3 kg). We are careful in adopting this interpretation, however, given that self-reported caloric intakes were numerically lower in GWP versus WP and MALTO subjects throughout the study. Hence, an alternative explanation of our

data could be that the observed loss in fat mass in GWP subjects occurred due to a higher caloric deficit relative to the other groups.

Greater disturbances of mood and higher levels of soreness occurred in MALTO, compared to subjects consuming either WP or GWP. The observation of better maintained mood states in whey protein-consuming subjects could be related to greater elevations in brain serotonin, since a decline in serotonin activity is involved in depressive mood (30). In this regard, Markus et al. (30) reported significant reductions in cortisol and depressive feelings under stress in subjects consuming a whey protein with high tryptophan content (a precursor of serotonin synthesis) compared to a placebo. Hence, it is possible that consumption of whey protein better maintained or increased brain serotonin levels and this could have affected mood disturbance scores. However, we are careful to speculate beyond our data given: 1) we did not explore in-depth mechanisms related to these variables, and 2) while effect sizes were greatest in MALTO, a significant group×time interaction was not observed. Regarding differences in soreness, a recent review by Pasiakos et al. (36) suggests this could be related to attenuated MPB and consequent reductions in prolonged soreness since greater amounts of exogenous amino acids were provided through supplementary sources reducing prolonged MPB and inflammatory-related processes.

Experimental considerations

Our study is limited in that only 31 subjects completed the intervention. As such, we were underpowered to detect small, but significant, effects and, as a result, a great deal of our findings were discussed in relation to effect sizes derived from mean differences and PRE-pooled

standard deviations rather than p-values derived from ANCOVA population-based inferential tests. Second, an unresolved limitation is that not all subjects adhered to the dietary self-reporting protocol. We felt that 2 to 4-day food logs would not entirely reflect what subjects consumed throughout the study. For this reason, we sought to implement a convenient and ecologically valid method of self-reporting dietary data which persuaded our utilization of daily mobile application entries. However, despite consistent verbal encouragement by research staff, only 60% of subjects were adherent. Thus, adopting strategies (e.g., additional monetary compensation on a per day entry basis) to increase mobile application self-reporting are needed moving forward. One methodological consideration is our reverence for DXA assessments reflecting whole-body muscle mass changes. While numerous forms of body composition assessment exist, recent technological advances in DXA persuaded its employment herein for measurements of body composition (38). Buckinx et al. (7) recently posited DXA as a reference standard (but not gold standard) method for measurement of LBM in research and clinical practice. As mentioned previously, our laboratory has observed excellent same-day reliability of the DXA during a test-calibrate-retest. Similar evidence also suggests DXA produces precise body composition readings (10, 15), and Aasen et al. (1) reported < 2% coefficients of variation for measurements of body composition between DXA and known phantom values. Notwithstanding, others have suggested a modest overestimation of fat mass using DXA compared to a 4-compartment model of body composition (37). Therefore, we acknowledge that DXA measurements of LBM and fat mass herein could have been under- or overestimated in an absolute sense. However, given the reliability of the measurement and the fact that subjects

served as their own controls in calculation of change scores, we feel DXA-related measurements were sufficient to reflect alterations in body composition. Finally, while a 6-week RT program seems rather abbreviated, we chose to implement this duration due to the concerns regarding subject safety. In spite of these limitations, we posit that our findings are novel in the sense that this is the highest implemented RT volume in humans to date in a 6-week timeframe and these findings point to the need to investigate dose-response relationships from greater than 6 weeks of RT involving a larger number of subjects.

CONCLUSIONS

Based on effect sizes, GWP subjects exhibited greater increases in DXA LBM, greater reductions in DXA fat mass, and a greater resiliency against reductions in VL fCSAs from weeks 1-3 with larger increases occurring from weeks 3-6 than both WP and MALTO subjects. These data imply a potential ergogenic role of graded whey protein consumption in context of graded increases in RT volumes (i.e., a proportional supplemental protein hypothesis). However, given that our study was limited in duration and subject number, this hypothesis needs to be clarified with longer-term interventions and larger sample sizes. Supplementation aside, the RT volumes investigated in this study are the highest formally studied in human subjects in a 6-week timeframe. Significant increases in LBM corrected for alterations in ECW were observed from weeks 1-3, although this response was dampened from weeks 3-6 suggesting that ~20 sets per exercise per week may approach a maximal adaptable volume in younger resistance-trained men.

ACKNOWLEDGEMENTS

Funding for subject compensation and assays were provided by a gift donation from Renaissance Periodization (Charlotte, NC, USA) and contract through Impedimed (Queensland, AU). We would like to thank the subjects for participating in this difficult training study. We would also like to thank Nick Shaw (CEO, Renaissance Periodization) for his generosity. Additionally, we would like to thank Dr. Rob Wildman from Dymatize Nutrition in coordinating the donation of nutritional supplement products. Finally, we would like to thank Dr. David Pascoe for his assistance with purchasing weight equipment and allowing us to use his laboratory space for testing procedures. We graciously acknowledge Casey Sexton, Jazzylyn Pepper, James Creel, Lauren Mussell, Brooke Anderson, Mclelland-Rae Johnson, Jacobs Brewer, Denico Jackson and JoAnna Roberts for their helpful assistance throughout the training portion of the study and/or data analysis.

CONFLICTS OF INTEREST

The results of the present study do not constitute endorsement by ACSM. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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