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The Effect of Protein Blend Supplementation and Resistance Exercise on Skeletal Muscle Growth and Adaptation

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The Effect of Protein Blend Supplementation and Resistance Exercise on Skeletal Muscle Growth and Adaptation

by

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Dedication

I dedicate this dissertation to my wife, Mollie, who provided me with constant support through our journey through life together.

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I owe all my life and talents to Jesus Christ, creator and reconciler of all. It is a humbling and awe inspiring experience attempting to gain the briefest glimpse into this vast world and complex beings that inhabit it.

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The goal of this project was to determine the efficacy of a new protein supplement to improve muscle growth and strength when ingested following exercise. The hypothesis is that novel supplementation of mixed dietary proteins (protein "blend") with different amino acid release profiles will prolong amino acid levels in the blood leading to increased muscle growth during resistance exercise-training (RET) as compared to a commonly used single protein source. I tested this hypothesis in the acute and chronic response to exercise and protein ingestion via two separate randomized double-blinded clinical trials. In the first clinical trial 20 young adults were randomly selected to ingest either the Blend or Whey protein 1 hour following high intensity resistance exercise. By combining a stable isotopic infusion with blood and muscle biopsy sampling I traced the acute post-exercise muscle protein synthesis response. In the second clinical trial I randomized ~60 young men into 3 supplement groups (Blend,Whey,Placebo) to undergo 12 weeks of RET. Outcomes of muscle mass and strength were measured pre/post 12 weeks of RET to determine efficacy and potential treatment differences in gains in muscle size and/or strength. To gain insight into potential mechanisms for these adaptations I assessed muscle protein expression, composition and RNA concentration.

I found that post-exercise protein blend supplementation was effective in stimulating post-exercise muscle growth following a bout of high intensity resistance exercise. This finding indicated that protein blend supplementation has a high potential to enhance muscle growth during resistance exercise training. I examined this further after chronic resistance exercise training and found that protein supplementation enhanced whole body lean mass gain, however this effect was not found in the leg, specifically, *vastus lateralis* muscle myofibers, indicating that this enhancement occurred in other areas, such as the upper body.

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CHAPTER 1

Introduction

(Pages 1-107 are part of a literature review on skeletal muscle and it's adaptability to exercise and amino acid nutrition.)

REGULATION OF PROTEIN METABOLISM

Human skeletal muscle protein metabolism is an intriguing and relevant area of investigation. The dynamic nature of this integrated system of physiology is challenged by the demands and consequences of human performance, nutrition, aging, inactivity and disease. Protein turnover is defined as the constant cellular processes of protein synthesis (using amino acids to make peptides and proteins) and protein breakdown (degrading proteins or peptides into amino acids) controlling the balance and quality of protein in a biological system. Examination into the nature of protein metabolism demonstrated that the proteins are subjected to constant chemical changes. Free-radical induced oxidation [1], glycation and random deamination of specific amino acids, among other modifications [2, 3] marks proteins and makes them more susceptible for degradation [1, 4]. By-products of these changes and the altered proteins themselves can impair protein function when they accumulate [5]. Accumulation of these damaged proteins impairs cellular function and if left unchecked, can lead to reduced function of the organ (such as muscle).

Therefore, skeletal muscle maintains muscle protein quality by recognizing and recycling damaged proteins to ensure proper physical function. Protein turnover is needed to keep the abundance and structure of specific proteins at the appropriate level and condition within in the correct time frame to effectively meet the next physiological

challenge. As we age this process is hampered by loss of motor neurons [6, 7], an accumulation of damaged proteins/DNA/lipids [3] and an impaired physiologic function [4]. Unfortunately, with aging, there is not an increase in protein turnover to replace these proteins [2, 8-10], suggesting desensitizing of the system and impairment in the defense/remodeling mechanisms. This is an important target of ongoing research.

An inequality between muscle protein synthesis and breakdown can lead to protein accrual/hypertrophy (e.g. exercise training and nutrition) or muscle loss (e.g. sarcopenia, inactivity, malnutrition and muscle wasting). Considering muscle contains approximately half of the body's protein, muscle loss is an important concern. Maintenance of muscle quality and mass is necessary for muscle to fulfill its adaptive roles in physical movement, energy metabolism, immunity and temperature regulation. Also, as the largest available protein source, skeletal muscle serves as a reservoir for water, minerals, vitamins and amino acids, which are essential in periods of stress.

Use of Stable Isotopes to Assess Muscle Protein Synthesis

Since Schoenheimer's use of isotopes and the gas-chromatograph mass spectrometry (GCMS) in 1943 to trace mammalian protein metabolism [11] methods have been developed to study whole body protein turnover using a pulse/bolus of tracer [12]. Later, the constant-infusion stable isotope technique utilizing a plasma plateau of infused tracer was adapted to study whole-body protein synthesis in humans [13, 14]. It was understood that various protein pools in the body have remarkably different rates of turnover, however, there was little information regarding the role of human muscle, which constitutes a large portion of total body mass. In 1975, Halliday and McKeran conducted the very first stable isotope study of human skeletal muscle [15]. It took 14 hours for their infused tracer, [^{15}N] Lysine, to reach steady state before they could start biopsy collection for the measurement of bound proteins to assess skeletal muscle

fractional synthetic rate (FSR). Several follow-up studies gave a small bolus of the tracer at the start of the infusion to "prime" the system and shorten the time for the tracer to reach steady-state [16-18]. The first study to define mixed-muscle protein synthesis, highlighted a more precise physiological role of muscle protein turnover and demonstrated that muscle contributes ~20-30% of whole body protein turnover [17]. The use of this technique was further complemented by development of a GCMS internal standard curve to improve the sensitivity and precision of this method [19, 20].

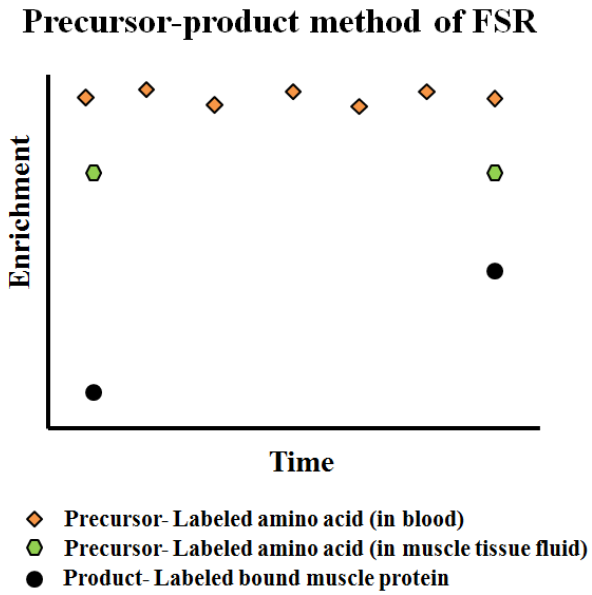
The precursor-product method is a central technique in the assessment of human protein turnover. This method for determining fractional synthetic rate (FSR) of a tissue or individual protein can be utilized in several forms - for a full review see [21, 22]. The most common form is the constant-infusion stable isotope technique for the assessment of human skeletal muscle FSR. The basic principle is that a stable isotope (tracer) is placed into the circulation and, at a steady-state level, it can be used to assess the rate that amino acids are incorporated into new proteins. FSR is calculated (**Figure 1.1.**) from the incorporation rate of a stable isotope such as L-[ring-$^{13}C_6$]phenylalanine into mixed muscle protein (the product, extracted from human muscle with a biopsy), and the free-tissue phenylalanine enrichment (the precursor) [21] (**Figure 1.2.**).

Figure 1.1. Equation for the calculation of muscle protein synthesis.

$$FSR\ (\%\ per\ hr) = \left\{ \frac{\frac{\Delta E_p}{t}}{\left(E_{m(1)} + E_{m(2)}\right)/2} \right\} \times 60 \times 100$$

Fig 1.1. $\Delta E_P/t$ is the slope of the straight line that fits the protein-bound phenylalanine enrichment across two sequential biopsies, *t* is the time interval encompassing the two biopsies and $E_{M(1)}$, and $E_{M(2)}$ are the phenylalanine enrichments (tracer/tracee) in the free muscle pool in the two biopsies. FSR in this case is presented as $\%{\cdot}h^{-1}$.

Figure 1.2. Schematic representation of the theory behind the precursor-product method of calculating muscle protein synthesis.



Over the past 40 years, the measurement of FSR has been used to study the effects of exercise, nutrition, aging and pathological conditions [2, 21, 23]. However, several assumptions and many different methodological approaches to the precursor-product method have resulted in divergent assessments of muscle protein synthesis leading to some confusion in the protein metabolism field [24]. Investigators have used diverse analytical techniques, several different tracers (e.g. phenylalanine vs. leucine), tracer labels ($^{2}H_5$, 1-^{13}C, $^{13}C_6$ to name a few), and precursors (enrichment in blood, muscle or tRNA) and varying amount of time between biopsies. All these methodological details are discussed elsewhere [21, 22, 24], but one of the most important facets to ensure validity of FSR is that the precursor enrichment must to be constant [25].

Regardless of the differences and variability in the literature, the ability to trace *in vivo* the muscle protein synthetic response *in vivo* has led to greater understanding of human muscle protein metabolism. The precision and sensitivity of this technique has enabled researchers to follow the effect of nutritional, exercise or other interventions on muscle. FSR is best used to assess change from an intervention and presents little

physiological and psychological trauma to subjects. Yet, the technique is limited in is its ability to make comparison across studies because of methodological differences. Comparison between groups is only valid if the two groups have similar pool sizes (muscle mass) of interest. Obviously, specialized procedures, training and equipment are needed to obtain, process and analyze the samples and assess FSR.

Protein Breakdown

The assessment of muscle protein breakdown (i.e. proteolysis) is needed along with FSR to calculate skeletal muscle protein turnover. However, due to either invasiveness and/or methodological/technological difficulties this outcome has been investigated less frequently and with less certainty [21]. Nonetheless, several approaches have attempted to estimate muscle protein breakdown by, 1) use of femoral-arterial balance across a limb using isotopic tracers to estimate endogenous release of AA from bound protein, 2) assessment of 3-methylhistidine release at the whole body level or across a limb or 3) more direct assessment of fractional breakdown rate (FBR) using muscle biopsies and endogenous dilution of tracer. These methods include the 3-pool (muscle, vein and artery) kinetic modeling or the various precursor – product methods (bolus, pulse or constant infusion) of FBR [21]. Disadvantages of these direct methods are that they can only be assayed over relatively short (<1h) time frames and present many more potential sources for analytical error compared to FSR.

BASIC MODEL OF SKELETAL MUSCLE ADAPTATION

Skeletal muscle comprises ~40-50% of the human body, by mass, and is the largest reservoir of protein/AA. Maintenance of muscle quality and mass is necessary for muscle to fulfill its adaptive roles in physical movement, energy metabolism, immunity and temperature regulation. Skeletal muscle is composed of numerous muscle fibers, (myofibers) organized in parallel via a series of connective tissues. The myofibers are connected in series and connected to bone at the origin and insertion points via tendons. Each fiber is multinucleated in order to maintain its long, but thin orientation. Regarding protein content, each myofiber contains ~50-60% myofibrillar protein, which is comprised, primarily, of actin and myosin, the two most abundant contractile proteins. These proteins run in parallel throughout the fiber anchored in structural repeats called sarcomeres, where the shortening and lengthening of muscle occurs.

A variety of stimuli (i.e. energetic, metabolite or ion flux, contraction or nutrients) or lack thereof, direct signal transduction to modulate a variety of molecular and cellular processes, mainly transcription of the genetic code and translation of the code into functional peptides and proteins. These events can initiate a myriad of processes, both anabolic and catabolic. It is generally thought that the immediate response to stimuli is to initiate signal transduction through post-translational modifications to signaling molecules in order to change the function of an enzyme or allow for translocation of a signaling molecule within a cell, etc. Often this is accompanied by processes allowing access to DNA and flux and activity of transcription factors in and out of the nucleus, which modulates the transcription of the genetic code to a single stranded nucleotide template called messenger mRNA or other RNA species. The mRNA template can be

directed toward ribosomal RNA / protein complexes where it is read (translated) directing the synthesis of peptides/proteins. The global level of mRNA or specific mRNA's is controlled by several RNA processing and stability mechanisms. The level of the translated proteins is further regulated by peptide/protein processing/stability and degradation processes.

The general process of muscle adaptation in response to exercise and nutritional interventions is thought to occur as a summation of alterations in these mechanisms over chronic exposure to stimuli. This is generally believed to initiate in a change in the level of specific mRNAs in the hours or 1-2 days following the stimuli, depending on the nature/novelty of the stimuli and the specific mRNAs being altered. An increase or decrease of a specific mRNA is generally thought to result in a gradual change in the protein encoded for that mRNA. However, a "law of diminishing returns" has left a mark in exercise biology demonstrating that as these events are repeated and adaptation occurs (i.e. the novelty of stimuli fades), signal transduction and transcriptional responses are attenuated, presumably due to improved ability or efficiency to respond to the stress stimulus, which may result in a new homeostatic set point and/or a genetically determined ceiling/restriction point.

ACUTE PHYSIOLOGICAL ADAPTATION TO RESISTANCE EXERCISE WITH AND WITHOUT PROTEIN AND/OR AMINO ACID FEEDING

In the past 30 years a dedicated effort has been made to study how an acute bout of resistance exercise (RE) can influence muscle protein metabolism during the early stages (hours to days) of post-exercise recovery. This early phase of adaptation in muscle protein metabolism involves a complex interaction of signal transduction, gene transcription, protein translation and protein degradation among many other changes [26]. The main focus will be to comprehensively examine the evidence characterizing the molecular and physiological response of human skeletal muscle growth and to determine whether muscle growth is enhanced when protein/amino acids (PRO/AA) are ingested in close proximity to acute RE or RE training. The evidence examining this physiological response measures protein metabolism *in vivo* with isotope tracers and mass spectrometry and investigates cellular mechanisms behind this response through the use of molecular techniques such as immunoblotting and qPCR. There are a host of transcriptional, translational and post-transcriptional responses to RE. We have tabulated all the available literature, to our knowledge, describing these responses in human skeletal muscle.

We now know that during these early stages (0-24hr) of post-RE recovery that muscle protein metabolism responds in several stages, which will be defined here as the immediate (0-1hr), intermediate recovery (1-6hr) and later (6-24hr) periods. The majority of the research in this area has focused on the immediate and intermediate responses; however, more recent studies, have descried the late adaptive periods.

The primary goal of skeletal muscle metabolism during RE is to maintain energy for contraction, which results in a reduction in the rate of the costly energic process of

muscle protein synthesis [27]. Yet it has also been suggested that muscle protein synthesis is reduced to divert the free amino acid pool to other fates (oxidation, etc) [27]. Regardless of the cause, this catabolic event of muscle metabolism results in drastic changes and flux of ATP, various ions (e.g. calcium, potassium, sodium, etc.) and metabolites (e.g. reactive oxygen and nitrogen species) among other changes that prompt decreased pH, increased blood flow and perfusion, glucose uptake, cell swelling, lactate release and amino acid flux [28, 29]. The mechanical (swelling, stretch) and various metabolic stresses (energy, metabolite, pH, RO/NS flux) during exercise are thought to initiate a complex web of signal transduction, gene transcription, translation and pre/post-translational changes throughout post-RE recovery. The duration, intensity/novelty and volume of RE have direct bearing on these responses [30-36].

The early muscle protein turnover response to RE is thought to be driven largely through translational and post-translational control [23, 37]. The increased translation of messenger RNA (mRNA) following RE is primarily controlled via the mechanistic target of rapamycin complex (mTORC1). This protein complex is a master growth regulator of translation initiation and elongation, among other processes and is activated following RE, through altered activity of several of its effectors, most prominently S6K1 (p70 ribosomal S6 kinase 1) (Table 1.1-1.3).

Signal Transduction Responses to Resistance Exercise in the Fasted and Fed State

Changes in the ADP:ATP ratio are known to direct AMPK activity, which then depresses anabolic action, partly through negative regulation of mTORC1 activity [27]. Although this action is well described in rodent models or during or shortly after aerobic exercise, its effects are less pronounced following RE in human exercise studies [29, 38, 39]. Dreyer et al. measured AMPK activity immediately after and in the first 2 hours following RE and found concomitant increases in AMPK activity and a decrease in muscle protein synthesis (MPS) during exercise [29]. Interestingly, during the post exercise recovery, muscle protein synthesis gradually increased even though AMPK activity was still increased, even in the presence of feeding [40]. In reviewing the literature, it appears that increased phosphorylation of AMPK does not always occur following RE in human skeletal muscle, however, in the few cases where this effect was present it only occurred in the immediate minutes post-exercise (**Table 1.1 & 1.2**). We did not observe an effect of PRO/AA on modulating post-exercise phosphorylation of AMPK in human skeletal muscle; however **Table 1.2** demonstrates a trend for less of an increase to be observed. Interestingly, phosphorylation of AMPK does not occur during the later time course post-exercise in young and older adults [9], yet when older adults are given a maximal post-exercise nutritional stimulus, 20g EAA, and demonstrate maximally activated MPS, they have prolonged and elevated phosphorylation of AMPK [41]. This may suggest that such a maximal stimulus of MPS is a novel and energy demanding process in older adults. A downstream target of AMPK, acetyl-CoA carboxylase (ACC) may be a better indicator of skeletal muscle energy status in human muscle (Eric Richter, personal communication).

The phosphorylation of an important upstream regulator of mTORC1, Akt (Protein Kinase B), has been extensively studied following RE (**Tables 1.1, 1.2, & 1.3**). Basic science models have linked Akt activation to muscle hypertrophy via contraction induced upstream activation via phosphatidylinositol 3-kinase and/or growth factors [42]. Akt can proceed to alter translation initiation via mTORC1 activation and/or GSK3-eIF2Bε. In the human literature, there is no clear pattern regarding the activation of Akt as many studies have not demonstrated increased phosphorylation, yet those studies that did show a change from resting values demonstrate a trend for an initial increase, concomitant with a rise in insulin, at Ser^{473} within 0-2 hours following RE followed by a decrease suggesting improved insulin sensitivity. Only 3 studies have demonstrated an effect of protein/AA on Akt phosphorylation at Ser^{473} over placebo [43-45] suggesting a minor or very transient effect of PRO/AA on this target. A downstream target of Akt signaling, glycogen synthase kinase 3 (GSK-3 α/β), is activated to modulate eIF2B as control point for global rates of protein synthesis via at the level of the 43S pre-initiation complex [46]. Phosphorylation of GSK-3 α/β only has been shown to be increased immediately post-exercise [47, 48], but does not appear to change in the post-exercise recovery period following RE regardless of feeding condition (**Tables 1.1, 1.2, & 1.3**).

Another upstream regulator of mTORC1, Tuberous Sclerosis Complex 2 (TSC2), provides an inhibitory role, until its phosphorylation allows for increased mTORC1 activity through interaction with Rheb binding [46]. The studies we found that probed for TSC2 phosphorylation in human skeletal muscle did not find increased phosphorylation as expected (**Tables 1.1, 1.2, & 1.3**), however, more recent data suggests that the Thr^{1462} phosphorylation site used in these studies may not be the ideal site to

asses TSC2 function (Troy Hornberger 2014 San Diego ACSM presentation). Thus re-examination of the role of TSC2 using the most appropriate phosphorylation site, is needed following RE in human skeletal muscle.

It is clear from a majority of the studies tabulated that phosphorylation of mTOR at Ser^{2448} is increased following RE in the fasted and fed conditions (**Tables 1.1, 1.2, & 1.3**). Because contraction and nutrient induced stimulation of mTORC1 are now thought to occur via independent mechanisms to illicit a synergistic response [49, 50] it would seem surprising to find that only a handful of studies demonstrate an additive effect of feeding on mTOR phosphorylation [45, 51-56]. However, the vast majority of studies investigating RE in the fed state demonstrate an elevation in post-exercise mTOR Ser^{2448} phosphorylation (**Tables 1.1, 1.2, & 1.3**). However, several studies, mostly from the same investigators [30, 32, 57-59], and a few others where feeding was not in close proximity to exercise [32, 60, 61] (e.g. were fed breakfast) did not demonstrate an increase in mTORC1 phosphorylation. However, it is now thought that the best readout of mTORC1 activity is via one it its effectors, p70 ribosomal S6 kinase 1 (S6K1), which may better reflect the additive effect of feeding. This kinase is partially activated at phosphorylation site $Thr^{421/424}$ and fully activated at phosphorylation site Thr^{389} [46]. A large mountain of evidence support post-RE activation of mTORC1 by demonstrating increased phosphorylation of S6K1 at Thr^{389} in the fasted condition and particularly with PRO/AA feeding. In the vast majority of studies containing a fasted (placebo) and PRO/AA fed groups, phosphorylation of S6K1 at Thr^{389} consistently demonstrates an additive effect of following RE [40, 43, 44, 51-56, 59, 62-66]. The activity of this target of mTORC1 is probably the best marker for the additive stimulation of PRO/AA in

human skeletal muscle. In support of this thesis, increased phosphorylation of the downstream target of S6K1, ribosomal protein S6 (rpS6) has also been demonstrated, albeit less frequently, with a similar effect at either $Ser^{240/244}$ or $Ser^{235/236}$ following RE in the fasted and particularly the PRO/AA fed condition [43, 51, 52, 55, 59, 67].

The increased phosphorylation of rpS6 and S6K1 are believed to prompt translation initiation (translational efficiency) and increase ribosomal biogenesis (translational capacity) [46, 68]. The increase in MPS following RE in humans has been suggested [69] and demonstrated to occur through increases in translational efficiency rather than translational capacity [37, 70], at least in the ensuing hours following one bout of RE. Based on these robust signal transduction events it was theorized that the repeated RE stimulus may gradually induce an increased translational capacity via cyclical regulation of ribosomal biogenesis. Since ribosomes constitute ~70-80% of the total RNA, investigators have assayed total RNA (ug RNA/ mg muscle) as a proxy for translational capacity. A few studies in human skeletal muscle have suggested that increases in total RNA are delayed since they are not seen at 2-6h [71] and 24h post-RE [72-74], but only after two exercise sessions [75, 76]. Increases have also been demonstrated 48hr post-exercise in the untrained, but not RE trained state in older men [77]. No effect at any time-point (acute or chronic) was observed in younger men [78] in a fasted or fed condition, however, others have shown increases in total RNA at 24h post-exercise [79]. Also, these same investigators have demonstrated that high responders have the largest changes in RNA 24h post-exercise in the untrained state [80], but following RET all participants demonstrate a similar ~40% increase in total RNA. In contrast to [80, 81], a comprehensive molecular investigation of RET adaptations has

suggested that enhancement of hypertrophy has demonstrated a down-regulation of ribosomal transcripts in high-responders to RET [82] which may suggest that hypertrophic adaptations to RET are most likely determined via the ability of high responders to improve translational efficiency rather than translational capacity [82].

Although pre-exercise feeding does not seem to impact total muscle RNA concentration [71], at least at 2 and 6h post-RE in young adults, there is little knowledge regarding changes in or the functional relevance of translational capacity in human skeletal muscle following PRO/AA feeding. Due to the robust additive stimulus that PRO/AA supposedly exerts on ribosomal biogenesis, it would seem intuitive that this would be an area modifiable by PRO/AA intervention. Indeed, translational capacity falls rather quickly in nutrient deprived conditions and tends to resist normalization [83-85]. It may be that responders to RET and/or PRO/AA demonstrate optimal translational plasticity via an enhanced interaction between translational efficiently and capacity. Future examinations should seek to determine the time course of translational capacity changes and the functional relationship between changes in total RNA and a physiologic outcome such as post-absorptive or post-exercise MPS.

Increased mTORC1 activity stimulates cap-dependent translation initiation through hyper-phosphorylation of 4E-BP1, which allows for a complex cascade of events leading to the binding of mRNA to the 43S pre-initiation complex and then the 48S pre-initiation complex, a rate limiting step in translation initiation [46]. Interestingly, the effect of RE in the fed or fasted condition on 4E-BP1 is less clear. This may stem from the lack of standardized methods or even a description of those methods across studies quantifying phosphorylation of 4E-BP1 and its various isoforms. This confusion is the

most probable reason why investigators have recently chosen to assay 4E-BP1 in its non-phosphorylated form [47, 86]. Even so, a strong trend suggests a decrease in 4E-BP1 activity in close proximity to the end of exercise [29, 40, 53, 87-90], especially in the fasted state. Also, a handful of studies demonstrate that hyper-phosphorylation gradually occurs within a few hours post-exercise [9, 34, 91] and is elevated the following morning [9, 30, 33, 92]. Some evidence suggests that exercise-induced activation of 4E-BP1 is altered with aging [9, 91] and modulated by exercise intensity/volume [30, 32-34]. In the fed condition, the immediate post-exercise induced hypo-phosphorylation is quickly removed and phosphorylation is increased with ingestion of PRO/AA has been observed [40, 45, 47, 54, 65, 67, 93]. In human skeletal muscle, phosphorylation of several other eukaryotic initiation factors, including eIF2Bε and the eIF4 family, have been examined following RE in the fasted and fed conditions. There is no clear consensus regarding the complex pattern of activation and time-course of these signals, however phosphorylation of eIF4E at Ser^{209} is up regulated following RE in most cases and in every case of PRO/AA feeding [47, 48]. These data taken together suggest that translation initiation is elevated following RE and an enhancement occurs with PRO/AA feeding in human skeletal muscle.

An alternate pathway to mTORC1, the mitogen-activated protein kinase (MAPK) pathway, is also upregulated by muscle contraction [33, 34, 41, 66, 86, 88-90, 94-105]. Many reports have demonstrated clear activation of several effectors (most commonly, ERK1/2 $Thr^{202/Tyr204}$, MNK1 Thr^{197}, p38 Thr^{180}/Tyr^{182} & p90RSK Thr^{573}) in this pathway within 0-1h post-exercise. Our compiled evidence suggests that the level and duration of activity of the MAPK signals is dependent on the novelty/intensity of the exercise [33,

34, 86, 101]. This occurs independent of whether the exercise was conducted in a fasted [32, 34, 55, 60, 87-90, 95, 97, 100-108] or fed [30, 32-34, 41, 55, 60, 61, 108-113] condition. It seems clear that trained individuals or those undergoing accustomed exercise do not illicit a response in this pathway suggesting activation of this pathway could be used as a marker of stress to unaccustomed exercise.

Generally, older adults have an attenuated signaling response to RE than young adults Tables (1.2, 1.2 & 1.3). This effect is seen with both mTORC1 [9, 31, 41, 91] and MAPK [41, 95, 114] signaling and is thought to be mediated by higher basal [95] and post-exercise levels of stress/inflammation [41, 95, 114-116] [117-120]. However, modification of exercise intensity/volume and also post-RE PRO/AA ingestion at higher doses is capable of restoring the mTORC1 signaling [9, 41, 56, 64, 121] to near maximal function. Interestingly, D'Souza et al. recently examined the post-RE dosing of whey protein on mTORC1 activity via S6K1 phosphorylation and discovered that the increase in S6K1 phosphorylation was correlated to changes in intracellular leucine [64]. This may represent a mechanism to by which mTORC1 can be activated to overcome anabolic resistance. However, 3 months of RET and whey protein supplementation attenuated the acute mTORC1 signaling response following a bout of RE and whey protein ingestion in older adults [56]. This data suggest a differential or adaptive role of mTORC1 signaling following resistance training.

CONCLUSION TO SIGNAL TRANSDUCTION SECTION

The overall conclusion from this compilation of evidence suggests that RE in the fasted state and that PRO/AA feeding enhances mTORC1 signaling in human skeletal

muscle. This is expected, since the main reason proposed for the increase in post-exercise MPS has been thought to be due to post-translational mechanisms activating translational initiation and elongation. However, these effects in signal transduction may be underestimated. Several investigators described an inherent variability in individual responses for certain markers of signal transduction and may have been underpowered to detect an effect. The exact reasons for these heterogeneous responses are not clear, but may be partially explained by exposure to unaccustomed exercise, extrinsic factors of the biopsy technique, dietary status and/or diurnal variation [86, 122]. Certainly, reasons for variability in the responses need further examination. It may be that some, but not all of these variable responses may underpin some of the divergent hypertrophic responses [82, 123]. The biological variability in these responses warrants further investigation. The activation or lack thereof and variability of select markers in these signal transduction pathways have yielded some inherent frustration. We are only just beginning to gain glimpses into the dynamic network of signal transduction and the biopsy technique can only capture a brief snapshot of a handful of markers in these pathways. With our limited observations it would seem imprudent to be over declarative or dismissive of the relevance of the signaling events occurring in human skeletal muscle. It is important to note that although a tremendous body of in work in cell, animal and human models has been complied over the past few decades; we still only have a limited understanding of these signaling events specific to the unique conditions in which they were studied. The good news is that there is vast opportunity and significant challenge for investigators to further unravel an extremely complex and beautifully designed dynamic network of signal transduction.

Table 1.1. Summary of intracellular signaling in vastus lateralis following acute resistance exercise conducted in fasted state, untrained and trained young humans.

Reference	SetsxReps; Mode	Intensity: Training Status	Norm	Time course (PEx)	AMPK	TSC2	Akt		mTOR	S6K1		4E-BP1	eEF2	rpS6		eIF4E	eIF2Bε	GSK-3a/β	ERK1/2	MNK1	p38	p90RSK	OTHER
Phosph Site (T=Threonine, S=Serine, Ty=Tyrosine)					T 172	T 1462	T 473	T 308	S 2448	T 389	T421/S424	T 37/46	T 56	S 240/244	S 235/236	S 209	S 539	S9	T202/Ty 204	T 197	T180/Ty 182	T 573	
Overal Pattern					↔↑	↓↔↑	↔↑	↓↔↑	↔,↑↑	↔,↑↑	↔,↑↑	↓↔↑	↓↔	↔↑	↔↑	↔, ↔↑	↓↔↑	↔	↔↑	↑	↑	↑	
Willoughby et al (2001, 2002)	3 leg Ex; 3x8-10	75-80% 1 RM		Rest, 30m, & 6h					-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑6 MyoD, ↑ SAPK/JNK
Thompson (2003)	50 Ecc contrations	biceps		48 hours	–	–	–	–	–	–	–	–	–	–	–	–	–	–	↑	–	–	–	
Williamson et al. (2003)	310 KE Young	70% 1RM, 3 min rest	T/PonS	0	–	–	–	–	–	–	–	–	–	–	–	↔	–	–	↑	↑	↔	↑	↔ SAPK/JNK
Karlsson et al. (2004)	4x10; LP	8x10; LP & 8x10; KE	none	0, 1 & 2h	–	–	–	–	–	↔	↑ 0, 2	–	–	↔	↔	–	–	–	↑ 0	–	↑ 0	–	-
Creer et al. (2005)	3x10; KE H-CHO	70% 1RM	T/PonS	0, 10 m	–	–	↑ 10	–	↑↔	–	–	–	–	–	–	–	–	–	↑ 10	–	–	↑ 10	-
	3x10; KE L-CHO				–	–	↔	–		–	–	–	–	–	–	–	–	–	↑ 10	–	–	↑ 10	-
Coffey et al. (2005)	8x5; KE ST	Maximal	None	Rest, 0, 3h	↔	↔	↔	–	–	↔	–	–	–	–	↔	–	↑3?	–	↑0	–	↔	-	↔PGC1α
	8x5; KE ET				↑0, 3	↓	↔	–	–	↑3	–	–	–	–	↑0	–	↓3?	–	↑0	–	↑0, 3	-	↔PGC1α
Dreyer et al. (2006)	11x10; KE	70% 1RM	None, T↔	Rest, 0 1 & 2h	↑ activity	↓ 1	↑	–	↑	↑ 2	–	↓EX, ↔1,2	↓1,2	–	-	–	–	–	–	–	–	–	-
Eliasson et al. (2006)	4x6; Con LP	Maximal	show T	0, 1 & 2h	–	–	↔	–	↔	↔	↔	–	–	–	↔	–	–	–	–	–	–	–	-
	4x6; Ecc LP				–	–	↔	–	↑↔	↑	↑	–	–	–	↑0,1,2	–	–	–	–	–	–	–	-
Deshmukh et al. (2006)	8x5; KE	Maximal	none	rest,imed post			↔	↔	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	60 m cycle	70% VO2 peak: ET					↑	↔	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Koopman et al (2006)	8x10; LP, 8x10; KE	75% 1RM: ET	a-actin, P/T	0, 30m & 2h	↑0	–	–	–	–	–	↑ (30)	↓,↔,↓	–	–	↑↔	–	–	–	–	–	–	–	-
Fujita et al. (2007)	1x30, 3x15; KE	20% 1RM 30s rest	Std, T↔	rest,3h	–	–	↔↑	–	↔	↔	–	–	↓	–	–	–	–	–	–	–	–	–	-
	W/ BFR				–	–		–	↔	↑	–	–	↓	–	–	–	–	–	–	–	–	–	-
Dreyer et al. (2008)	10x10; KE	70% 1RM	Std, T↔, PonS	1,2h	–	↔	↑1	–	↑	↑	–	↔↓ex	↔ex,↓1, 2	–	–	–	–	–	–	–	–	–	-
Drummond et al. (2008)	8x10; KE	4x10 (80%), 4x15 (65%); LP		1, 3 & 6h	↔	–	↑ 3	–	↑	↑	–	↑ 3, 6	~↓ 3,6	–	–	–	–	↓ 6	↑ 1	↑ 1	–	–	↓ 1 eIF2Bα
Harber et al. (2008)	6x10 KE	70% 1RM		1 & 2h	↔	–	–	–	–	–	–	–	–	–	–	–	–	–	↑	–	↑ 1	–	↑ 1 SAPK/JNK
Glover et al. (2008)	4x10; LP, 4x10; KE	10RM	sT	6h	↔	–	↑	–	↔	↑	–	–	–	↑	↑	–	↓	↔	–	–	–	–	↔FAK
Mascher et al. (2008)	4x10, LP	~80% 1RM	sT	15 m, 1, 2h	–	–	↔	–	↑ ↔Ser2481	↑	↑	–	↓	–	↑1	–	–	↔	–	–	–	–	-
		2nd day			–	–	↑↔	–	↑&Ser2481	↑↑	↑↑	–	↓↓	–	↑	–	–	↔	–	–	–	–	-
Deldicque et al. (2008)	10x10; LE	80% 1RM	sT	rest, 0, 24h	–	–	↓0,↔24	↓	-	↑24	↑	↓0,↔24	↔	–	–	–	–	–	↑ 0	–	↑ 0	–	-
Deldicque et al. (2008)	10x10; LE	80% 1RM	sT	rest, 0, 24,72 hr	-	-	↔	↓0	-	↔	-	↓0	-	-	-	-	-	-	↑↔	-	↑	-	↑MEF2
	10x10; LE + creatine				-	-	↔	↓0	-	↔	-	↓0, ↔	-	-	-	-	-	-	↑↔	-	↑	-	↑MEF2
Spiering et al. (2008)	5x5KE	90-95% 1RM: active	T w/ ELISA	Rest, 10m,3h	↑10		↔		-	↔↑ 3	-	↓10	-	-	-	-	-	↔	-	-	-	-	-
	4x10 4 upper body + lower	90-95% 1RM, 10RM: active			↑10		↔		-	↔	-	↓10	-	-	-	-	-	↔	-	-	-	-	-

Reference	SetsxReps; Mode	Intensity: Training Status	Norm	Time course (PEx)	AMPK	TSC2	Akt		mTOR	S6K1		4E-BP1	eEF2	rpS6		eIF4E	eIF2Bε	GSK-3a/β	ERK1/2	MNK1	p38	p90RSK	OTHER
Phosph Site (T=Threonine, S=Serine, Ty=Tyrosine)					T 172	T 1462	T 473	T 308	S 2448	T 389	T421/S424	T 37/46	T 56	S 240/244	S 235/236	S 209	S 539	S9	T202/Ty 204	T 197	T180/Ty 182	T 573	
Overal Pattern					↔↑	↓↔↑	↔↑	↓↔↑	↔,↑↑	↔,↑↑	↔,↑↑	↓↔↑	↓↔	↔↑	↔↑	↔,↔↑	↓↔↑	↔	↔↑	↑	↑	↑	
Drummond et al. (2009)	8x10; KE	70% 1RM	p/T	1,2h	–	–	–	–	↑, ↔2	↑	↑ 1,2	↓1	↓2	↑1	↑1	↑	–	–	↑	↑ 1,2	–	–	↑ eIF4G (Ser1108)
	rapamycin				–	–	–	–	↔,↑2	↔,↑2	↔	↔	↔	↔,↑2	↔,↑2		–	–	↔	↔	–	–	
Fujita et al. (2009)	11x10; KE	70% 1RM	Std, T↔	0, 1 & 2h	↔	–	↔	–	↑1,2	↑1,2	–	↓ 0	↓	–	–	–	–	–	–	–	–	–	-
Mayhew et al. (2009)	3sets each LP, KE, S	8-12 RM	PonS?	rest, 24h	–	–	↑	–	–	NM	↑↔	↑	–	↔	–	↑	–	–	–	–	–	–	↔ eIF4G (Ser1108)
Kumar et al. (2009)	3x9 (60%), 3x8 (75%), or 6x3 (90%); LE	60-90% 1RM (groups mixed)	GABDH ?	rest,10m,1,2&4h	–	–	–	–	–	↑1	–	↑1	↔↓10, ↑1	–	–	–	–	–	–	–	–	–	-
Coffey et al. (2009)	8x5 LE, 30m cycle	80% 1RM, 70% Vo2peak	α-Tub	15m post 1st EX, 15m post 2nd & 3h last	↔	↑3	↔	–	↓3	↑ 1x, 2x	↔	–	–	–	↑ 1x	–	–	–	–	–	–	–	-
	30m cycle, 8x5 LE				↔	↔	↔, ↑ 2x	–	↔	↑ 1x	↔	–	–	–	↑ 1x	–	–	–	–	–	–	–	-
Coffey et al. (2009)	8x5 LE, 10x6s sprints	80% 1RM, max, 54 sec rest	α-Tub	15m post 1st EX, 15m post 2nd & 3h last	↔	↔	↔	–	↔	↑ 1x	–	–	–	–	↑ 1x	–	–	–	–	–	–	–	-
	10x6s sprints, 8x5 LE	max 54 sec rest, 80% 1RM			↔	↔	↔, ~↑ 2x	–	↔	↔	–	–	–	–	↔	–	–	–	–	–	–	–	-
Holm et al. (2009)	10x36, U-KE	16% 1RM	none, T↔	rest,0.5, 3 & 5h	↑ 0.5	–	↑0.5	–	–	↔	–	↔	↔	–	–	–	–	–	↔	–	↔	–	-
	10x8, U-KE	70% 1RM			↑ all	–	↑ 30m, 3	–	–	↑↔	–	↑3	↔	–	–	–	–	–	↑0.5, 3	–	↑0.5	–	-
Hulmi et al. (2009)	5x10; LP (n=9)	10RM (~75% 1RM)	s PonS	rest, 1,48h, 21wk (3d)			↓21		↔	↑1	-	↔↓1	↔	-	↑1	-	-	-	-	-	-	-	↓1 Myostatin, ↔AR or ↓AR, ↓ total (AKT,p70S6k1
	(n=11) control	none					↔		↔	↔	-	↔	↔	-	↔	-	-	-	-	-	-	-	
Farnfield et al. (2009)	3x12; KE cybex	Maximal	none?	rest, 2,4 & 24h			↔		↔ 4?	↔ ~↑ 2	-	↔	-	-	↔ ~↑ 4	-	-	-	-	-	-	-	-
Dreyer et al. (2010)	11x10; KE	70% 1RM	β-tub?	0, 1 & 2h	–	–	↑1	–	↑	↑	–	↔	↓	–	–	–	–	–	–	–	–	–	-
Camera et al. (2010)	8x5; LE	80% 1RM	α-Tub	0, 15, 30 & 60 m	↔	↔	↑ 30,60	↑ 30,60	↑ 30	↑ 60	–	↔↑ Thr70	–	–	–	–	↔	↔	–	–	–	–	↓ GS (Ser641)
Apro and Blomstrand (2010)	4x10 (80%), 4x15 (65%); LP	80% and 65% 1RM	none	rest, 0 & 1h	↔	–	↔	–	↑	↔	–	–	↓1	–	↑↑	–	–	–	–	–	–	↑	↔PKD1
	no ex				↔	-	↔	–	↑	↔	-	-	↓1	-	↑	-	-	-	-	-	-	↑	↔PKD1
Burd et al. (2010) Fed breakfast	4x5 to fail	90% 1RM	α-actin, p/t	rest, 4, 24h	–	–	↑ 24	–		↔	–	↑24	↔	–	–	–	–		↔	–	–	-	-
	4x~14 to WM	30% 1RM			–	–	↑ 24	–	↑4	↔	–	↔	↔	–	–	–	–	–	↔	–	–	-	-
	4x~28 to fail	30% 1RM			–	–	↔	–		↑ 4	–	↑4,24	↔	–	–	–	–	–	↑ 4	–	–	-	-
Hulmi et al. (2010)	5x10; LP	10RM (~75% 1RM)	α-actin, PonS	rest, 0.5h	–	–	–	–	↑↔, ↔Ser2481	↑	↑↑	–	–	↑↑	↑↑	–	–	–	↑	–	↑	-	-
	15x1; LP	1RM			–	–	–	–		↔	↑	–	–	↑	↑	–	–	–	↔	–	↑	-	-
	1x6, LP				↔	–	↔	–	↑	↔	–	–	–	–	↔	–	–	–	↔	–	↑	-	-
Terzis et al. (2010)	3x6 LP	6RM	sT, ↔	rest, 0.5h	↔	–	↔	–	↑	↑	–	–	–	–	↑	–	–	–	↔	–	↑	-	-
	5x6; LP				↔	–	↔	–	↑	↑↑	–	–	–	–	↑↑	–	–	–	↔	–	↑	-	-

Reference	SetsxReps; Mode	Intensity: Training Status	Norm	Time course (PEx)	AMPK	TSC2	Akt		mTOR	S6K1		4E-BP1	eEF2	rpS6		eIF4E	GSK-3a/β	ERK1/2	MNK1	p38	p90RSK	MuRF-1	MAFbx	Foxo3a	STAT3	OTHER
Phosph Site (T=Threonine, S=Serine, Ty=Tyrosine)					T 172	T 1462	T 473	T 308	S 2448	T 389	T421/S424	T 37/46	T 56	S 240/244	S 235/236	S 209	S 9	T202/Ty204	T 197	T180/Ty182	T 573					
Overal Pattern					↔↑	↓↔↑	↔↑	↓↔↑	↔,↑↑	↔,↑↑	↔,↑↑	↓↔↑	↓↔	↔↑	↔↑	↔,↔↑	↔	↔↑	↑	↑	↑	↔↑	↔	↔	↑	
Glynn et al (2010)	1h fasted	10x10; KE 70% 1RM	Std, T↔	rest, 1h	↑	–	↑	–	–	–	–	–	–	–	–	–	–	–	–	–	–	↑	↔	↔	-	↔, LC3B1/2, Foxo3a
Reitelseder et al. (2011)	10x8, U-KE	80% 1RM	p/t, GABDH	rest, 1, 3 & 6h	–	–	↔	↔	–	↔, ↑time	–	↔, ↑6	–	–	–	–	↔	–	–	–	–	-	-	-	-	-
Borgenvik et al. (2011)	3x5 warm, 4x10, U-KE	80% 1RM	α-Tub	rest, 1&3h	-	-	-	-	↑1,3	↑1,3	-	↔	-	-	-	-	-	-	-	-	-	↑	↔	-	-	-
	non-ex	N/A			-	-	-	-	↑1,3	↑1,3	-	↔	-	-	-	-	-	-	-	-	-	↑	↔	-	-	-
		Unilateral; 70% 1RM, Normoxia			↔	-	↔	-	↔	↑	-	↔	↔	-	-	↔	-	↔	-	-	-	-	-	-	-	↔REDD1
Etheridge et al. (2011)	6x8; Young	Unilateral; 70% 1RM, Hypoxia	β-actin	0,3.5h (NR)	↔	-	↔	-	↔	↑	-	↔	↔	-	-	↔	-	↔	-	-	-	-	-	-	-	↔REDD1
Drummond, 2010; Fry et al. (2011) walker 2012	10x10 Young	70 % 1RM, 3 min rest	sT	0, 3, 6, 24	↔	-	↑ 3, ↑24	-	↑3, 6, 24	↑3, 6, 24	↑ 6, 24	-	-	↑3, 6, 24	↑3, 6, 24	-	-	↑ 6, 24	-	-	-	-	-	-	↑3,6	↑3,6,24; ↔eIF2Bα (Ser52) SNAT2;
Roschel et al. (2011)	Slow (20deg/s): 5x8reps Ecc	Maximal: active	Total	rest, 0 & 2h	-	-	↑0,2	↑0,2	↑0	↑0,2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Fast (210deg/s): 5x8reps Ecc				-	-	↑0,2	↑0,2	↑0	↑0,2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kumar et al (2012)	3x14 (40%)	40% 1RM	none	10m, 1, 2 & 4h	-	-	-	-	-	↑↔1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3x8 (75%)	75% 1RM			-	-	-	-	-	↑1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6x14 (40%)	40% 1RM			-	-	-	-	-	↑↔1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6x8 (75%)	75% 1RM			-	-	-	-	-	↑1,2,4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Farnfield et al. (2012)	3x8; U-KE cybex	Maximal, UT	p/t	rest, 2h	-	-	↔	-	↔	↔	-	↔	-	-	↔	-	-	-	-	-	-	-	-	-	-	-
		Maximal, TR 12wk			-	-	↔	-	↔	↔	-	↔	-	-	↔	-	-	-	-	-	-	-	-	-	-	-
Camera et al. (2012)	8x5; LE	80% 1RM (low Gly)	α-Tub	0, 1, 4h	↔	-	↔	-	↔	↔	-	-	-	-	↑ 1,4	-	-	-	-	-	-	-	-	-	-	-
		80% 1RM (norm Gly)			↔	-	↑ 1	-	↑ 1,4	↑ 1,4	-	-	-	-	↑ 1,4	-	-	-	-	-	-	-	-	-	-	↔eIF4G (Ser1108)
Burke et al. (2012)	8x10; KE	80% 1RM, RT	α-Tub	rest, 1, 5h	-	↔↑~	↑ 1	-	↔↑~	↔	↑ 1	-	-	-	↔↑~	-	-	-	-	-	-	-	-	-	-	-
Gundermann et al. (2012)	1x30, 3x15 w/ BFR	20% 1RM 30 sec rest	none	rest,1,3h	-	-	-	↑ 1	↑ 1,3	↑ 3	-	↔	↔	↑ 1,3	-	-	-	~↔↑3	↑ 3	-	-	-	-	-	-	-
	1x30, 3x15 w/ SNP				-	-	-	↔	↔	↔	-	↓3	↔	↔	-	-	-	↔	↔	-	-	-	-	-	-	-
Taylor et al. (2012)	4x18-20	60-65% 1 RM	ELISA	Rest, 0, 2 & 6h	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	-	-	-	-	-	-	-	↑ IRS-1, ELK-1 (0,2,6); MEK1 (0,2)
	4x8-10	80-85% 1 RM			-	-	-	-	-	-	-	-	-	-	-	-	-	↑	-	-	-	-	-	-	-	↑ IRS-1 (2,6);ELK-1; MEK1 (0,2)
Apro et al. (2013)	RE: 4x8-10, 4x10-12, 2x fatigue	85%,75, 65% 1RM	α-Tub	rest, 1,3h post RE	↓3	-	↓	-	↑	↑,1,↑↑3	-	↓1	↓	-	-	-	-	↔	-	↑↑1,↑3	-	-	-	-	-	↔CaMKII; REDD1 ↔↑
	RE+AE: 4x8-10, 4x10-12, 2x fatigue+ 30 m cycle	85%,75, 65% 1RM; 70% VO2 max		rest, 1,3h post RE (15m, 165m post AE	↓3	-	↔	-	↑	↑,1,↑↑3	-	↓1	↓	-	-	-	-	↔	-	↑↑1,↑3	-	-	-	-	-	

Reference	SetsxReps; Mode	Intensity: Training Status	Norm	Time course (PEx)	AMPK	TSC2	Akt		mTOR	S6K1		4E-BP1	eEF2	rpS6		eIF4E	GSK-3a/β	ERK1/2	MNK1	p38	p90RSK	MuRF-1	MAFbx	Foxo3a	STAT3	OTHER
	Phosph Site (T=Threonine, S=Serine, Ty=Tyrosine)				T 172	T 1462	T 473	T 308	S 2448	T 389	T421/S424	T 37/46	T 56	S 240/244	S 235/236	S 209	S 9	T202/Ty204	T 197	T180/Ty182	T 573					
	Overal Pattern				↔↑	↓↔↑	↔↑	↓↔↑	↔,↑↑	↔,↑↑	↔,↑↑	↓↔↑	↓↔	↔↑	↔↑	↔,↔↑	↔	↔↑	↑	↑	↑	↔↑	↔	↔	↑	
Vissing et al. (2013), Lamon et al. (2013), Moller et al. (2014)	4x12 of 3 thigh Ex	12RM: after 10wk Training	GABDH, total?	Rest, 0, 2.5,5,22h	↑0	-	↔	↔	↑2.5,5,22	↑5	-	-	-	-	-	↑5	↑0	↔	-	↑ 0;↓2.5,5	-	-	-	-	-	↔ in STARS proteins, IR(Tyr1361), IRS1(Tyr612); ↓ TSC1(Ser511)@22
	2h cycle	60% VO2 peak: after 10wk Training			↑0	-	↔↑,↓5	↔	↔	↔	-	-	-	-	-	↔	↔	↔	-	↔	-	-	-	-	-	
	Control: rested for 2 h	-			↔	-	↔	↔	↔	↔	-	-	-	-	-	↔	↔	↔	-	↔	-	-	-	-	-	
Trenerry et al. (2007), Della Gatta et al. (2014)	3x8-12; 3 leg Ex	~80% 1RM	Total, EL	Rest, 2,4,24h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑2	↑ IL-6 (2), IL-8 (2), MCP-1 (2&4h)
Della Gatta et al. (2014)	3x12; KE	Maximal: Young	ELISA	Rest, 2h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑ IL-6,IL-8, MCP-1; IL-10 ↑PT;↔IL-13, TNFa ND
Gundermann et al. (2014)	1x30, 3x15 w/ BFR	20% 1RM 30 sec rest	none	rest,3,6,24h	-	-	-	-	↑3,6,24	↑3,6,24	-	-	-	-	-	-	-	~↔↑24	↑24	-	-	-	-	-	-	-
	1x30, 3x15 w/ BFR + Rapamycin				-	-	-	-	↔	↓6,24	-	-	-	-	-	-	-	↔	↔	-	-	-	-	-	-	-	-
Stefanetti et al. (2014)	4X12, 3Ex	12RM: TR 10Wk	GABDH	Rest, 0,2.5,5 & 22h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↔	-	↔	-	↔FOXO1,EIF3F,MHC, MyoD,MyoG, PKM; ↓22 FBXO4O
Stefanetti et al. (2014)	10YM	3x14, 60% 1RM	GABDH	Rest, 2h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↔	↔	↔	-	↔FOXO1,EIF3F,MHC, MyoD,MyoG
Ternerry et al (2010)	3x12; KE	Maximal: TR	T	rest, 3h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	↑ IL-6, PDGF-BB,VEGF
		Maximal: UT			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	
Vella et al. (2012)	3x8-12, KE	~80% 1RM	T	rest,2,4h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	p65↑2, IkBa↓2
Areta et al. 2014	6x8; KE; energy decifit	80%1RM, TR	α-Tub?	Rest, 1,4	↔	-	↑1	-	↔	↔	-	↔	↔	-	↑4	-	-	-	-	-	-	-	-	-	-	-
Camera et al. 2014	8x5; KE & 30m cycle	80% 1RM, 63% cont, peak power output	α-Tub	Rest, 1,4h	-	-	↔	-	↑1,4	↔	-	-	↓1	-	-	-	-	-	-	-	-	-	-	-	-	-
Ferreira et al. (2014) (placebo)	4x8-12 LP, LE	~75-80 % 1RM, 2.5 min rest	ELISA	Rest, 0.5, 2 & 6h	-	-	↑0.5,2	-	↑0.5,2	↑6	-	↔	-	-	-	-	-	-	-	-	-	-	-	-	-	IRS-1↔
Markworth et al. (2014)	3x8-10 Squat, LP, KE + Ibuprofen	~80% 1RM. 1 min rest	Total	Rest, 0,3, 24h	-	-	~↔↑3.24	-	-	↑3.24	↑0	-	-	↑3	↑3	-	-	↔	↔	↑0	-	-	-	-	↑3	↑ RSK (0)
	3x8-10 Squat, LP, KE + Placebo				-	-	~↔↑3.24	-	-	↑3	↑0,3	-	-	↑3	↑3	-	-	↑0,3,24	↑0,3	↑0	-	-	-	-	↑3	↑ RSK (0)
Kakigi et al. (2014)	6x4; KE	Max	P/T	rest, 1h	-	-	↔1	-	↔1	↑1	-	↓1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D'Souza et al. (2014)	3x8-10 Squat, LP, KE	~80% of 1RM, Untrained	ERK1/2	Rest, 2 & 4h	-	-	-	-	-	↔	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: Signaling molecules were recorded above if included in two or more studies. Arrows denote direction of phosphorylation: Signaling molecules were recorded above if included in two or more studies. Arrows denote direction of phosphorylation. ↑, significantly increased; ↓, significantly decreased; ↔, no change; ↔↑, trend to increase; ↔↓, tend to decrease; ↔. Red color arrows represent a group diference. Blue arrows represent an effect of feeding. Arrows reresent change from rest (where available), Underlined arrows indicate a change from the fed condition. Underlined exercise sets, reps and mode, represents an aging comparison. RM, repetition maximum; LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Ecc, eccentric contractions; Con, concentric contractions; BRF, Blood flow restriction; O, old; Y, young; M, men; W, women; h, hour; m, minutes; s, seconds; RT, resistance trained, SNP, sodium nitoprusside; RE, resistance exercise; ST, strength trained; ET, endurance trained.LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Si, Single leg; TR, Trained; UT, Untrained; α-Tub, alpha-Tubulin; sT, show total protein but not correct for.

Table 1.2. Summary of intracellular signaling in vastus lateralis following acute resistance exercise conducted in the fed state, untrained and trained young humans.

Reference	Feeding	SetsxReps;Mode	Intensity:TR Status	Norm	Time course (PEx)	AMPK	TSC2	Akt		mTOR	PRAS40	S6K1		4E-BP1	eEF2	rpS6		eIF4E	eIF2Bα	eIF2Bε	ERK1/2	p38	p90RSK	Other
Phosph Site (T=Threonine, S=Serine, Ty=Tyrosine)						T 172	T 1462	T 473	T 308	S 2448	T 246	T 389	T421/S424	T 37/46	T 56	S240/244	S235/236	S 209	S 52	S 539	T202/Ty204	T180/Ty182	T 573	
Overal Pattern						↔↔↑	↔↑	↔↑, ↑	↑	↔,↑↑, ↑	↓↔↑	↑↑	↑,↑	↔,↑,↑	↓↔	↑,↑	↓↔↑	↔↑	↓↔↑	↓↔	↔↑	↔↑	↔↑	
Karlsson et al. (2004)	BCAA ~77 mg/kg BW	4x10; LP	80% 1RM	none	0, 1 & 2h	–	–	–	–	↑1	-	↑0, 1,2	↑ 1,2	–	–	–	↑ 1,2	–	–	–	↑ 1	↑ 1	–	-
Cuthbertson et al. (2006)	45 g EAA	12 min stepping	ECC & CON	p/T PonS	3, 6 & 24h	–	–	↑ at all		–	-	↑ at all	–	–	–	–	–	–	–	–	–	–	–	-
Koopman et al. (2007). Use IHC also	CHO: 0.3 g/kg	8x10; LP & 8x10; KE	75% 1RM	a-actin, P/T	0, 1 & 4h	–	–	–	–	–	-	↑0	↑	↓ 0 ,↑1,4	–	–	↓ 0	–	–	–	–	–	–	-
	CHO+PRO:0.3 g/kg					-	-	-	-	-	-	↑0,1,4	↑	↓ 0,↑1,4	-	-	↑0,1,4	-	-	-	-	-	-	-
Dreyer et al. (2008)	EAA 0.35 g/kg + CHO 0.5 g/kg	10x10; KE	70% 1RM	Std, T↔	rest, 2h (fed @ 1h)	–	↔↑	↑	–	↑↑	-	↑↑↑	–	↑, 2	↓	–	–	–	–	–	–	–	–	-
Drummond et al. (2008)	20g EAA	10x10; KE	70% 1RM	Std, T↔	rest,1h (fed) 3, 6h	↔	–	↑ 3 y	–	↑	-	↑	–	↑, 3,6	↓	-	-	-	↓1	–	↑1	–	–	↑1, ↔3 MNK1; ↓6GSK-3β
Glover et al. (2008)	3x; 10g PRO, 41g CHO	4x10; LP & 4x10; KE	10 RM	P/T	6h (rest vs ex)	–	–	↑	–	↔	-	↑	–	–	–	↑	↑	-	↓	↓	–	–	–	↔FAK,GSK-3β
Terzis et al. (2008)	**Breakfast 2h pre**	6x6, LP	6RM, UT	p/T	rest, 30 min	–	–	↓	–	↑	-	↑	↑	–	–	–	–	–	–	–	–	–	–	–
Wilkinson et al. (2008)	1.1 g protein/kg	5x8-10; U-KE	80% 1RM UT	p/T	0, 4h	↑ 0	–	↑ 0,4	–	↑ 0	-	↑ 0,4	–	–	–	–	↑ 4	~↑ 4	–	–	–	–	–	↑ 0,4 FAK; ↑0 GSK-3β
			TR 12 wk			↑ 0	–	↑ 0,4	–	↑ 0, 4	-	↑ 0	–	–	–	–	↔	↑↑0,4	–	–	–	–	–	↑ 0 FAK, GSK-3β
Moore et al. (2009)	egg PRO 5-40g	4x8-10, LP, LC, KE	Failure each set UT	actin	1 & 4h (no rest)	–	–	–	–	–	-	↔	–	–	-	↔	–	–	↔	–	–	–	–	-
Fujita et al. (2009)	EAA 0.35 g/kg + CHO 0.5 g/kg	11x10; KE	70% 1RM	None, T↔	rest, pre-Ex, 0, 1 & 2h	↑1,2	–	–	–	↑pre	-	↑	–	↑pre,0,1	↓	–	–	–	–	–	–	–	–	-
Farnfield et al. (2009)	Whey:27g AA,as 3.6 Leu	3x12; KE cybex	Maximal	none	rest, 2,4 & 24 h	-	-	↔	-	↑2	-	↑2	-	↑2	-	-	↔ ~↑ 2	-	-	-	-	-	-	-
Holm et al. (2008), (2009)	Constant feeding, SOY,Milk,fat CHO,1300 kcal	10x36, U-KE	16% 1RM	none	rest,0.5, 3 & 5h	↔	–	↓ 3, 5	–	–	-	↑0.5	–	↔	↓	–	–	–	–	–	↔	↔	–	-
		10x8, U-KE				↔	–	↔	–	–	-	↑0.5, 3,5	–	↔	↔,↓vs fast	–	–	–	–	–	↑0.5,↓3	↔	–	-
Hulmi et al. (2009)	15gx2 Whey after 3h fast & EX	5x10; LP (n=9)	10RM(~75%1RM)	s PonS	1 & 48h, 21wk (3d)	-	-	↔↑1, ↓21	–	↑ 1,48, 21	-	↑1	–	↔↑1	↔	–	↑1, 21	–	–	–	–	–	–	↔AR or total (AKT,p70S6k1 , rpS6, Myostatin
	Control	(n=11)	none			-	-	↔	-	↔	-	↔	-	↔	↔	-	↔	-	-	-	-	-	-	
Ahtiainen et al (2011)	YM (N=8), 3h fast	5x10, 4x10; LP, squats	10RM(~75%1RM): TR	Ponceau	rest & 48h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↔AR
West et al. (2009)	25g Whey, **Fed breakfast?**	4x10 arm	90-95% 10RM	α-actin	rest & 4h	-	-	↔	-	-	↔	↑	-	↔	↓↔	-	-	-	-	-	-	-	-	↔ ACC, JAK2, ↔↑STAT3
		4x10 arm + Heavy leg Ex	95% 10RM			-	-	↔	-	-	↔	↑	-	↔		-	-	-	-	-	-	-	-	
Apro and Blomstrand (2010)	BCAA~ 84 mg/kg BW	4x10, 4x15; LP	80% & 65% 1RM	?	rest, 0 & 1h	↔	–	↔	–	↑	-	↑	–	–	↓1	–	↑↑	–	–	–	–	–	↑	↔PKD1
		no ex leg				↔	-	↔	-	↑	-	↑	-	-	↓1	-	↑	-	-	-	-	-	↑	
Burd et al. (2010)	20g Whey, **Fed breakfast?**	1 set LE to fatique	70% 1RM TR	none	5F, 29F h	–	–	↔	–	↔	-	↑5	–	–	–	↑29	–	–	-	↔	–	↔	↔	
		3 sets LE to fatique				–	–	↔	–	↔	-	↑5,29	–	–	–	↑5,29	–	–	-	↓5	–	↔	↔	↔GSK-3β
		1or3 sets LE, volitional fatique	70% 1RM		24h fast	–	–	↔	–	↔	-	↔	–	–	–	↔↑	–	–	–	↔	–	↔	↑	
Witard et al. (2009)	**50%kcal Breakfast < 2h before EX**	8x10; LE, LP	70% 1RM,	α-actin	NO REST,0 & 6h	↔?↑	–	–	–	↔?↑	-	↔?↑	↔?↑	↑ 0, 6	↓?↔	-	↔?↑, 0 ↑	–	↑ 0, 6	-	–	–	-	-

Reference	Feeding	SetsxReps;Mode	Intensity:TR Status	Norm	Time course (PEx)	AMPK	TSC2	Akt			mTOR	PRAS40	S6K1		4E-BP1	eEF2	rpS6		eIF2Bα	ERK1/2	p38	p90RSK	Other
	Phosph Site (T=Threonine, S=Serine, Ty=Tyrosine)					T 172	T 1462	T	473	T 308	S 2448	T 246	T 389	T421/S424	T 37/46	T 56	S240/244	S235/236	S 52	T202/Ty 204	T180/Ty 182	T 573	
	Overal Pattern					↔↔↑	↔↑	↔↑, ↑		↑	↔,↑↑,↑	↓↔↑	↑↑	↑,↑	↔,↑,↑	↓↔	↑,↑	↑,↑	↓↔↑	↔↑	↔↑	↔↑	
Glynn et al (2010) ?	EAA + 30g CHO	10x10; KE	70% 1RM	Std, T↔	rest, 2h	↑	–	↑		–	–	-	–	–	–	–	–	–	–	–	–	–	↔(LC3B1,MaFbx, Foxo3a), ↓LC3B2; ↑ MuRF-1
	EAA + 90g CHO					↔	–	↑		–	–	-	–	–	–	–	–	–	–	–	–	–	
Moore et al. (2011)	25g Whey, **Fed breakfast?**	4x8-10, LP, LC, KE	Failure each set	α-actin	rest, 1, 3 & 5h	–	–	↑ 1		–	↔	-	↑1,3,5	–	↑	↔	–	–	–	↑1	–	↑1,5	-
		no ex				-	-			-	↔	-	↑ 1	-		↔	-	-	-	↔	-	↔	-
Ahtiainen et al (2011)	3h fast (ould and Young)	5x10RM, Lp	10RM (~80% 1RM)	Ponceau	Pre 21wk RT	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	↔AR
Cooke et al. (2011)	10g Whey (pre-Exercise)	4x8-10, LP & KE	80% 1RM	ELISA,	Rest, 15m, 2h	-	-	↔		-	↑15	-	↑15	-	↓15	-	-	-	-	-	-	-	-
	CHO (pre-Exercise)					-	-	↔		-		-		-		-	-	-	-	-	-	-	-
Reitelseder et al. (2011)	Whey 0.3 g/kg LBM	10x8, U-KE	80% 1RM	p/t, GABDH	1, 3 & 6h	–	–	↑1		↑1, ↓6	–	-	↑1,3, 6?	–	↑1,3, 6	–	–	–	–	–	–	–	↔GSK-3β
	Casein 0.3 g/kg LBM					–	–	↑1		↑1, ↓6	–	-	↑1,3	–	↔?	–	–	–	–	–	–	–	
Borgenvik et al. (2011)	BCAA (45%,30% & 25%, Leu, Val, Ileu)	3x5 warm, 4x10, U-KE	80% 1RM	α-tub	rest, 1&3h	-	-	-		-	↑1,3	-	↑1,3	-	↔	-	-	-	-	-	-	-	↔ MaFbx, MuRF-1 (< PLA)
		non-ex	N/A			-	-	-		-	↑1	-	↑1,3	-	↔	-	-	-	-	-	-	-	
Staples et al. (2011)	25g Whey	4x8-10, LP, LC, KE	to Fail	α-actin	1 & 3h (no rest bx)	–	–	↑		–	–	-	↔	–	↔	↔	–	–	–	↔	–	–	↑ACC
		no ex leg				-	-	↔		-	-	-	↔	-	↔	↔	-	-	-	↔	-	-	↔ACC
	25g Whey + 50g CHO	4x8-10, LP, LC, KE	to Fail			-	-	↑		-	-	-	↔	-	↔	↔	-	-	-	↔	-	-	↑ACC
		no ex leg				–	–	↔		–	–	-	↔	–	↔	↔	–	–	–	↔	–	–	↔ACC
West et al. (2011)	25g Whey Bolus	8x10; KE	10RM, 2m rest, Act	α-tub	1, 3 & 5h	–	–	↑ 1		↑ 1	↑ 1,3	↑↑1, ↓3,5	–	↑↑1,↑3, ↓5	↔	↔	–	↑↑1,↓3, 5	–	–	–	–	-
	Given as 10 Pulse (2.5g)					–	–	↑ 1		↑ 1	↑ 1,3	↔	–	↑ 1,3	↔	1↓, 5	–	↑ 1,3,5	–	–	–	–	-
Burd et al. (2012)	20g Whey	Slow Sets (6s Con/ECC) ~25 reps Conn Set (1s CON/ECC) ~25 reps	TR, 30% 1RM	P/T	Rest, 6,24,30h	-	-	↔		↔	↔	-	↑24	-	↑30	-	↔	↔	↔	↔	↔	↑ 24	-
						-	-	↔		↔	↔	-	↔	-	↑ 6,24,30	-	↔	↔	↔	↔	↔	↔	-
Farnfield et al. (2012)	Whey:27g AA,as 3.6 Leu	3x8; U-KE cybex	Maximal, UT	P/T	rest, 2h	-	-	↔		-	↑2	-	↔	-	↔↑	-	-	↔?↑	-	-	-	-	↔?↑ eIF4G
			Maximal, TR 12wk			-	-	↔		-	↑2	-	↔↑	-	↔↑	-	-	↔?↑	-	-	-	-	
West et al. (2012)	25g Whey Bolus	8x10; KE	10RM, 2m rest,M	α-tub	1, 3 & 5h	-	-	↑ 1		-	↑ 1,3,5	-	↑ 1,3,5	-	-	-	-	-	-	-	-	-	-
			10RM, 2m rest, W			-	-	↔↑1		-	↑ 1,3,5	-	↑ 1,3,5	-	-	-	-	-	-	-	-	-	-
Camera et al. (2012)	20g Whey + 40g CHO (2x)	8x5; LE	80% 1RM (low Gly)	α-tub	0, 1, 4h	↔	↑ 1	↑ 1		-	↑ 1,4	-	↑ 1,4	-	-	-	-	↑ 1,4	-	-	-	-	-
			80% 1RM (norm Gly)		0, 1, 4h	↔	↑ 1,4	↑ 1		-	↑ 1,4	-	↑ 1,4	-	-	-	-	↑ 1,4	-	-	-	-	-
Burke et al. (2012)	25g Whey + 5g Leu Bolus	8x10; KE	80% 1RM, RT	α-tub	rest, 1, 5h	-		↑ 1,5		-	↔↑~	↔	↑ 1	↔↑1	-	-	-	↑ 1, ~5	-	-	-	-	-
	Given as 13 pulses					-		↑↑ 1		-	↑ 1, 5	↑ 1	↑↑ 1	↑ 1	-	-	-	↑↑ 1,~5	-	-	-	-	-
Churchward-Veene 2012	Whey 25g, w/ 3g leu	4x10-12 reps SiLE & press, (underlined if EX-fed is different that Fed	95% 10RM, active	α-tub	rest, 1, 3, 5h	-	-			-		-	↑ 3,5	-		-	-	-	-			-	-
	AA 8g, w/ 3g leu					-	-	↑ 1		-	↑ 1,3,5	-	↑ 3,5	-	↑ 1,3,5	-	-	-	-	↑ 1,3,5	↑ 1	-	-
	AA 12g, 9g EAA & 1 g leu					-	-			-		-	1↔,↑5	-		-	-	-	-			-	-

Reference	Feeding	SetsxReps;Mode	Intensity:TR Status	Norm	Time course (PEx)	AMPK	TSC2	Akt		mTOR	PRAS40	S6K1	4E-BP1	eEF2	rpS6		eIF4E	ERK1/2	p38	p90RSK	Other
	Phosph Site (T=Threonine, S=Serine, Ty=Tyrosine)					T 172	T 1462	T 473	T 308	S 2448	T 246	T 389	T421/S424	T 37/46	T 56	S240/244	S235/236	S 209	T202/Ty204	T180/Ty182	T 573	
	Overal Pattern					↔↔↑	↔↑	↔↑, ↑	↑	↔,↑↑,↑	↓↔↑	↑↑	↑,↑	↔,↑,↑	↓↔	↑,↑	↑,↑	↔↑	↔↑	↔↑	↔↑	
Reidy et al. (2013+2014)	~17.5g Whey	8x10 Young	70% 1RM, 3 min rest	P/T	rest, 3, 5h (2,4hpi)	-	-	-	↑ 3	↑ 3,5	-	↑3, ↔5	-	↑ 3,5	↓3	-	↑ 3,5	-	-	-	-	-
	~19g Protein Blend					-	-	-	↑ 3	↑ 3,5	-	↑ 3,5	-	↑ 3,5	↓3,5	-	↑ 3,5	-	-	-	-	
Rahbeck et al. (2014), Stefanetti (2014)	Whey+ CHO	CON: 6x10reps Max	Maximal	GABDH	rest,1,3,5h	-	-	↑1 ↓3,5	-	↑ *1,3,5*	-	↑1	-	↑ 1	-	-	↑1	↓1,↑3	-	-	-	↑ *1,3,5 (ACC);* ↓*5 (Foxo3a, MuRF-1 & eIF3F);* ↓↔*5(FOXO1)*
	CHO					-	-	↑1 ↓3,5	-	↑ *1,3,5*	-	↑1	-	↑ 1	-	-	↑1	↔	-	-	-	↑ 1,3 (ACC); ↓5 (Foxo3a, MuRF-1 & eIF3F); ↓↔5(FOXO1)
	Whey+ CHO	ECC: 6x10reps Max				-	-	↑1 ↓3,5	-	↑ 1,3,5	-	↑ 1,3,5	-	↑ 1	-	-	↑ 1,3,5	↓1,↑3	-	-	-	↓*3,5 (ACC), ;* ↔*5 (Foxo3a, MuRF-1 & eIF3F);* ↓*5 (FOXO1)*
	CHO					-	-	↑1 ↓3,5	-	↑ 1,3,5	-	↑ 1,3,5	-	↑ 1	-	-	↑ 1,3,5	↔	-	-	-	↓3 (ACC); ↔5 (Foxo3a, MuRF-1 & eIF3F); ↓5 (FOXO1)
Donges et al 2012	20g PRO	8 × 8 leg ext	70% 1RM		Fast, 1, 4h	↔	-	↑1	↑1	↔↑~	↔	-	-	↔	-	-	↑1	-	-	↔	-	↑1,4 (PAS160)
	20g PRO	40 min cycle	55% PPO	P/T	Fast, 1, 4h	↔	-	↔	↔	↔↑~	↑1	-	-	↔	-	-	↔	-	-	↔	-	↔↑~ (PAS160)
	20g PRO	Both	Both		Fast, 1, 4h	↔	-	↑1	↑1	↔↑~	↑1	-	-	↔	-	-	↔	-	-	↔	-	
Churchward-Veene et al . 2013	Whey 6g, w/ 0.75g leu, 35g CHO, ~6g fat	8x10-12; U-KE & LP, (underlined if EX-fed is different that Fed	~80% 1RM, REC	α-tub	rest, 1.5 & 4.5h	-	-	4.5	-	↔	-	↔	-		↔		-	-	↑ 1,3,5	↑ 1,3,5	-	-
	Whey 6g, w/ 3g leu, 35g CHO, ~6g fat					-	-	↑ 1.5, 4.5	-	↑ 1.5	-	↔	-		↔		-	-	-	-	-	-
	Whey 25g, w/ 3g leu, 35g CHO, ~6g fat					-	-	↑ 1.5	-	↑ 1.5	-	↔	-	↔, main effect ↑ 1.5	↔	main effect ↑ all, ↑ 1.5	-	-	-	-	-	-
	Whey 6g, w/ 5g leu (~8g BCAA), 35g CHO, ~6g fat					-	-	↔	-	↔	-	↔	-		↔		-	-	↑ 1,3,5	↑ 1,3,5	-	-
	Whey 6g, w/ 5g leu, 35g CHO, ~6g fat					-	-	↔	-	↑ all	-	↔	-		↔		-	-	↑ 1,3,5	↑ 1,3,5	-	-
Wernbom et al. (2013)	**Breakfast 3h** pre, 24 &48h	BFR: 5xfail	30% 1RM	stain kit	rest, 1,24,48h	↔	-	↔	-	↔	-	↑1,24	-	-	↔	-	-	-	↔	↑1	↔	-
		Con: work matched	30% 1RM			↔	-	↔	-	↔	-	↑24	-	-	↔	-	-	-	↔	↔	↔	↔eIF4G (Ser1108)
Walker et al. (2013)	**Breakfast 3h** pre	Variable resistance, 5x10RM, Lp	~80% 1RM: TR	PonceuS, α-actin	rest, 1h	-	-	↓	-	↔	-	↑1	-	-	-	↑1	↑1	-	↑1	↑1	-	↑1 MAPKAPK-2; ↔total protein
			~80% 1RM: UT			-	-	↓	-	↔	-	↑1	-	-	-	↑1	↑1	-	↑1	↑1	-	
		Constant Resistance, 5x10RM, Lp	~80% 1RM: TR			-	-	↓	-	↔	-	↑1	-	-	-	↑1	↑1	-	↑1	↑1	-	
			~80% 1RM: UT			-	-	↔	-	↔	-	↑1	-	-	-	↑1	↑1	-	↑1	↑1	-	
Churchward-Veene et al . 2014 (elderly)	Whey 45g, w/ 5.4g leu	6x10-12; U-KE & LP, (underlined if EX-fed is different that Fed	80% 1RM, REC	α-tub	rest, 2.5, 5h	↔	-	↑2.5,5	-	↑	-	↑	-			↑all	-	-	↑ 1,3,5	↑ 1,3,5	-	-
	Whey 15g, w/ 1.8g leu + 10g Citrulline					↔	-	5	-	↑	-	↑	-	↑2.5,5	↔↑~	↑2.5	-	-	↑ 1,3,5	↑ 1,3,5	-	-
	Whey 15g, w/ 1.8g leu + NEAA					↔	-	5	-	↑	-	↑	-			↑2.5,5	-	-	↑ 1,3,5	↑ 1,3,5	-	-
Morberg et al. (2014)	~16g EAA (2.6g Leu)	4x10, 4x1 ; LP	80% & 65% 1RM	α-tub?	rest, 1,3h	-	-	↔	-	↑1,3	-	↑1,3	-	-	↓	-	-	-	-	-	-	↔ MaFbx, MuRF-1
	~13g EAA (no Leu, L-Gly instead)					-	-	↔	-	↑3	-	↑3	-	-	↓	-	-	-	-	-	-	

Reference	Feeding	SetsxReps;Mode	Intensity:TR Status	Norm	Time course (PEx)	AMPK	TSC2	Akt		mTOR	PRAS40	S6K1		4E-BP1	eEF2	rpS6		eIF4E	ERK1/2	p38	p90RSK	Other
Phosph Site (T=Threonine, S=Serine, Ty=Tyrosine)						T 172	T 1462	T 473	T 308	S 2448	T 246	T 389	T421/S424	T 37/46	T 56	S240/244	S235/236	S 209	T202/Ty204	T180/Ty182	T 573	
Overal Pattern						↔↔↑	↔↑	↔↑, ↑	↑	↔,↑↑,↑	↓↔↑	↑↑	↑,↑	↔,↑,↑	↓↔	↑,↑	↑,↑	↔↑	↔↑	↔↑	↔↑	
Areta et al. 2014	Whey Bolus 40g 2x	4x10; KE.	80% 1RM, TR	α-tub?	Rest, 1,4,6,7,12h	-	↑1,7	↑1	-	↑1,7 peak	↑1, 7	↑1,7 peak	-	↔	↔	-	↑1, 7	-	-	-	-	-
	Intermediate Whey 20g 4x					-	↓4-12	↑,↓12	-	↑	↑7	↑	-	↔	↔	-	↔	-	-	-	-	-
	Pulse Whey 10g 4x					-	↔	↑, ↓7	-	↑	↓7	↑1	-	↔	↔	-	↔	-	-	-	-	-
Areta et al. 2014	ED - 15g Whey	6x8; KE;	80% 1RM, TR	α-tub?	Rest, 1,4	↔	-	↑1	-	↑1	-	↑1,4	-	↔	↔	-	↑1,4	-	-	-	-	-
	ED - 30g Whey					↔	-	↑1	-	↑1,4	-	↑1,4	-	↔	↔	-	↑1,4	-	-	-	-	
Parr et al. 2014	PRO (25g whey 2x)	8x5; KE & (30min & sprint (10x30) cycle	80% 1RM, 63% cont, 110% interval (peak power output, PPO)	α-tub	Rest, 2,8h	↔	-	-	-	↑↑2	-	↑2	-	↔	↓↔2	-	-	-	-	-	-	-
	ALC-PRO (25g whey 2x)					↔	-	-	-	↑2,8	-	↑2	-	↔	↓	-	-	-	-	-	-	-
	ALC-CHO (25g maltodextrin 2x)					↔	-	-	-	↑2,8	-	↔	-	↔	↓	-	-	-	-	-	-	-
Camera et al. 2014	25g Whey +	8x5; KE & 30min cycle	80% 1RM, 63% cont, PPO	α-tub	Rest, 1,4h	-	-	↑1	-	↑1	-	↑1	-	-	↓1	-	-	-	-	-	-	-
Ferreira et al. (2014) (sup 30min & imed & after RE)	BCAA+CHO (~5g leu) (120g CHO)	4x8-12 LP, LE	~75-80% 1RM	ELISA	Rest, 0.5, 2 & 6h	-	-	↑0.5,2	-	↑0.5,2	-	↑6	-	↔	-	-	-	-	-	-	-	IRS-1↑ 0.5,2
	CHO 120g					-	-	↑0.5,2	-	↑0.5,2	-	↑6	-	↔	-	-	-	-	-	-	-	IRS-1↑ 0.5,2
D'Souza et al. (2014).	10g Whey	3x8-10 Squat, LP, KE	~80% 1RM, UT	ERK1/2	Rest, 2 & 4h	-	-	-	-	↔	-	↔	-	-	-	-	-	-	-	-	-	-
	20g Whey					-	-	-	-	-	-	↑2,4	-	-	-	-	-	-	-	-	-	-
	30g Whey					-	-	-	-	-	-	↑2,4	-	-	-	-	-	-	-	-	-	-
	40g Whey					-	-	-	-	-	-	↑↑2,↑4	-	-	-	-	-	-	-	-	-	-
Mitchell et al. (2014)	30g milk Protein	4x8, LP,LC,KE, CP	8RM	α-Tub	rest, 1, 5h	-	-	-	-	-	-	↑5	-	-	-	-	-	-	-	-	-	↔AR
Mitchell et al. (2014)	30g milk Protein	4x8, LP,LC,KE, CP	8RM	α-Tub	rest, 1,3,6h	-	-	↑1	-	↑1,3	-	-	-	↔	-	↑1,3,6	-	-	-	-	-	-
Kakigi et al. (2014)	10g WH	6x4; KE	Max	P/T	rest, 1h	-	-	↑1	-	↑1	-	↑1	-	↑1	-	-	-	-	-	-	-	-
	20g WH					-	-	↑↑1	-	↑↑1	-	↑↑1	-	↑↑1	-	-	-	-	-	-	-	-
Mitchell et al. (2012)	**breakfast**, 2h fast and then imed PE 30g milk Protein + 33g Cho, 11g fat	1xfatigue	80%1RM	total	rest, 1h	-	-	↔	-	↑1	-	↑1	-	-	-	-	-	-	-	-	-	-
		3xfatigue	80%1RM			-	-	↔	-	↑1	-	↑1	-	-	-	-	-	-	-	-	-	-
		3xfatigue	30%1RM			-	-	↔	-	↑1	-	↔	-	-	-	-	-	-	-	-	-	-

Signaling molecules . Arrows denote direction of phosphorylation. ↑, significantly increased; ↓, significantly decreased; ↔, no change; ↔↑, trend to increase; ↔↓, tend to decrease; ↔. Red color arrows represent a group diference. Blue arrows represent an effect of feeding. Arrows reresent change from rest (where available), Underlined arrows indicate a change from the fed condition . RM, repetition maximum; LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Ecc, eccentric contractions; Con, concentric contractions; O, old; Y, young; M, men; W, women; h, hour; m, minutes; sec, seconds; TR, trained; UT, Untrained; RT, resistance trained; RE, resistance exercise; ST, strength trained; ET, endurance trained.LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Si, Single leg; BCAA, Branch Chain Amino Acids; EAA, Essential Amino Acids; CHO, Carbohydrate; PRO, Protein; LBM, Lean body mass; pi, post-ingestion; sT, show total protein but not correct for. Threonine

Table 1.3 Summary of intracellular signaling in vastus lateralis following acute resistance exercise conducted in the fasted/fed state, untrained and trained older human adults.

Reference	Fed/Fasted	SetsxReps; Mode	Intensity	Norm	Time course (PEx)	AMPK	Akt		mTOR		S6K1		4E-BP1	eEF2	rpS6		eIF4G	eIF4E	eIF2Bα	GSK-3β	ERK1/2	MNK1	other
Phosph Site (T=Threonine, S=Serine, Ty=Tyrosine)						T 172	S 473	T 308	S 2448	S 2481	T 389	T421/S424	T 37/46	T 56	S240/244	S235/236	S 1108	S 209	S 52	S 9	T202/Ty204	T 197	
Overal Pattern						↔↑	↔	↔↑	↑,↑	↔↑	↔,↑,↑	↑	↓,↔, ↑,↑	↓↔	↓↔	↔↑	↓↔ ↑	↑	↔↑	↔	↔	↔	
Williamson et al. (2003)		3 x 10 KE old	70% 1RM, 3 min rest	T/PonS	0	-	-	-	-	-	-	-	-	-	-	-	-	↔	-	-	↔↓	↔↓	↔JNK. P38; ↑p90RSK; ↔↑MKP 1
Mayhew et al. (2009)		3sets eac, LP, KE, S	8-12 RM	PonS?	rest, 24h	–	↔	-	–	–	NM	↑↔	↔	↑	↑	-	↑	↑	-	-	-	-	-
Fry et al. (2010)		1x30, 3x15	20% 1RM 30 sec rest	p/T	0, 1 & 3h	↔	–	↔	↔,↓1,↑3	–	↔	–	↓0, ↔	↔	↔	↓0,↔,↑3	–	-	-	-	↔	↔	↔FAK, eIF2Bε
		W/ BFR				↔	–	↑ 3	↔,↑3	–	↑1,3	–	↔	↔	↑ 1, 3	↑1,3	–	-	-	-	↔, ↑1	↑	↔FAK, eIF2Bε
Kumar et al (2009)		3x9 (60%), 3x8 (75%), or 6x3 (90%); LE	60-90% 1RM (combined)	GABDH	10min, 1, 2 & 4h	–	–	–	–	–	↔	–	↔	↔	–	–	–	–	-	-	-	-	-
Fry et al. (2011)		~8-10x10 Old	71 % 1RM, 3m rest	sT	0, 3, 6, 24	↔	↔	–	↔	–	↔	–	↔	–	↑3	↑3, 6	–	-	↑3, 6,24	-	↔	-	↔< LAT1, SNAT2, CD98, ATF4; ↓6,24 CDK2,p27kip1,CyclinD1; ↑3, 6,24 STAT3
Kumar et al (2012)	Fasted	3x14 (40%)	40% 1RM	None	10min, 1, 2 & 4h	–	–	–	–	–	↔	–	–	–	–	–	–	-	-	-	-	-	-
		3x8 (75%)	75% 1RM			–	–	–	–	–	↑↔10m	–	–	–	–	–	–	-	-	-	-	-	-
		6x14 (40%)	40% 1RM			–	–	–	–	–	↑1,4	–	–	–	–	–	–	-	-	-	-	-	-
		6x8 (75%)	75% 1RM			–	–	–	–	–	↑1,2,4?	–	–	–	–	–	–	-	-	-	-	-	-
Stefanetti et al. (2014)		10OM	3x14, 60% 1RM	GABDH	Rest, 2h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↔FOXO1,EIF3F,MHC, MyoD,MyoG, MURF-1, MAFbx. FOXo3a
Della Gatta et al. (2014)		3x12; KE	Maximal: Old	ELISA	Rest, 2h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑ IL-6,IL-8, MCP-1; IL-10 ↑PT;↔IL-13, TNFa ND
Farnfield et al. (2012)	Whey:27g AA,as 3.6 Leu	3x8; SiKE cybex	Maximal, UT	P/T	rest, 2h	–	↔	–	↔	–	↔	–	↔	–	–	↔	↔	-	-	-	-	-	-
			Maximal, TR 12wk			–	↔	–	↔↑	–	↔	–	↔	–	–	↔?↑	↔	-	-	-	-	-	-
			Maximal, UT			–	↔	–	↑2	–	↑2	–	↑2	–	–	↔?↑	↑2	-	-	-	-	-	-
			Maximal, TR 12wk			–	↔	–	↔↑	–	↔↑	–	↑2	–	–	↔	↔?↑	-	-	-	-	-	-
Drummond et al. (2008)	Fed 20g EAA & 40g CHO	8x10; KE	~70% 1RM	Std, T↔	rest,1(fed) 3, 6h	↑ 1,3	↔	-	–	↑ all	↑1,3,6	–	↑ 3	~↓ 3,6	–	–	–	–	↔	↔	↔	↔	–
Churchward-Veene et al . 2014 (elderly)	Whey 45g, w/ 5.4g leu	6x10-12; U-KE & LP, (underlined if EX-fed is different that Fed	80% 1RM, REC	α-tub	rest, 2.5, 5h	↔	↑2.5,5	-	↑	-	↑	-			↑all	-	-	-	-	-	↑ 1,3,5	-	
	Whey 15g, w/ 1.8g leu + 10g Citrulline					↔	5	-	↑	-	↑	-	↑2.5,5	↔↑~	↑2.5	-	-	-	-	-	↑ 1,3,5	-	p38 ↑ 1,3,5
	Whey 15g, w/ 1.8g leu + NEAA					↔	5	-	↑	-	↑	-			↑2.5,5	-	-	-	-	-	↑ 1,3,5	-	
Dickinson et al. (2014)	10g EAA (3.5Leu)	8x10 old	70 % 1RM, 3 min rest	none	Rest, 1, 6, & 24h	-	-	-	↑2,5	-	↑2	-	↑2,5	↔	-	-	-	-	-	-	-	-	-
	10g EAA (1.8Leu)					-	-	-	↑2	-	↑2	-	↔	↓2	-	-	-	-	-	-	-	-	-
	Placebo					-	-	-	-	-	↔	-	-	-	-	-	-	-	-	-	-	-	-
D'Souza et al. (2014)	10g Whey	3x8-10 Squat, LP, KE	~80% of 1RM, Untrained	ERK1/2	Rest, 2 & 4h	-	-	-	-	-	↔	-	-	-	-	-	-	-	-	-	-	-	-
	20g Whey					-	-	-	-	-	↑2,4	-	-	-	-	-	-	-	-	-	-	-	-
	30g Whey					-	-	-	-	-	↑2,4	-	-	-	-	-	-	-	-	-	-	-	-
	40g Whey					-	-	-	-	-	↑↑2,↑4	-	-	-	-	-	-	-	-	-	-	-	-

Signaling molecules . Arrows denote direction of phosphorylation. ↑, significantly increased; ↓, significantly decreased; ↔, no change; ↔↑, trend to increase; ↔↓, tend to decrease; ↔. Red color arrows represent a group diference. Blue arrows represent an effect of feeding. Arrows reresent change from rest (where available), Underlined arrows indicate a change from the fed condition . RM, repetition maximum; LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Ecc, eccentric contractions; Con, concentric contractions; O, old; Y, young; M, men; W, women; h, hour; m, minutes; sec, seconds; TR, trained; UT, Untrained; RT, resistance trained; RE, resistance exercise; ST, strength trained; ET, endurance trained.LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Si, Single leg; BCAA, Branch Chain Amino Acids; EAA, Essential Amino Acids; CHO, Carbohydrate; PRO, Protein; LBM, Lean body mass; pi, post-ingestion; sT, show total protein but not correct for.

Transcriptional Response to Resistance Exercise in the Fasted and Fed State

A host of metabolic, energetic and mechanical biological events initiated during or following RE prompt flux and activity of transcription factors and/or other molecular regulators modulating mRNA expression in an adaptive effort to maintain homeostasis. Exercise physiologists have relied heavily on the use of the muscle biopsy and qPCR to quantify a diverse range of transcriptional responses to exercise. Here we have tabulated a summary of mRNA responses following RE directed at modulating human muscle growth and protein turnover in both the fasted and fed condition. An enormous body of literature has utilized qPCR and microarrays to examine transcriptional responses to resistance training [82, 92, 119, 120, 124-128]. We were unable to provide complete coverage of the literature regarding RE, but still provide a comprehensive examination of muscle growth/turnover literature assessing acute changes in mRNA abundance of inflammatory, protein breakdown markers, growth factors and those regulators of myogenesis. This served as a body of comparison for examining the effect of PRO/AA feeding on transcriptional post-RE changes.

In regards to the inflammatory mRNA response, there appears to be an up-regulation of TNFα [107, 129-131], IL-8, IL-15, IL-1β and IL-6 in the first few hours and up to ~24h post-exercise [107, 115-117, 129, 130, 132-138], but not 3 days later [139]. The exact time course and magnitude of the inflammatory response, if any, seems to be dictated by the nature of the stress response and is likely influenced by training status [107, 131, 135, 138] and age [115, 116, 124, 131, 140]. Of all the mRNA markers examined with human skeletal muscle biopsies, the inflammatory markers are the most susceptible to influence from repeated biopsy sampling or factors in the technique itself

and that additional procedures, such as a control group or extra precaution should be considered [107, 137, 141, 142]. Another concern with these transcripts, is that the muscle may not be the sole source of inflammatory mRNA markers, as they also may arise from infiltration of other immune cells [116, 117, 139]. Regardless of these factors, we were not able to discern any effect of PRO/AA feeding on modulating these inflammatory responses in skeletal muscle. Because inflammation plays a pivotal role in the adaptability to RET [128], especially in older adults [119, 128] future research could benefit from examine the role of coupling anti-inflammatory mediators as a means to improve inflammation-induced amino acid insensitivity.

Several studies have demonstrated a wide range of responses in post-exercise mRNA abundance of insulin like growth factor 1 (IGF-1) following RE [35, 73, 74, 80, 89, 119, 124, 143-160] independent of PRO/AA feeding. These responses may be impacted by exercise intensity and aging [153, 154]. The role for IGF-1 in the normal physiological response to RE is not entirely clear and are likely reflective of the alternative splicing patterns on this mRNA [148]. Although chronic PRO/AA feeding alters the IGF axis in humans [161, 162] this evidence suggests that exercise may override this stimulus, and does not have an additive interaction. Recent observations have demonstrated that physiological IGF-1 responses do not impact the early growth response to exercise [163]. A growing body of evidence, in human models, has recently suggested that the basal or post-exercise hormonal response does not influence MPS [164, 165] or enhance muscle strength or hypertrophy [166-168]. Also, basal levels of testosterone do not alter the molecular response to RET [145]. However, IGF-1 and other

growth factors may pose a regulatory role with satellite cells [169, 170] or play another role at other time periods during muscle hypertrophy.

Although circulating growth factors may not directly mediate hypertrophic responses in humans [167], it is possible that variations in the amount of hormonal receptors, such as the androgen receptor (AR), may interact with the circulating hormonal milieu to modulate hypertrophic responses [171, 172]. However, there are mixed reports as to whether RE increases AR mRNA and protein content [145, 149, 150, 157, 159, 165, 171], yet it is clear that PRO/AA feeding does not influence this response [150]. It is likely that the androgen receptor content is genetically determined.

Due to the methodological difficulties of assessing muscle protein breakdown via stable isotopic methods (discussed later) many investigators have selected to examine two key E3 ubiquitin ligases essential for muscle atrophy [173, 174]; Muscle RING (Really Interesting Novel Gene) Finger 1 (MuRF-1) and Muscle Atrophy F-box (MAFbx, Atrogin-1) as markers of protein breakdown. However, the role of these atrogenes in human muscle hypertrophy is less clear. These mRNAs are thought to be down-regulated through increased Akt/FOXO signaling (discussed elsewhere) caused by phosphatidylinositol 3-kinase, or possibly growth factors, but more particularly the insulin secretion from carbohydrate or protein feeding [174]. Increased Akt activation modulates FOXO translocation and activity on proteolytic gene expression to reduce protein breakdown [174]. The evidence suggests no change [175, 176] in post-RE FOXO1 or FOXO3a [133, 152, 175-177] whereas other have shown an early increase [151] followed by a decrease [35, 151] in FOXO3a concomitant with an increase in FOXO1 and no change in FOXO40 [35, 151]. This is in contrast to the data from others

showing an increase [176] or a decrease [175] in FOXO40 mRNA following RE. The inconsistency of responses may be due totraining status [175, 176] or exercise type [178]. Contrary to the hypothesis that added nutrition would potentiate the Akt/FOXO interaction; none of the studies we found demonstrated an effect of PRO/AA feeding on the FOXO's mRNA response [35, 151, 152, 178].

To examine the downstream targets of this mechanism, many investigators have examined skeletal muscle MURF-1 and MAFbx following RE. Examination of the evidence from human skeletal muscle following RE discovered either an increase [176, 179], decrease [35, 52, 89, 132, 151, 152, 179-182], or no change [63, 103, 124, 133, 177, 180, 183-187] in MAFbx mRNA. Although many studies have included a feeding condition we were able to find only one study demonstrating a main effect for feeding to reduce MAFbx mRNA and this occurred independent of the exercise stimulus [63]. Regarding the MuRF-1 mRNA response to RE, examination of the evidence from human skeletal muscle clearly suggest an increase [35, 63, 103, 132, 133, 151, 176, 177, 179, 180, 184-186, 188], not affected by aging [133, 176, 187] that is intensity/mode/stress dependent [35, 103, 175, 186]. However, a few studies did not see an increase in the fasted condition [152, 182]. Dablo et al. gave either pre-exercise CHO or PRO and found MAFbx did not change with PRO, but had decreased at 2 and 6h post-exercise in CHO and only 6h post-exercise in PLA [152]. Although we suggest an effect (~2fold) of RE+PRO on reducing post-exercise Murf-1 expression [189], similar to the literature on MAFbx, we were unable to find a statistical effect in the many studies examining an effect of PRO/AA on reducing Murf-1 [35, 51, 52, 63, 110, 151, 152, 178] following RE. However, following concurrent exercise an effect was recently demonstrated [44], similar

to that demonstrated with aerobic exercise and feeding [190]. More recently, several investigators have sought to examine changes in protein levels of the atrogenes and their substrates. Total MuRF-1 protein was found to be unchanged [175, 176, 178, 191] or upregulated [63, 110] following RE and one study demonstrated an effect of feeding, independent of exercise, on reducing total MuRF-1 protein [63]. Total MaFbx protein is also unchanged following RE regardless of feeding [63, 110, 191]. Collectively, these findings suggest that timing of nutrition in proximity to exercise has little bearing on enhancing the response of these markers of protein breakdown/proteolysis.

MUSCLE SATELLITE CELLS

As a result of the amplified level of translation in response to resistance exercise [50, 192], myofiber growth occurs over the course of repeated exposure to RE stimulus [170]. The prevailing theory for contraction induced myofiber growth posits that as acute elevations in protein synthesis accumulate myocellular protein, or some other stimuli, causing myofiber expansion [170, 193-195]. This expansion strains the myonuclear domain, the area of a myofiber maintained by one myonucleus to regulate essential cell function [170]. Concurrent with this response, transcriptional regulation [196], myogenic proliferation and differentiation occur in dormant satellite cells (SC). These dormant satellite cells become active and are fused as nuclei to myofibers to meet the demands of the enlarged myofiber. Several stimuli during this process activate satellite cells, which have several functions, self-renewal, and maintenance of the myofiber environment, repair/remodel myofibers and to undergo terminal differentiation and fuse to current myofibers as myonuclei, (i.e. myonuclear addition) to facilitate additional hypertrophy [193]. Although satellite cells may not be necessary for hypertrophy to occur [170], it is

possible that they modulate the magnitude of muscle hypertrophy [197, 198] or direct influence on areas of muscle maintenance and quality [170, 199, 200]. A host of evidence has suggested increased SC activation and content following RE in human skeletal muscle [170], yet we and others have demonstrated a blunting of [201] or a delayed ability [202] to activate and increase the SC pool in older men compared to a younger cohort. The current research suggests that reductions in the ability to stimulate muscle protein synthesis [192, 203] and promote proliferation and differentiation of muscle stem cells [195] are primary contributors to the development of sarcopenia. In addition, muscle stem cells (aka satellite cells) may also play an important role in the maintenance of muscle quality [200], which is especially relevant during aging [199]. It is well known that exercise and amino acid/protein (AA/PRO) nutrition, in particular the amino acid leucine, are important stimulators of muscle protein synthesis through activation of mTORC1 regulatory role on peptide translation [50].

AA and leucine provision has also been shown to up regulate SC activity though the mTORC1[204-206]. The literature is dominated by reports of how supplemental protein/AA may influence the early muscle growth response (i.e. muscle protein synthesis) yet, very little is understood regarding the effect of protein type and/or the influence of protein supplementation on further mediation of muscle growth and adaptation over chronic resistance exercise training through expansion of the satellite cell (SC) pool and via myonuclear addition. Some evidence has suggested increased SC activation and content following RE in human skeletal muscle [170]. In addition, AA provision has been shown to up regulate SC activity though mTORC1 [204-206], yet the

combined effects of RE and PRO/AA on SC activity and content has not been well examined.

Changes in mRNA transcripts involved in cell cycle progression, proliferation and differentiation (MyoD, MyoG, Cdks, MFRs and Cyclins) have been extensively investigated following RE [207]. Besides modulating SCs, these myogenic regulatory factors are involved in several other processes, including the transcription of many skeletal muscle contractile proteins and potentially fiber type transition [207].

The amount of literature on this topic is too numerous to discuss in detail [207], so we will highlight a few studies depicting the general trend of these markers in response to RE in the fasted state and focus our examination for any additive effects of PRO/AA ingestion. Following RE in the fasted state the response of MyoD is not directly clear, it appears that mRNA transcripts either do not change or, for the most part, are increased during 24h post-exercise (**Table 1.4**). A similar pattern is seen in the fed condition, with no potentiation of feeding apparent (**Table 1.5**). Regarding MyoG (myogenin), a delayed, but readily apparent increase is seen beyond ~5-6h to ~24h post exercise that may be intensity and/or age dependent (**Table 1.5**). Once again, an effect of PRO/AA was not readily apparent, however Hulmi et al. demonstrated decreased MyoG at 1h post-exercise and in resting conditions after 21 weeks of RET in a placebo group, but not a PRO fed condition [78]. Several other markers of satellite cell activity (p27, p21 and various cdks and cyclins) have also been investigated following RE in human skeletal muscle. For the most part, p27kip mRNA expression has been shown to be unchanged or in one instance down-regulated [208] following RE. However, p21 tends to demonstrate a clear increase in the hours following RE returning to baseline 24h post

exercise and then again up-regulated at 48h (see tables). However, no effect of feeding is apparent in the post-exercise condition. A few investigators have examined the mRNA response of CDK2 and found no change [78, 156, 209], however, following ingestion of whey protein in both younger and older men, a several fold up-regulation of CDK2 mRNA at 1 and 48 hours post-exercise, respectively [78, 209] that was not evident on the placebo condition.

The evidence suggests that RE up-regulates mRNA expression of markers of satellite cell activity and although there is limited dual examination of RE + PRO/AA feeding, there is some evidence, albeit very limited, to suggest that satellite cell activity [78, 209] and content [210] could be enhanced. This theory is supported by evidence discussed in the chronic section.

The TGF-β superfamily negative regulator of muscle growth, myostatin, has been extensively examined following RE. Following RE in the fasted condition, myostatin has demonstrated a very clear downdown-regulation in all but 3 studies [156, 183, 211] and there may be a divergence by age [156] in this response. In the PRO/AA fed condition, a similar trend for a decrease in seen, except during glycogen depletion [52] and in 2 studies by Hulmi et al. where they showed that post-exercise PRO feeding actually caused no change or increased mRNA/protein expression myostatin [53, 78]. This finding is interesting as it could suggest a role for myostatin to limit myofiber expansion occurring through the potent mTORC1 signaling and growth response occurring with PRO/AA and RE. This is an interesting finding that warrants further in-depth investigation. Indeed, much attention has been focused on the pronounced changes in the mRNA levels of myostatin, however, the myostain protein remains unchanged or

increases [53, 77, 81, 212] in response to RE suggesting a negative feedback mechanism keeping this process in check as a potential homeostatic mechanism to limit human muscle hypertrophy. In support of this concept, recent work demonstrated that a very low protein diet (11g/d) attenuated the post-exercise myostatin protein expression and type 2 fiber co-localization compared to the post-exercise response on a normal protein diet [213]. The authors suggested this was a compensatory mechanism to low protein intake.

The target of myostatin, the activin 2b receptor, has only been investigated in a handful of studies, but it is clear that mRNA expression of this target is either unchanged or down-regulated by exercise and unchanged by age and/or feeding (**Table 1.5**). Interestingly, folistatin was shown to be up regulated in placebo, but unchanged in PRO condition, suggesting protein delayed or inhibited [152] its action.

Table 1.4. Summary of mRNA responses to resistance exercise in the fasted state

Reference	Subjects	SetsxReps; Mode	Intensity	Norm	Time (PEx)	TNFα	IL-#	MaFbx	MuRF-1	FOX01	FOX03a	FBXO40	PGC1α	MyoD	MyoG	Mrf4	Myf5	p21	Myostatin	IGF-1, MGF	MCH I	MCH II	OTHER
Overal Pattern						↔↑	↑	↓↔↑	↓↔↑	↔↑	↓↔↑	↓↔↑	↔↑	↔↑↑	↔↑↑	↔↑↑	↔↑	↔↑	↓	↓↔↑	↓↔↑	↔↑	
Greiwe et al. (2001)	12 old (81±1 yr) and 12 Y (23±1 yr) M and woM for comparison	3 mon RET (30)/wk of 50-90 min of mixed intensities and repetitions)			Rest before & after exercise (3 biopsies?, immediately after?)	↑PE O; ↓ PT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Willoughby et al (2001, 2002)	M	3 leg Ex; 3x8-10	75-80% 1 RM	GABDH	Rest, 30m, & 6h	-	-	-	-	-	-	-	-	0,↑6	0,↑6	-	-	-	-	-	↑6	0,↑6	-
		Control	none			-	-	-	-	-	-	-	-	↔	↔	-	-	-	-	-	↔	↔	-
Bickel et al (2003)	7 M & F,Rec	2 bouts KE	Max- E stim	18S	Pre, 24h post 2nd bout	-	-	-	-	-	-	-	-	-	↑24	-	-	↑24	-	↔, ↑ BP-4 , ↓ BP-5	-	-	↔ IGFR1, ↔↑ Cyclin D1, ↔ RNA
Psilander et al (2003)	6 M, REC	4x6-14, 4x6-14; LP, KE	to fail	GABDH	Pre, 0, 1, 2, 6, 24, 48h post	-	-	-	-	-	-	-	-	↑0	↑6, ↔ ↑24	↑2	-	-	-	Eabc ↓ 1,6	-	-	↔ IGF-IEbc
Hameed et al (2003)	8 M UT	10x6; KE	80% 1RM	Total RNA, a-actin	Pre, 2.5h post	-	-	-	-	-	-	-	-	↔↑2.5	-	-	-	-	-	↑young	-	-	-
Hameed et al (2003)	19 OM UT	3-5x8-12; RT	?	Total RNA, a-actin	Pre, 24h post last session																		
Willoughby (2003)	9 UT Y M; 21.0 ±2.6 yr	2 RE bouts (7x10 Ecc KE) 3wk apart	150% concentric 1 RM		Pre, Post 6 & 24h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑ caspase-3 activity & protein 6 & 24h for both bouts; ↑ 1st bout > 2nd; ↓ DNA &
Jones (2004)	9 UT Y M; 18-30yr	2 wk limb immobilization followed by 6 wk			a. Pre (48 before immobilization),	-	-	↑0, ↓↔24	MuRF-1 ↑ 34% at	-	-	-	-	-	-	-	-	-	-	-	-	-	Calpain-1 ↑ 24h, 1 wk RE & 6 wk RE; Calpain-2 ↑ 24h & 1 wk RE; b. ↔0 2nd RE & 6 wk RE
Nieman (2004)	30 RT M	4x10; 10 Ex CHO or placebo drinks during RE	Mixed	18S	Pre & Post Exercise (immediately?)	↑	↑ 6,8 & 1 B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Willoughby et al (2004)	18UT M	3 leg Ex; 3x8-10 (3 bouts)	75-80% 1 RM	GABDH	Rest,48h each bout	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑androge-R mRNA & protein after each Ex bout
		Control	none			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bickel et al (2005)	9 M&F, Rec	KE with E-stim, 2 bouts	Max- E stim	18S	Pre, 12, 24, & 48h post 1st bout, 24 & 72 h post 2nd bout	-	-	-	-	-	-	-	-	↑12	↑12,24, 48	-	-	↑12,24, 48	-	1↓ @12, BP-4 ↑ 12, 48 & 96	-	-	↑ Cyclin D1 @12, RNA @ 96h
Coffey et al. (2005)	7M RT	8x5; KE	Maximal	control sample	0, 3h	-	-	↔	-	-	-	-	↔~↑	↔	↔	-	-	-	↔	-	-	-	-
	6M ET					-	-	↔↓	-	-	-	-	↔	↔~↑	↔	-	-	-	↔↓	-	-	-	↑PDK-4
Kim et al (2005)	M & F	3x8-12; KE	70% 1 RM		Pre & 24h post	-	-	-	-	-	-	-	-	↑24	↑24	↓24	-	↔24	↓24h	↑24	-	-	↑ RNA , cyclinD1
Yang et al (2005)	T M & F	3x10 @ 70% 1 RM			Pre, 0, 1, 2, 4, 8, 12, & 24h post	-	-	-	-	-	-	-	-	↑8	↑8,12	↑2,4,8	-	-	-	-	-	-	-
Coffey et al (2006)	M	8x5; KE	max dyna		Pre, 3h post	-	-	↔	-	-	-	-	-	↔3	↔3	-	-	-	↔	-	-	-	-
Kosek et al (2006)	49 M&W	4x10; 3 leg Ex	100% 10RM	18S	Pre, 4, & 24h post	-	-	-	-	-	-	-	-	↔24 UT, ↑24 RT	↑24 Y, ↑24 RT	-	↑24 RT	-	-	-	-	-	↔MRf6
Przybyla et al (2006)	34M Y&O	4x8, 3 leg Ex	80% 1RM	18S	Pre & 72h post	-	↔ 6, 10, AMAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↔ old
Yang et al (2006)	8 M, Sed	3x10; KE	65% 1 RM	GABDH	Pre, 4 & 24h post, MHC I or IIa	-	-	1 > 2a	Pre: 1 > 2a; both ↑4	-	-	-	-	-	-	-	-	-	-	-	-	-	Calpain-1&2, Caspase-3, 1>2a ; Bax/Bcl-2 1<2a; PDK-4 ↑4
Churchley et al (2007)	7M RT	8x5; KE	80% 1 RM	cyclophilin	Pre, 3h post	-	-	↔↓3	↔	-	-	-	-	↔3	↔↑3	-	-	-	↔	↔	-	-	
Costa et al (2007)	15M, UT	6 bouts, 6x15; KE	Max ECC	β-actin	Pre, 24h post 3rd & 6th bout	-	-	-	-	-	-	-	-	↓ 3rd	↑ 3rd	-	↔	-	↓ both	-	-	-	Ki-67 ↑ both; p21cip ↑ 3rd
Jensky et al (2007)	21 Y &O , Rec	6x12-16; KE	max ECC isokinetic	18S	Pre, 24h post	-	-	-	-	-	-	-	-	↔24	-	-	-	-	↔24h	-	-	-	↔ SGT, Follistatin
Kim et al (2007)	66 M&F Y&O	3x8-12; 3 leg Ex	80% 1 RM	18S	Pre & 24h post (RT and UT)	-	-	-	-	-	-	-	-	-	-	-	-	-	↓24 Both	-	-	-	↑ RNA , cyclinD1 in high responders in UT, all with RT; ↔ ActRIIB, p27kip1 or p21cip1
Kostek et al (2007)	5M, Rec	1 leg CON, 1 Leg ECC	Max?	GABDH	Pre, 3, 6, & 24h post	-	-	↓3,6,24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Reference	Subjects	SetsxReps; Mode	Intensity	Norm	Time (PEx)	TNFα	IL-#	MaFbx	MuRF-1	FOX01	FOX03a	FBXO40	PGC1α	MyoD	MyoG	Mrf4	Myf5	p21	Myostatin	IGF-1, MGF	MCH I	MCH II	OTHER
		Overal Pattern				↔↑	↑	↓↔↑	↓↔↑	↔↑	↓↔↑	↓↔↑	↔↑	↔↑↑	↔↑↑	↔↑↑	↔↑	↔↑	↓	↓↔↑	↓↔↑	↔↑	
Kvorning et al (2007)	26 M, Rec	4x10; KE	80% 1 RM	GABDH, 28S,	Pre, 4 &24h post	-	-	-	-	-	-	-	-	↔4,24	↑4,24	-	-	-	↓4	↔ Eb,Ec, Ea	-	-	↔AR, IGF-lea
Louis et al. (2007)	6 M & F, Rec	3x10: KE	70% 1RM	GABDH	Pre, 0, 1, 2, 4, 8, 12, & 24h post	↑0,2,4,8,24	IL-6 ↑4-24; IL-8↑	↓8,12	↑1,2,4	-	↔↓8,12	-	-	-	-	-	-	-	↓1-24	-	-	-	-
Nedergaard et al (2007)	20M, UT	1 leg CON, 1 Leg ECC	Max?	28S	Pre, 3 & 24 h, & 7d post	-	-	CON ↑3; ECC	↑3	-	-	-	-	-	-	-	-	-	-	-	-	-	other ubiquitin-proteosome mRNA"s
Raue et al (2007)	8Y, 6 O F	3x10, KE	70% 1RM	GABDH	Pre, 4h post	↔	-	Y↔, ↑O	↑MuRF-1	-	↔	-	-	↑4	↔	↑4	↔↓4	-	↓4	-	-	-	-
Deldicque et al. (2008)	9M, UT	10x10; LE	80% 1RM	β-2M	rest, 30s, 24,72 hr	-	-	↑0, ↓24	-	-	-	-	↑0	↔	-	-	-	-	↓24	-	↔	↔	↓24 GLUT4; ↑0,72 PCNA; ↑72 Calplain-1, ↔ Collagen-1
		10x10; LE + creatine				-	-	↑0, ↓24	-	-	-	-		↔	-	-	-	-	↓24	-	rest	↑0	rest,↓24 GLUT4; ↑0,72 PCNA, ; ↑72 Calplain-1
Dennis et al (2008)	M	4x8; KE	80% 1 RM		Pre & 72h post	-	-	↔72	-	-	-	-	-	-	-	-	-	-	↓72	-	-	-	-
Dennis et al (2009)	8O W & M	4x8 KE, LC, LP; 2 min rest	~80% 1RM		Pre & 72h post	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Drummond et al. (2008)		1x30, 3x15	20% 1RM 30 sec rest	β-2M	rest, 3h	-	-	↔	↑	-	-	-	-	↑	↔	-	-	↑	↓	↔	-	-	↔CyclinD1,S6K1, mTOR,, ↓REDD1, ↑HIF-1α
		W/ BFR				-	-	↔		-	-	-	-		↔	-	-			↔	-	-	
Mascher et al (2008)	8M UT	2 bouts, 4x10; KE	80% 1RM		Pre, 2, (48, 50 = b4 and after 2nd bout)	-	-	↓48,50	↑2,50	-	-	-	-	-	-	-	-	-	↓2,48, 50	-	-	-	-
Trenerry et al (2008)	Young	3x8 maximal KE	Maximal: young	cyclophillin	rest, 2h	-	↔	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↔↑ c-FOS,c-Myc, VEGF, JUNB
	Old		Maximal: old			-	↑	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑ c-FOS,c-Myc, VEGF, JUNB
Buford et al. (2009)	24 Pm W	3x10 RE on 3Ex	~80% 1RM	β-actin	rest, 3h	↑	↑ (1β, 6,8)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑ JUNB, COX2, c-FOS, IKKB, SOCS2, SAA1, SAA2; ↔IL-(2,5,10,&12)
Coffey et al. (2009)		8x5 LE, then 30 min cycling	80% 1RM, 70% Vo2peak	GABDH	rest, 3h	-	-	↔↑	↑	-	-	-	↑	↑	-	-	-	-	-	↔	-	-	↔PGC1β
		30 min cycling, then 8x5 LE				-	-		↔	-	-	-			-	-	-	-	-	↔, ↓	-	-	
Coffey et al. (2009)		8x5 LE then 10x6sec sprints	80% 1RM, max, 54s rest	GABDH	15min post 1st EX, 15min post 2nd & then 2.5 h	-	-	↔	↑	-	↔	-	↑	↔ ↑	-	-	-	-	-	↔ ↓	-	-	↔↑MTFa,CS
		10x6sec sprints then 8x5 LE	max 54s rest, 80% 1RM			-	-	↔	↑	-	↔	-	↔↑		-	-	-	-	-	-	-	-	-
Roberts et al. (2009)	11 YM	3x10 LP, Squat, KE	~80% of 1RM, Untrained	β-actin	Rest & 24h	-	-	-	-	-	-	-	-	-	↔	-	-	↔	-	↔	-	-	↔AR, MHCemb, IGF-lea
	13 OM					-	-	-	-	-	-	-	-	-	↔	-	-	↔	-	↔	-	-	
Burd et al. (2010)		4x5 to fail	90% 1RM	GABDH	rest, 24h	-	-	-	-	-	-	-	-	↔	↑	↔	↔	-	-	-	-	-	↑ Pax7
		4x~14 to WM	30% 1RM			-	-	-	-	-	-	-	-	↑	↔	↔	↔	-	-	-	-	-	↑ Pax7
		4x~28 to fail	30% 1RM			-	-	-	-	-	-	-	-	↔	↑	↔	↔	-	-	-	-	-	↑ Pax7
Glynn et al (2010)		1h fasted	10x10; KE	GABDH	70% 1RM	-	-	↔	↑	-	-	-	-	-	-	-	-	-	-	-	-	-	↔Caspase3
Roberts et al. (2010); Dalbo et al. (2013)	M	3x10 LP, Squat, KE	~80% of 1RM, Untrained	β-actin	Rest, 2,& 6h	-	-	↓6	↔	-	↔	-	-	↔	-	-	-	-	↓	↔	-	-	↑cip1; ↔kip1, ACTB, ACRV2B; ↑6 (CDK4), 2 (follistatin, SMURF1))
Ternerry et al (2010)	Trained	3x12 maximal KE	Maximal	Cyclophilin	rest, 3h	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑ JUnNB, c-FOS, SCOC3, c-Myc, VEGF
	UT						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Borgenvik et al. (2011)		3x5 warm, 4x10, SiKe	80% 1RM	α-tub	rest, 3h	-	-	↔	↑	-	-	-	-	-	-	-	-	-	-	-	-	-	↔hVPS34,↓↔REDD2,↔↑Rheb
		non-ex	N/A			-	-	↔	↔	-	-	-	-	-	-	-	-	-	-	-	-	-	↔hVPS34,REDD2,↔↑Rheb
Fry et al. (2011), Drummond 2010		10x10 Young	70 % 1RM, 3 min rest	B2M	Rest, 0, 3, 6, 24	-	-	↔	↑3,6	-	-	-	-	-	-	-	-	-	-	-	-	-	↓3~6 GABARAP, ↔ LC3
		10x10 old				-	-	↔	↑3,6	-	-	-	-	-	-	-	-	-	-	-	-	-	↓3~6 GABARAP, ↔ LC3

Reference	Subjects	SetsxReps; Mode	Intensity	Norm	Time (PEx)	TNFα	IL-#	MaFbx	MuRF-1	FOX01	FOX03a	FBXO40	PGC1α	MyoD	MyoG	Mrf4	Myf5	p21	Myostatin	IGF-1, MGF	MCH I	MCH II	OTHER
Overal Pattern						↔↑	↑	↓↔↑	↓↔↑	↔↑	↓↔↑	↓↔↑	↔↑	↔↑↑	↔↑↑	↔↑↑	↔↑	↔↑	↓	↓↔↑	↓↔↑	↔↑	
Roberts et al. (2011, 2011), Dalbo et al. (2011)	10M Young	3 leg Bouts, 9x10 LP, Squat, KE	~60-80% 1RM	B2M + 28S	Rest, 48h each bout	-	-	-	-	-	-	-	-	↑1 (48h)	-	-	-	-	↔	↔	↑4 MHC_{emb}	-	↔ (ACRV2B,CDK2,CDK4, CyclinD1, p21kip, p21cip1, IGF-Iea, FSTL3, FLST, SMURF1); ↓ SGTA
	10M Old					-	-	-	-	-	-	-	-	↔	-	-	-	-	↓ T4	↑all	↔MHC_{emb}	-	
Roschel et al. (2011)		Slow (20deg/sec): 5x8reps Ecc	Maximal: active	RPLPO	rest, 0 & 2h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑2	-	-	-
		Fast (210deg/sec): 5x8reps Ecc				-	-	-	-	-	-	-	-	-	-	-	-	-	-	↔	-	-	-
Wilborn et al. (2009), Taylor et al. (2012)	13 UT M	4x18-20	60-65% 1 RM	B-actin	Rest, 0, 2 & 6h	-	-	-	-	-	-	-	-	↑	↑	↑	↑	-	↓6	-	↑2,6	↑2,6	p27kip ↓6
		4x8-10	80-85% 1 RM			-	-	-	-	-	-	-	-	↑	↑	↑	↑	-	↓6	-	↑2,6	↑2,6	p27kip ↓6
Camera et al. (2012)		8x5; LE	80% 1RM (low Gly)	GABDH	0, 1, 4h	-	-	↓4	-	-	-	-	-	-	-	-	-	-	↓4	-	-	-	-
			80% 1RM (norm Gly)			-	-	↓4	-	-	-	-	-	-	-	-	-	-	↓4	-	-	-	-
Mathers et al. (2012)	OM	3x12 maximal KE	Maximal	GABDH	rest, 2h	-	↑IL-6	-	-	-	-	-	-	↑2	↓2	-	-	-	↓2	-	-	-	↑ MCP1 & MIP-1B;↔ MCP-3, Mac1
	OW		Maximal			-	-	-	-	-	-	-	-	↑2	↓2	-	-	-	↓2	-	-	-	↑ MCP1 & MIP-1B;↔ MCP-3, Mac1
Gundermann et al. (2012)		1x30, 3x15 w/ BFR	20% 1RM 30 sec rest	GABDH	rest,1,3h	-	-	↔	↑ 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		1x30, 3x15 w/ SNP				-	-	↔	↔	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Snijders et al. (2012)	8M	Cycle 4x5m w/ 4x2.5m; 5x10	65% & 45% watt max; 55,65 &75% 1RM	GABDH	Rest, 0, 9h	-	-	-	-	-	-	-	-	↔	↔↑9	↑9	↑↔	-	↓0	-	-	-	↔ (DLK1, Follistatin)
Vella et al. (2012)	Active M	3x8-12	~80% 1RM	GABDH	rest,2,4h	-	IL-6: ↑↑2, ↑4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	same w/ MCP-1 & IL-8
	Control	none	none			-	↔↑	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↔↑
Agergaard et al. (2013)		10x36, SiKE	16% 1RM		Rest, 3h	-	-	↓	↓	↑	↓	↔	-	↑	↔	↑	-	↔	-	↑Eb,↓Ea	↓	-	-
		10x8, SiKE	70% 1RM			-	-	↓	↑	↑	↓	↔	-	↑	↑	↑	-	↑	-	↑Eb,↓Ea	↓	-	-
Apro et al. (2013)		RE: 4x8-10, 4x10-12, 2x fatigue	85%,75, 65% 1RM	GABDH	rest, 1,3h post RE	-	-	-	-	-	-	-	↑3	-	-	-	-	-	-	-	-	-	↑ Rheb, PDK (1,3), ↓ mTOR, hVps34, TSC1, REDD2 (3), ↑ TSC2, S6K1 (1); REDD1 (↑1, ↓3), cMyc, PRC (↑1,↑↑3)
		RE+AE: 4x8-10, 4x10-12, 2x	85%,75, 65% 1RM; 70% VO2 max		rest, 1,3h post RE (15m, 165m post AE	-	-	-	-	-	-	-	↑3	-	-	-	-	-	-	-	-	-	
Reitelseder et al. (2014)		10x8, SiKE	80% 1RM	GABDH, RPLPO	1, 3.5 & 6h	-	-	↓3.5,6	↑1,3.5	↑1,3.5	↑1, ↓3.5,6	↔	-	-	-	-	-	-	-	-	-	-	↑6, RPLP0, ↑1↓3.5 REDD1
Vissing et al. (2013), Lamon et al. (2013), Stefanetti et al. (2014), Moller et al. (2014)	9M	Strength: 4x12 of 3 thigh Ex	12RM: after 10wk Training	RPLPO	Rest, 0, 2.5,5,22h	↑0	↑0 IL-8,6	↔	↔↓0,2.5, 22	↔	↔	↓5	-	-	-	-	-	-	-	-	-	-	↑2.5,5 STARS/36B4; ↓0,2.5,22 SRF, MRTF-A/36B4
	9M	Endurance: 2h cycling	60% VO2 peak: after 10wk Training			↑0	↑0 IL-8,6,1	↑2.5,5,22	↑2.5	↑5	↑ 5	↑ 0,2.5,5,2	-	-	-	-	-	-	-	-	-	-	↓0,2.5,22 SRF, MRTF-A/36B5; ↔STARS/36B4;
		Control: rested for 2 h				↔0	↔0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	repeated biopsy sampling alters myokine mRNA
Stefanetti et al. (2014)	10YM	3x14	60% 1RM	Cyclophilin	Rest, 2h	-	-	↑	↑	↔	↔	↑	-	-	-	-	-	-	-	-	-	-	-
	10OM					-	-	↑	↑	↔	↔	↔	-	-	-	-	-	-	-	-	-	-	-
Ternerry et al (2007), Della Gatta et al. (2014)	Y active M	3x8-12 of 3 leg Ex	~80% 1RM	GABDH	Rest, 2,4,24h	↔	↑IL-6,8 (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑MCP-1,FKN, SOCOC3, c-MYC, , c-FOS, JUNB, (2);↑↑Upa,VEGF (2,4h), ↔LIF, IL-6R

Note: mRNA targets were recorded above if included in two or more studies. Arrows denote direction of change. ↑, significantly increased; ↓, significantly decreased; ↔, no change; ↔↑, trend to increase; ↔↓, tend to decrease; ↔. Red color arrows represent a group difference. Blue arrows represent an effect of feeding. Arrows reresent change from rest (where available) . RM, repetition maximum; LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Ecc, eccentric contractions; Con, concentric contractions; O, old; Y, young; M, men; W, women; h, hour; m, minutes; sec, seconds; Tr, trained, RT, resistance trained, SNP, sodium nitoprusside; RE, resistance exercise; ST, strength trained; ET, endurance trained; BFR, Blood flow restriction.

Table 1.5. Summary of mRNA responses to resistance exercise in the fed state

Reference	Feeding	Sets x Reps; Mode	Intensity:Training Status	Norm	Time course (PEx)	MaFbx	MuRF-1	FOX01	FOX03a	AR	MyoD	MyoG	p21	CDk2	Myostatin	Activin IIb	FLRG	REDD1	REDD2	IGF-1	Rheb	cMyc	hVPS34	Other
		Overal Pattern				↔↓	↑	↑	↓↔↑	↔	↔↑	↔↑	↑	↔↑	↔↓	↔↓	↔↑	↔↓	↔↓	↓↔↑	↔↑	↑	↔	
	20g EAA young					-	-	-	-	-	↑6	↑3,6	-	-	↓	-	-	↔	↓	↑6	↑3,6	↑6	↔	↔ mTOR, s6K1, TCTP, MAP4K3, PRAS40, pri-miR-1-1, miR-133a,TCTP, miR-206, Drosha,, Exportin, TSC1/2; ↓6 pri-miR-1-2, miR-1, pri-miR-133a-1; ↑6 mib2; ↔; ↓6 pri-miR-133a-2, ↑3 pri-miR-206
Drummond et al. (2008)		10x10; KE	70% 1RM	GABDH, B2M; 5SrRNA	rest, 1(fed) 3,6h																			
	20g EAA old					-	-	-	-	-	↑6	↔	-	-	↓	↔	-	↓6	↓	↔	↔	↑6	↔	↔ mTOR, s6K1, TCTP, MAP4K3, PRAS40, pri-miR-1-1, miR-133a,TCTP, miR-206, mib2, pri-miR-1-2, pri-miR-133a-1, miR-1, ; ↓ 3,6 TSC1/2; ↑Drosha, exportin; ↓3 pri-miR-133a-2, ↑6 pri-miR-206
Hulmi et al. (2008, 2009)	15gx2 Whey Old	5x10; LP, 2m	10RM(~75%1RM) TR 5mo, 2x/wk	GABDH/18s	rest,1 & 48h						↔	↑48	↔↑1,48	↑48	↔	↔	↑48							
	brkfast 3h Placebo Old										↔	↑48	↑1, ↔↑48	↔	↔↓1,↓48	↔	↔							↔↑p27
	brkfast 3h Placebo Old	5x10; LP	10RM(~75%1RM) TR		rest,1 & 48h					↔	↔	↔			↓48	↔↓1								↔p27
Hulmi et al. (2007), Ahtiainen et al (2011)	brkfast 3h Placebo Old	5x10; LP	10RM(~75%1RM) UT	GABDHor18s	rest,1 & 48h					↔	↔↑48	↑			↔	↓1								↔p27
	brkfast 3h Control	Control	no ex		rest,1 & 48h					↔	↔	↔		↔	↔	↔	↔							↔p27
Hulmi et al. (2009)	15gx2 Whey YM (N=11)	5x10; LP	10RM(~75%1RM)		rest,1 & 48h, 21wk		-	-	-	↔↑	↔	↔	↑1	↑1,48,21	↔	↓48	↔	-	-	↔	-	-	-	
Hulmi et al. (2007, 2009)	YM Placebo (N=10)	5x10; LP	10RM (~75% 1RM)	GABDH/18s	rest,1 & 48h, 21wk		-				↔	↓1,21	↑1,48	↓21	↓1	↓48	↔		-	↔				
Hulmi et al. (2009)	Young Control (N=10)	5x10; LP	10RM(~75%1RM)		rest,1 & 48h, 21wk		-	-	-	↔	↔	↔	↑1	↔	↔	↔	↔	-	-	↔	-	-	-	
Glynn et al (2010)	EAA + 30g CHO	10x10; KE	70% 1RM	GABDH	2h	↔	↑1,2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↔ Caspase3
	EAA + 90g CHO					↔	↑1,2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↔ Caspase3
Roberts et al. (2010), Dalbo et al. (2013	Whey 25g	3x10 LP, Squat, KE	~80% of 1RM, Untrained	β-actin, ACTB	Rest, 2,& 6h	↔	↔		↔		↑6		↑cip1, ↔kip1	↑6 (CDK4)	↓					↔				↔ACTB, follistatin;↔ RNA, ↑DNA 6, ↑6 (SMURF1), ↔↑2 CyclinD1
	CHO 25g maltodextrin					↓2,6	↔		↔		↑6			↑6 (CDK4)	↓					↓				↔ACTB ,SMURF1 follistatin;↔ RNA, ↑DNA 6, ↓ Cyclin D1
Reitelseder et al. (2011)	Whey 0.3 g/kg LBM	10x8, SiKE	80% 1RM	GABDH, P/T some	rest, 1, 3.5 & 6h	↓3.5,6	↑3.5,6	↑1,3.5	↑1, ↓3.5,6	-	-	-	-	-	-	-	-	-	-	Variants	-	-	-	↑6, RPLP0, ↔ FOXO4
	Casein 0.3 g/kg LBM									-	-	-	-	-	-	-	-	-	-	Variants	-	-	-	
Borgenvik et al. (2011)	BCAA (45%,30% & 25%, Leu, Val, Ileu)	3x5 warm, 4x10, SiKe	80% 1RM	GABDH	rest, 1&3h	< PLA	↑	-	-	-	-	-	-	-	-	-	-	↔	↓↔	-	↔	-	↔	
	non-ex		N/A				↔	-	-	-	-	-	-	-	-	-	-	↔	↔	-	↔	-	↔	
Burd et al. (2012)	20g Whey	Slow Sets (6s Con/Ecc) ~25 reps	TR, 30% 1RM	GABDH	Rest, 6,24,30h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	PGC1α ↑6
		Conn Set (1s CON/Ecc) ~25 reps				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
West et al. (2012)	25g Whey Bolus	8x10; KE	10RM, 2m rest,M	GABDH	1, 3, 5, 26, 28h	↓ 5, 26h	↑ 28h	-	-	↔?↑ @28	-	-	-	-	-	-	-	-	-	-	-	-	-	
			10RM, 2m rest, W			↓ 5,26h	↑ 1,3,5	-	-	↔?↑ @28	-	-	-	-	-	-	-	-	-	-	-	-	-	
Camera et al. (2012)	20g Whey + 40g CHO (2x)	8x5; LE	80% 1RM (low Gly)	GABDH	0, 1, 4h	↓4	-	-	-	-	-	-	-	-	↔	-	-	-	-	-	-	-	-	
			80% 1RM (norm Gly)		0, 1, 4h	↓4	-	-	-	-	-	-	-	-	↓4	-	-	-	-	-	-	-	-	

Reference	Feeding	SetsxReps; Mode	Intensity:Training Status	Norm	Time course (PEx)	MaFbx	MuRF-1	FOX01	FOX04	FOX03a	PGC1α	PGC1β	MyoD	MyoG	p21	Myostatin	REDD1	REDD2	IGF-1	Rheb	Other
		Overal Pattern				↔↓	↑	↑	↔	↓↔↑	↔↑	↔↑	↔↑	↔↑	↑	↔↓	↔↓	↔↓	↓↔↑	↔↑	
Reidy et al. (2013)	~17.5g Whey	8x10 Young	70 % 1RM, 3 min rest	B2M	rest, 3, 5h (2,4hpi)	↔	↑ 5	-	-	-	-	-	-	-	-	-	-	-	-	-	
	~19g Protein Blend					↔	↑ 3,5	-	-	-	-	-	-	-	-	-	-	-	-	-	
Morberg et al. (2014)	~16g EAA (2.6g Leu)	4x10 (80%), 4x15 (65%); LP	80% and 65% 1RM	GABDH	rest, 3h	↔	↔	-	-	-	-	-	-	-	-	-		↔	-		
	~13g EAA (no Leu, L-Gly instead)					↔	↔	-	-	-	-	-	-	-	-	-	↓	↔	-	↑	↑ cMyc, ↔ hVPS34
Donges et al 2012	20g PRO	8 × 8 leg ext	70% 1RM	GABDH	Fast, 1, 4h	-	-	-	-	-	↔	↔	↑4	↑4	-	-	-	-	-	-	
	20g PRO	40 min of cycling	55% peak aerobic power output		Fast, 1, 4h	-	-	-	-	-	↑ 1,4	↑4	↔	↔	-	-	-	-	-	-	↔ GLUT4
	20g PRO	Both	Both		Fast, 1, 4h	-	-	-	-	-	↑ 1,4	↑4	↔	↔	-	-	-	-	-	-	
Areta et al. 2014	Whey Bolus 40g 2x	2 warm-up sets and 4x10reps w/ 3 min rest	80%1RM, Trained	GABDH	Rest, 1, 7,12h	-	↑1	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Intermediate Whey 20g 4x					-	↔	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Pulse Whey 10g 4x					-	↑1	-	-	-	-	-	-	-	-	-	-	-	-	-	
Areta et al. 2014	EB	2 warm-up sets and 6x8reps @ 80%1RM w/ 3 min rest	80%1RM, Trained	GABDH	no effect at baseline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	ED					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	ED - Placebo					↑4	↔	-	-	-	-	-	-	-	-	-	-	-	-	-	
	ED - 15g Whey				Rest, 1,4	↑4	↑ 1	-	-	-	-	-	-	-	-	-	-	-	-	-	
	ED - 30g Whey					↑4	↔	-	-	-	-	-	-	-	-	-	-	-	-	-	
Parr et al. 2014	PRO (25g whey 2x)	(8x5 reps leg ext) & (30 min & high intensity interval (10x30) cycling	80% 1RM, 63% cont, 110% interval (peak power output, PPO)	GABDH	Rest, 2,8h			-	-	-	-	-	-	-	-	-	-	-	-	-	
	ALC-PRO (25g whey 2x)					↓8	↑2	-	-	-	-	-	-	-	-	-	-	-	-	-	
	ALC-CHO (25g maltodextrin 2x)							-	-	-	-	-	-	-	-	-	-	-	-	-	
Camera et al. 2014	Placebo	8x5 reps leg extension & 30 min cycling	80% 1RM, 63% cont, peak power output	GABDH	Rest, 1,4h	↑1	↑1	-	-	-	↑4	-	-	-	-	↓1,4	-	-	-	-	↑all VEGF
	25g Whey					↔	↑1	-	-	-	↑4	-	-	-	-	↑all	-	-	-	-	↑all VEGF
Agergaard et al. (2013)	Constant feeding, SOY,Milk,fat CHO,1300 kcal	10x36, SiKE	16% 1RM: Sed	GABDH/RPLPO	rest, 3h	↓	↓	↑	↔	↓			↑	↔	↑	↔			↑Eb,↓Ea		↑ MRF, ↔RPLP0
		10x8, SiKE	70% 1RM: Sed			↓	↑	↑	↔	↓			↑	↑	↑↑	↓			↑Eb,↓Ea		↑ MRF, p21, mrf6, ↔RPLP0
Nieman (2004)	30 resistance T M; CHO: 21.6 ±0.5 yr, Placebo: 21.3±0.5 yr	2h RE; 10 exercise; 4x10 for each exercise of mixed intensities with CHO or placebo drinks during RE			Pre & Post Exercise (immediately?)																↑ TNF-α, ↔ between groups
Mikkelsen et al. 2010	18–23g PRO & 26–34g CHO w/in an 1h Pex, 2h Pex a sandwitch was given	**200 reps, NSAID INF**	Max Ecc: AE TR	GABDH	Rest, 5h, 8d																↑ IL's 5h, less 8d, COX2 5h, ↔COX1, many others
		201 reps, PLA INF																			↑ IL's 5h, less 8d, ↔COX1, many others
Rahbeck et al. (2014), Stefanetti RJ (2014)	Whey+ CHO	CON: 6x10reps Max	Maximal	RPLPO	rest,1,3,5h	↔	↑1	↑3,5		↔											
	CHO																				
	Whey+ CHO	ECC: 6x10reps Max				↓3,5	↑1,3,↔5	↔		↑3,5											
	CHO																				

Note: mRNA targets were recorded above if included in two or more studies. Arrows denote direction of change. ↑, significantly increased; ↓, significantly decreased; ↔, no change; ↔↑, trend to increase; ↔↓, tend to decrease; ↔. Red color arrows represent a group diference. Blue arrows represent an effect of feeding. Arrows reresent change from rest (where available) .AR, androgen receptor; RM, repetition maximum; LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Ecc, eccentric contractions; Con, concentric contractions; O, old; Y, young; M, men; W, women; h, hour; m, minutes; sec, seconds; Tr, trained, RT, resistance trained, SNP, sodium nitoprusside; RE, resistance exercise; ST, strength trained; ET, endurance trained.LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Si, Single leg; TR, Trained; UT, Untrained; BCAA, Branch Chain Amino Acids; EAA, Essential Amino Acids; CHO, Carbohydrate; PRO, Protein; LBM, Lean body mass; pi, post-ingestion.

AMINO ACID TRANSPORTERS

As discussed in later sections, the increased blood flow, microvascular perfusion and or other possible changes such as electrolyte flux are thought to be involved in prompting the increased amino acid flux seen to occur during and following RE. Amino acids move across gut and muscle membranes through specific membrane bound amino acid transporter mechanisms [214]. We have recently investigated skeletal muscle amino acid transporters (AAT) in a series of studies. The results of these studies has been reviewed elsewhere [215] and are summarized in **Table 1.6**, thus we will not go into intensive detail here regarding this mechanism. In brief, it is very clear that RE is a potent stimulus for the pronounced post-exercise increases in mRNA expression of several select amino acid amino acid transporters in human skeletal muscle (**Table 1.6**). Interestingly, when individuals conduct RE while undergoing energy deficit, we see an opposite pattern with a decrease in *LAT1* mRNA following RE in the fasted or PRO fed condition [51]. At the protein level, changes in these AAT are much less obvious, but the literature thus far suggests that only younger adults, but not older adults demonstrate slight increases in a few select AATs following RE in the fasted and fed states. It is obvious that exercise is the more potent stimulus on these AAT's in that the effect of exercise prolongs the increase in several AAT (compared to nutrition only) and there exists no clear tendency for feeding to enhance the mRNA expression of these markers. If anything, following RE in the PRO/AA fed state, in young adults, there was no change [51, 189] or even a decrease [216] in LAT1 protein compared to the fasting condition where an increase was seen [140, 216]. However, older adults demonstrate a different time course of the select AAT mRNA's and do not have increases in AAT protein

following RE in the fasted and fed state, but may start to demonstrate an trend for an increase in the fed state (**Table 1.6**). Although feeding may not have a clear additive effect beyond that of the fasted condition alone, the type of feeding may, in certain circumstances [121, 217], but not others [189] alter the time course of the mRNA expression of these markers. Basic science studies have suggested that these AAT's are involved in mTORC1 signaling and AA sensing [214]. However the distinct increases in the mRNA expression of select AAT's following RE is a fascinating finding, in that very little is known regarding the functional significance of these AAT in human skeletal muscle. Only one study, to our knowledge, has linked post-exercise AAT induction with adaptations in muscle size and strength following RET [128]. Thus future research should continue to investigate the functional relevance of these AAT's in human exercise biology.

Table 1.6. Summary of AAT responses (protein & mRNA) to resistance exercise in the fasted and fed state

Table 1.6.						Protein					*mRNA*				
Reference	**Feeding**	**SetsxReps; Mode**	**Intensity: Training Status**	**Norm**	**Time course (PEx)**	**ATF4**	**GNC2**	**SNAT2**	**LAT1**	**CD98**	**SLC38A2 (SNAT2)**	**SLC7A5 (LAT1)**	**SLC3A2 (CD98)**	**SLC36A1(PAT1)**	**SLC7A1(CAT1)**
		Overal Pattern				↔↑	↑	↔↔ ↑	↔↑	↔↑	↔↑	↑	↑	↑	↑
Fry et al. (2011), Drummond 2010	Fasted	10x10 Young	70 % 1RM, UT	B2M	Rest, 0, 3, 6, 24	↔↑6	-	↔	↑6,24	↑24	↔	↑3	↑6	↑6	↑6,24
		10x10 old				↔	-	↔	↔	↔	↑3	↑3,6	↑6,24	↑3,6	↑3,6
Churchward-Veene 2012	Whey 25g, w/ 3g leu	4x10-12 reps SiLE & press ((underlined if EX-fed is different than Fed)	95% 10RM, active	GABDH	rest, 1, 3, 5h	mRNA ↑ 1,3,5	mRNA ↑5	-	-	-	-	↑ 1,3,5	↑ 3,5	↑1,3,5	-
	Leu: AA 8g, w/ 3g leu							-	-	-	-	↑ 1,3,5	↑ 3,5	↑1,5	-
	EAA-Leu: AA 12g, 9g EAA &1g leu							-	-	-	-	↑ 1,3,5	↑ 3,5	↑1,5	-
Dickinson et al. (2013)	20g EAA+Leu	10x10 Young	70 % 1RM, UT	B2M	Rest, 3, 6h	-	-	↑3,6	↔↓3	-	↔↑3	↑3,6	↔↑6	↔↑6	↑6
		10x10 old				-	-	↔	~↔↑	-	↑3	↑3,6	↔	↔	↑3,6
Dickinson et al. (2013)	Fasted	10x10 Young	70 % 1RM, UT	B2M	Rest, 3, 6h	-	-	↔	↔↑ 3,6	-	↔	↑3	↔↑6	↔↑6	↑6
		10x10 old				-	-	↔	↔	-	↑3	↑3,6	↑6	↑6	↑3,6
Reidy et al. (2013)	~17.5g Whey ~19g Protein Blend	8x10 Young	70 % 1RM, REC	B2M	rest, 3, 5h (2,4hpi)	-	-	↔	↔	-	↑ 3	↑ 3	↑ 3,5	↑ 3,5	↑ 3,5
						-	-	↔	↔	-	↑ 3	↑ 3,5	↑ 3,5	↑ 3,5	↑ 3,5
Areta et al. 2014	Whey Bolus 40g 2x	2 warm-up sets and 4x10reps w/ 3 min rest	80%1RM, TR	GABDH	Rest, 1, 7,12h	-	-	-	-	-	↑1	-	-	-	-
	Intermediate Whey 20g 4x					-	-	-	-	-	↔	-	-	-	-
	Pulse Whey 10g 4x					-	-	-	-	-	↔	-	-	-	-
	EB				no effect at baseline	-	-	-	-	-	↔	↔	-	-	-
	ED					-	-	-	-	-	↔	↔	-	-	-
Areta et al. 2014	ED - Placebo	2 warm-up sets and 6x8reps @ 80%1RM w/ 3 min rest	80%1RM, TR	GABDH		-	-	-		-	↔	↓, (4h,↓4ED)	-	-	-
	ED - 15g Whey				Rest, 1,4	-	-	-	↔LAT1	-	↔	↓, (1h,↓4ED)	-	-	-
	ED - 30g Whey					-	-	-		-	↔	↓	-	-	-
Dickinson et al. (2014)	10g EAA (3.5Leu)	8x10 KE old	70 % 1RM, UT	GABDH	rest. 1,5, 24	-	-	-	-	-	↑2	↑2,24	↑2,5,24	↑2,5,24	-
	10g EAA (1.8Leu)					-	-	-	-	-	↑2	↑2	↔	↑5	-

Note: mRNA and Protein targets were recorded above if included in two or more studies. Arrows denote direction of change/[hosphorylation. ↑, significantly increased; ↓, significantly decreased; ↔, no change; ↔↑, trend to increase; ↔↓, tend to decrease; ↔. Red color arrows represent a group diference. Blue arrows represent an effect of feeding. Arrows reresent change from rest (where available) . RM, repetition maximum; LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Ecc, eccentric contractions; Con, concentric contractions; O, old; Y, young; M, men; W, women; h, hour; m, minutes; sec, seconds; Tr, trained, RT, resistance trained; RE, resistance exercise; ST, strength trained; ET, endurance trained.

CONCLUSION TO TRANSCRIPTION SECTION

The effect of PRO/AA feeding on the muscle growth response to exercise seems to be limited to the level of signal transduction in post-translational regulation. In the plethora of available studies we could not identify a clear interaction of PRO/AA feeding with RE on transcriptional responses. Rather, it appears that at the transcriptional level PRO/AA feeding is independently regulated [35, 63]. As suggested elsewhere [35], the compiled literature indicates that mRNA abundance of these growth transcripts is not potentiated by feeding in close proximity to exercise. Rather modulation of exercise mode, intensity, and training are more likely candidates for transcriptional modification. There are very few exceptions to this overall pattern [63, 78, 189, 216]. Because of the additive effects seen in mTORC1 activity, future investigations should examine transcriptional modulation of amino acid sensing mechanisms in clinical trials containing a fasted and fed condition.

Human Muscle Protein Turnover following Resistance Exercise in the Fasted and Protein and /or Amino Acid Fed State

See **tables #1.7 and 1.8** for full disclosure of all available studies, to our knowledge.

The aforementioned, energetic, metabolic and mechanical stresses during and following RE play dynamic roles in the control of protein turnover. It seems intuitive that during exercise the primary goal of skeletal muscle metabolism during RE is to maintain energy for contraction, thus prompting a reduction [27] or no change [28], from basal values, in the energy costly process of muscle protein synthesis in human skeletal muscle. However, this inhibition of MPS may be intensity dependent [27] and specific to certain muscle protein sub-fractions. As expected, during high-intensity RE, muscle blood flow is increased and secondary to that, muscle perfusion, shunting and AA flux are as well [28]. In one study, Fujita et al. maximally stimulated MPS with EAA ingestion before exercise and failed to demonstrate an increase during exercise beyond that of the fasted post-absorptive state value [40]. However, the aforementioned reduction in MPS during RE did not occur in the presence of this feeding. This suggests that EAA ingestion may attenuate the reduction in muscle protein synthesis. However, this was a short ~30min session of intermittent (3 min rest periods) high-intensity RE. During a 2 hour session of concurrent AE+RE Beelen and colleagues gave participants CHO or PRO+CHO and assessed MPS [218]. They found that provision of PRO+CHO offered a greater stimulus over CHO during exercise [218, 219], but not in the in the overnight recovery thereafter [219]. Although the authors did not include a resting, post-absorptive assessment of MPS, their values for the CHO condition (~0.05%/h) suggest that MPS was not increased. This data suggests that the increased flux of and provision

of amino acids may attenuate the exercise induced reduction in MPS during exercise. It is possible that MPS could actually be stimulated, at least during rest intervals, or if the exercise is of low intensity. However, this effect is difficult to determine given the rapid changes in pool size inherent with exercise.

Many reports indicate during the immediate (0-1h) period following RE, the metabolic milieu switches from catabolic to anabolic as demonstrated by release of AMP-activated protein kinase (AMPK) inhibition of translation initiation and MPS [9, 28, 29, 40, 220]. During this time, blood flow and lactate levels normalize as the muscle sensitizes for nutrients, presumably due to the increased amino acid flux driven through amino acid transport [69, 140, 189], mTORC1 signaling, particularly though S6K1 [29, 140, 220], and increased insulin sensitivity. Although, contrary to animal literature [221], studies in human models demonstrate that increased AMPK activity and reduced skeletal muscle energy status [51, 52] has less or no inhibitory effect on human post-RE MPS regardless of feeding status. Also, studies at UTMB and by others [34] have repeatedly demonstrated elevated MPS during periods of elevated AMPK phosphorylation [110] and kinase activity [29, 40].

Several other endocrine responses also occur during this period [222], some of which seem to have no impact on immediate protein turnover [164, 167, 168, 223]. Because of this finding we did not examine the effect of feeding on endocrine responses to RE.

The majority of the literature examining protein metabolism with RE and PRO/AA has studied the immediate hours following RE, but in the past few years more investigations have extended their focus to later time periods ~12-24h post-exercise. One

reason why so much of the research has dominated the immediate hours (0-3, 4, 5 or 6h) post-exercise is because of methodological convenience and an initial investigation in the laboratory of Dr. Wolfe at the University of Texas Medical Branch, where participants conducted RE with post-exercise EAA supplementation or no exercise/ supplementation and underwent 24 hours of invasive monitoring of protein turnover as inpatients. It was determined that the phenylalanine uptake assessed in the 3h post-exercise period was similar to the cumulative 24h uptake value [224]. This was convenient at the time, because there are methodological and practical difficulties in measuring FSR over a 24h period. This study utilized assessment of amino acid balance across the leg, not the precursor-product method of MPS. Although, many other investigations have used the later method predominantly to assess the early post-exercise response. In the intermediate post-exercise recovery (1-6 h), skeletal muscle is highly anabolic and sensitive for nutrients as evidenced through elevations in mTORC1 signaling, amino acid transport mechanisms and MPS [9, 31, 140, 189, 225] (**Table 1.8**).

Several assumptions and many different methodological approaches explain some of the inherent variability with the in vivo assessment of human MPS [24]. Investigators have used different analytical techniques, several different tracers (such as phenylalanine vs. leucine), tracer labels ($^{2}H_5$, 1-^{13}C, $^{13}C_6$ to name a few), and precursors (enrichment in blood, muscle or tRNA) or had varying number of and amount of time between biopsies. Because of methodological constraints comparison of across labs restricts meta-analysis. Thus, direct comparisons of qualitative values across laboratories should be interpreted with caution. Nonetheless, some general trends can be taken from examining the literature examining MPS following RE with or without PRO/AA feeding. For mixed-

muscle protein synthesis, increases from a resting value of ~0.05-0.07%/h to ~0.07-0.12%/h are common (**Tables 1.7 & 1.8**). It is rare to see a value for MPS above 0.15% per hour following RE, but these values are highly dependent on several methodological choices. For myofibrillar MPS, it is common to find a maximal stimulation ~0.07-0.09% per hour following RE compared to a resting value ~0.02-0.05% per hour. However, the duration and magnitude of post-RE MPS is highly dependent on the exercise intensity and volume [32, 33, 226, 227]. These "maximal" values appear to only stay elevated for about 1-3 hours before starting to decline, depending on the exercise intensity, precursor and muscle fraction studied and the type and timing of the PRO/AA feeding.

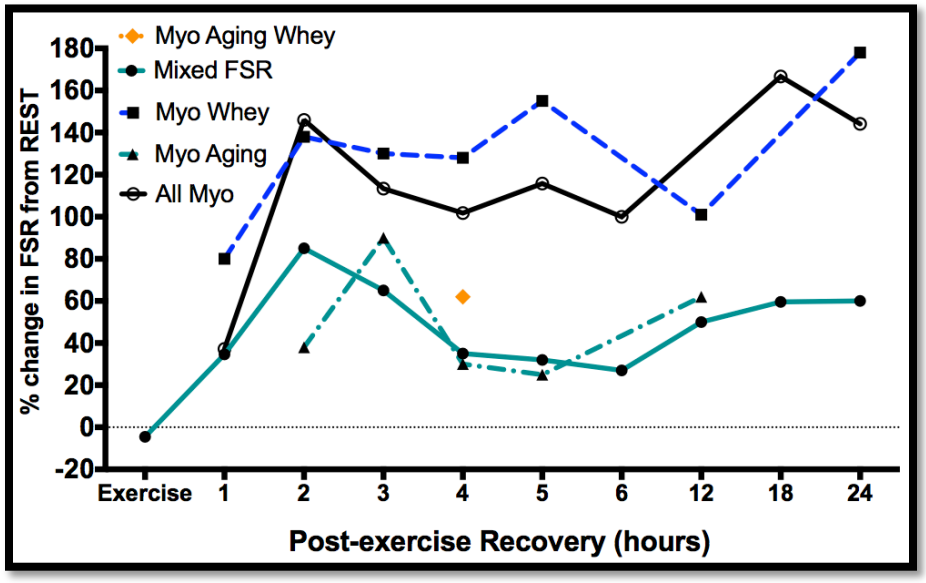
Because of methodological differences, absolute values should not be compared across laboratories; however, some information can be gleaned from the changes occurring in each investigation. In order to provide a comprehensive view of the effect of PRO/AA on post-exercise MPS we examined all the literature and estimated the percent change in MPS in studies with PRO/AA feeding and RE. The following comparisons, of estimated mean responses, if present, were highlighted in these the studies; 1) fasted post-exercise MPS vs basal resting values (Ex-Fast vs rest) 2) vs PRO/AA fed resting values vs basal resting values (Fed vs rest) 3) PRO/AA fed post-exercise MPS vs basal resting values (EX-Fed vs rest) 4) PRO/AA fed post-exercise MPS vs fasted or CHO placebo post-exercise values (Ex-Fed vs Ex-PLA/CHO) 5) PRO/AA fed post-exercise MPS vs fed resting values (Ex-Fed vs Fed) 6) Fasted post-exercise MPS vs fed resting values (Ex-Fast vs Fed). These comparisons were examined over early late and entire post-exercise periods of varying duration (**Table 1.9**).

Resistance exercise alone exerts an obvious increase in post-exercise MPS (**Tables 1.7, 1.8 & 1.9**), and although the magnitude may vary between investigations, it appears that post-exercise mixed MPS increases ~49% from resting basal values. Myofibrillar MPS increases to similar extent, ~57%, and the collagen fraction is most sensitive with a ~107% increase. 2 & 3pool methods appear to be less responsive in this condition, with only ~23% increases from basal resting values. The average fasted state post-RE increase in MPS for all studies and methods across all time periods suggests a post-exercise increase of 52%. Although the magnitude and duration of MPS response is highly dependent on exercise intensity/volume [31, 34] and the status of the population studied [31, 91], it appears that a fatiguing bout of RE, studied in the fasted state results in multi-phasic post-exercise MPS responses. A sluggish increase in MPS peaks somewhere 2-3h post-exercise (~60-70%) declining ~4h, slightly increasing in the ensuing hours, decreasing during sleep and then rebounding the following morning. The prevailing theory is that provision of exogenous AA during the post-RE periods can further increase and prolong MPS.

The maximal MPS response following nutrition alone (no exercise) is rather transient in that it is in only captured in the first few hours post-ingestion, when MPS typically doubles (~0.10%/h) [228-231]. Indeed, examination of the literature suggests increases during the first hour or two post-ingestion with Fed vs Rest MPS of ~92%, 56%, 81% and 73% for Myofibrillar, Mixed, 2 or 3 pool and Sarcoplasmic MPS, respectively. There is no information regarding this comparison with the mitochondrial muscle protein fraction.

Throughout the 1-6h post-exercise period, the muscle may reap additional benefit from elevated [56, 108, 111, 232-234] and, end of this 3-6h period, prolonged aminoacidemia [65, 235-237]. The AA may be provided in the form of crystalline EAA, protein hydrolysate, or isolate, such as whey protein or other high quality protein (see Figure 1.3 for a theoretical construct). The additional increase in MPS with ingestion of whey [9, 44, 51, 52, 57, 65, 108, 111-113, 151, 167, 189, 225, 232-235, 237-245], casein [65, 232, 235, 239, 241], soy [241, 245, 246], milk [151, 246, 247], egg [233] and beef [248, 249] following exercise are driven through elevations in insulin and amino acids, which enhance mTORC1 signaling and translation initiation and elongation [52, 56, 237] through combined, yet presumably independent mechanisms.

Figure 1.3. Percent change approximation in human skeletal mixed-muscle and myofibrillar FSR over the course of a 24h recovery period following a bout of moderate-hard intensity resistance exercise in the fasted and whey protein fed state in young and aged subjects.



The majority of research has been confined the Ex-Fed vs Fast comparison, which elicits the highest rates of post-exercise MPS as evidenced by changes of ~129%, 108%,

47%, 170% and 54% for Myofibrillar, Mixed, Mitochondrial, 2 or 3 pool and Sarcoplasmic MPS, respectively. The average increase in MPS for all studies and methods across all time periods suggests a post-exercise increase of 123%.

To determine the effect of protein ingestion on enhancing the MPS response, a comparison to exercise in the fasting or carbohydrate fed condition is clearly required (Ex-Fed vs Ex-PLA/CHO). This comparison has been made [9, 29, 34, 40, 41, 43, 44, 47, 51, 52, 54, 65, 69, 151, 232, 233, 235, 238, 240, 242, 244, 245, 248, 250-257], albeit in restricted conditions, due to the logistic difficulty of procuring additional subjects or biopsy samples. Interestingly, only two studies, from the same laboratory, have examined the effect of PRO/AA feeding on collagen post-RE MPS. They found no effect, and even a slight (non-statistical) decrease in collagen MPS was evident in young adults [34], yet an effect (~50% post-exercise increase) was discovered in older adults [235]. With myofibrillar MPS a consistent post-exercise additive effect (~45%) of PRO/AA on MPS has been demonstrated. This effect has been demonstrated regardless of glycogen depletion [52], energy deficit [51] or inclusion of concurrent AE with RE [44] suggesting this effect is rather robust. As further challenge to the dogma of a post-exercise "anabolic window"; examination of the various time periods, of 2h or more, when myofibrillar/mixed MPS was assessed, does not seem to indicate an optimal time for ingestion of PRO/AA to maximize the effect. Indeed, anabolic sensitivity to PRO/AA following RE has been shown to be similar at 1 and 3h post-RE [258] and is obvious all the way out to 24h post-exercise in the myofibrillar protein fraction [250]. These data highlight the ability of exercise to sensitize the muscle to amino acids during post-exercise recovery. However, given the multi-phasic response of MPS in the fasted state,

the additive effect of PRO/AA should be tested at various post-exercise time points to determine the most effective synergism/interaction of PRO/AA feeding and MPS. A recent investigation examined the repeated timing and dosing of PRO/AA for optimizing the post-RE MPS [259]. They suggested that repeated periods of AA flux from post-exercise ingestion of 20g of PRO every 3h was more effective than 40g every 6h or 10g every 1.5h at maximizing myofibrillar MPS throughout a 12h period [259]. However, the optimal timing and dosing of protein supplements around the typical meal patterning is unknown. From the available literature, it seems that protein dose [51, 233, 238, 242, 245, 248] rather than exercise intensity [34] mediates this additive effect. Intriguingly, for the Ex-Fed vs PLA/CHO comparison, older adults tend to demonstrate a greater change (~84-168%) in myofibrillar MPS [238, 245, 248] than young adults (~37-58%) [51, 242], when a maximal dose of protein is given. With mixed-muscle MPS a consistent additive effect (~50-70%) of PRO/AA on MPS has been demonstrated, illustrating a similar pattern to the myofibrillar fraction, except that at a maximal dose, young adults can reach a >100% change in MPS [233] with this comparison. Interestingly, only 1 recent study did not demonstrate an additive effect of PRO following RE [47]. A potential explanation is that the subjects were accustomed to the exercise bout via an exercise habituation period preceding the metabolism study. Most investigations examining this comparison have used untrained, recreationally active or older participants (**Table 1.7 & 1.8**). This theory could be questioned with the observation that resistance trained participants have also demonstrated this PRO/AA effect [44, 51, 52, 233, 242]. Yet, even resistance trained participants do not habitually train higher volumes of knee extension exercise as conducted during these metabolic

studies and one could infer that these "trained" participants are still experiencing a novel stimulus. Future examination of this comparison should determine if the additive effect of PRO/AA may be more beneficial on post-RE MPS during a novel vs habituated stimulus. When 2 or 3-pool models were utilized a slightly higher effect is seen (~110% change) with this comparison, yet this additive effect is much more transient, similar to any change in MPS with this methodology, lasting only 1-2 hours post-ingestion [69, 224, 252-255, 257, 258, 260, 261]. The potential explanations for this phenomenon are highlighted later.

When examining the literature, myofibrillar MPS demonstrates an interesting trend in response to RE compared to mixed-muscle MPS in the fed state. If you look at studies giving a maximal dose of PRO/AA containing an 1-3h early and 3-5h late incorporation periods, myofibrillar FSR tends to peak in the later period [108, 111, 113, 234], a phenomenon which we also have seen (unpublished findings). However, this effect is not seen when the later assessment is extended into the 6h post exercise [65, 243]. As mentioned earlier, energy status and AMPK activity do not seem to affect mixed-muscle protein synthesis. However, it is interesting to speculate that energy status or other fiber type specific mechanisms may control this delayed increase in myofibrilar MPS, which seems to be intensity dependent, at least in the fasted condition [34]. Also, there is no information regarding this comparison on the sarcoplasmic or mitochondrial muscle protein sub-fractions.

Several studies have demonstrated an additive effect of feeding, in some cases, with PRO/AA [44, 52, 54, 262], but not carbohydrate ingestion during an early post-exercise time frame (0-4h) post-exercise. However, others have shown that following

exercise, the effect of a maximal dose of nutrition on maximizing MPS is similar to that of to that of nutrition only in some studies [108, 111] but not others [240, 241, 263], yet exercise may serve to prolong the duration of MPS. Interestingly, older adults tend to display a marked ability to separate this combined effect of feeding with exercise vs. feeding alone [108, 111-113, 238, 239, 245], at least for the myofibrillar muscle protein fraction. In this muscle protein sub-fraction the EX-Fed vs fed response is ~33% greater on average. For a maximal PRO/AA dose, this effect is non-existent in the first 3 hours post-exercise [108, 111, 113] and when PRO/AA is co-ingested with carbohydrate and fat [113]. With a maximal dose, this effect is evident at 3-5h [108, 111] and 24h [32, 224, 250, 263, 264] following high-intensity RE. Also, low-intensity RE may potentiate this effect even out to 8-10h post-exercise [265]. Yet, this effect of exercise in the fed state is altered with resistance training [48, 263, 266]. At the same absolute intensity, a decrease in MPS is observed and at the same relative intensity the magnitude is increased [263] or unchanged [48], but the time course of the MPS response is shorted [263].

Many have highlighted the transient effect of nutrients (AA) on muscle protein synthesis, while extracellular AA is maintained; a phenomenon termed the "muscle full" effect [228]. We believe that this effect is largely dependent on the sensitivity of the muscle to nutrients and is most often regulated by physical activity (exercise) or lack thereof [34, 108]. We propose that in exercise-stimulated muscle, this “full effect” is attenuated and it is more likely for prolonged amimoacidemia to have an effect on extending a higher rate of MPS. It is interesting to speculate that attenuation of this “full effect” is partly a consequence of the muscle perfusion and swelling that transiently enhances myofiber size in the hours or days following resistance exercise. Further, the

"full effect" first postulated in [228] is most likely to occur when the muscle AA pools are rapidly filled with a large bolus of a quickly digested protein, such as whey. Thus blending protein sources with different digestion rates may confer a potential benefit have just enough AA pool expansion to signal additional MPS while delaying this "filling" and subsequent effect by not overfilling the pool. This effect may be especially relevant in exercise-sensitized muscle as AA flux is increased (**Figure 1.4** for a theoretical construct).

Following RE, both MPS and muscle protein breakdown (MPB) are increased compared to rest; yet net balance is less negative [69, 104, 267]. In the fed state, FSR increases to a greater extent, FBR is thought to slightly decrease, presumably due to insulin and/or AA mediated effects, and net balance becomes positive [110, 253, 266]. It is clearly obvious that carbohydrate (CHO) ingestion does not cause pronounced stimulation in post-exercise MPS [109, 110, 254, 261, 268], however, many have suggested, based on limited evidence from early studies, that CHO may further enhance muscle protein anabolism by causing further, but slight, reduction in estimates of MPB [257, 261]. More recently, several studies have shown that no further reduction in estimates of MPB is evident by adding CHO to PRO [109], increasing the CHO dosage [110] or altering the timing of the CHO dose [269]. Due to the inherent difficulties in obtaining a precise assessment of MPB, it is unsurprising investigations have been unable to find nutritional intervention or age related effects on MPB. One chronic RET study has demonstrated that adding CHO to Pro has no additional effect on long term outcomes [270] although, another study indicated that addition of CHO to EAA was more effective than EAA alone in enhancing long term outcomes [271], presumably due to a CHO

induced blunting of cortisol and 3MH estimated myofibrillar protein degradation [271, 272]. More research needed to be conducted to resolve these conflicting findings.

Until more recently, less was known concerning the MPS response in the later period (6-24 h) [224], when it was demonstrated that a single bout of RE improves the MPS response to nutrition during sleep [244] and 24 hours post-exercise, in the morning after [9, 10, 37, 224, 250, 267, 273]. These changes are likely due to increased amino acid transporter mechanisms, improved insulin sensitivity and elevated MTORC1 signaling [9, 140, 250].

Table 1.7. Summary of human skeletal MPS & MPB responses after RE in the fasted state

Author	Subjects	Study	Tracer	# reps	Exercise	Protein Fraction	FSR Bx Time PEx	Group	MPS (fasted, %/hr) Rest	Ex	PEx	Net Bal	Age Dif	Other Notes
MacDougall et al 1995	23±2	Effect of Ex on MPS	2-[^{13}C] Leu	lots	4xfail @80%1RM, 12 set to fail 3 bicep Ex, 3-4m rest	Mixed	31-41h infusion	Ex vs Cntl	0.041	-	0.047	-	-	Non ExArm served as control, BB Muscle
Biolo et al. 1995	UT, 5MW, 24±5	Effect of RE on protein turnover	[$^{13}C_6$] Phe	146	LP, squats, KCs, KE 4,5X8,10 75%	Mixed	During 3hr PEx	Ex vs Cntl	0.045	-	0.11	↑	-	100% ↑ MPS & 50% MPB
				~120	3x6-10 @ 65-80%1RM WB RE, 60-90s rest			RE	~0.04	-	0.05	-	-	
Tipton et al. 1996	Swim-TR, 7W, 20±1	Effect of Swim, RE & SW+RE on PEx MPS	[$^{13}H_6$] Phe	lots	Swim=4600m	Mixed	1.5-6.5h	Swim	-	-	0.065	-	-	4 visits: Rest, Swim, RE, SW+RE; Post Deltoid muscle
				lots	Both			Swim+RE	-	-	0.085	-	-	
Phillips et al. 1997	Active, UT, 4M, 4F, 23±1	To examine the time course of PEx MPS & MPB	[$^2H_5$] Phe	64	10m cycle warmup KE 8x8 @80%con1RM, 2 grps con/ecc; no diff b/w	Mixed	Day1: Rest Day2: 0-3h Day3: 21-24h Day4: 45-48h	Rest 0-3h 21-24h 45-48h	~0.06 - - -	- - - -	- ~0.13 ~0.09 ~0.08	- ↑ ↑ ↑	- - - -	just exercise, 4 visits: rest, 3, 24, 48h, FBR increased, but net bal less negative
Biolo et al 1999	UT, 5M, 29±5	Effect of insulin INF on resting & PEx MPS	[$^{13}C_6$] Phe	146	10m cycle warmup, Inc LP: 5 x10 @12RM, Squat, KC, KE: 4x8 @10RM, 2m rest	Mixed	1-4h 4-7h, Ex: 1-4h	Rest Insulin INF	0.048 -	- -	0.095 0.075	↔↑ ↑	- -	Also use 3-pool Model. Insulin no added effect on PEx MPS, ↔↓ MPB,
Fowles et al. 2000	Rec, 8M	Effect of stretching on MPS	1-[^{13}C] Leu	ISO	IM Active ISO ~= to max passive stretch of 40% MVC ~27m volitional fatigue	Mixed	10-22h	isometric stretch	0.049 0.067	- -	0.074 0.086	- -	- -	2 separate trials isometric versus stretch, same leg versus control; Soleus Muscle
Trappe et al. 2002	Rec, 8M, 25±3	Effect of NSAID & Ecc Ex on PEx MPS	[$^2H_5$] Phe	100-140	Unilateral: KE 10-14x10ecc of 120%con, 60s rest	Mixed	24h	PL AECT IBU	~0.08 0.08 0.085	- - -	~0.14 0.085 0.105	- - -	- - -	unilateral ex versus non ex leg as control
Pitkanen et al. 2003	Active, fit, 6M, 26±5	Effect of RE on protein turnover & AA []	[$^2H_5$] Phe	129	4-5 leg exercises 1-3x1-10reps @10RM or control	3-Pool	1,~3h PEx	Exercise	-	-	↔,↑	↔	-	21% ↑ MPS & 17% MPB, non-significant, via 3-pool
Durham et al. 2004	Rec, 5M, 2W 27±3	Protein turnover during RE	[$^2H_5$] Phe	144	LP, 8x10 @70% 1RM; KE, 8x8 @~80%1RM	3-Pool	Pre & imed post	Exercise	↔	↔	-	↔	-	No change via 3-pool
Trappe et al. 2004	Rec, 8M, 27±4	Effect of exercise on soleus PEx MPS	[$^2H_5$] Phe	180	Unliateral: 4x15 70%1RM of st&/bent/seated calf	Mixed	0-3h	control	0.051	-	0.069	-	-	Soleus Muscle
ShefMoore et al. 2005	Rec, 6M, 22±2 Rec, 6M, 69±1	Effect of aging on early PEx MPS in the fasted state	[$^2H_5$] Phe	48	KE 6x8 @80% 1RM, 2warmup sets	Mixed	0-10min, 0-1, 0-3 hour	Young Old	0.072 0.076	-	0.072,0.091,0.102 0.12,0.089,0.079	↔ ↔	yes, minor	crossover design, Rest & Recovery same infusion, 3-pool not much change
Dreyer et al. 2006	UT, 7M,4W, 27±2	Explore effect of MPS time course during & ealry recovery after MPS	[$^2H_5$] Phe	100	KE 10x10 @70% 1RM, some subjects 60-65%, 3m rest	Mixed	Rest, dur Ex, 0-1 &1-2h PEx	N/A	~0.063	0.045	0.085,0.095	↔	-	MPS ↓ during Ex & rebound @ 1 & 2h post
Carrithers et al. 2007	Active, 6M, 6W, 6M, 26±2	Effect of adding AE to RE on PEx MPS	[$^2H_5$] Phe	>80 >80	SiL RE+AE 90m @ 60% 4x10@80%1RM KE,LP	Myo	0-4h	AE+RE RE	- -	- -	0.01 0.092	- -	- -	1leg RE only, 1 leg RE+AE
Fujita et al. 2007	UT, 6M, 32±2	Effect of low intnesity RE & BFR on PEx MPS in young men	[$^{13}C_6$] Phe	75	20%1RM 1x30, 3x15, 30s rest, BFR 1x30 bilateral KE	Mixed	0-3h	RE only RE+BFR	~0.055 ~0.06	- -	~0.06 ~0.085	- -	- -	2 visits: RE or restrict blood flow +RE
Drummond et al. 2009	UT, 8M, 29±2	If Effect of RE on MPS is inhibited by Rap	[$^2H_5$] Phe	110	bilateral cybex KE 11x10 @70%1RM, 3m rest	Mixed	0-2h	Control Rap	0.06 0.061	-	0.095 0.058	-	-	Rap blocks contraction-induced increase in human MPS
Fujita et al . 2009	13M, 9F, 26±3	Effect of EAA timing on time course of EX & early PEx MPS	[$^2H_5$] Phe	100	KE 10x10 @70%1RM	Mixed	Rest, dur Ex, 0-1, 1-2, 0-2h PEx	Fast	0.06	0.047	0.08,0.09,0.073	↔	-	2 groups: EAA+CHO 1h before Ex & Control (Ex + no fed)
Mayhew et al. 2009	UT, 8, 28±1 UT, 7O, 5±1	Effect of age on novel PR MPS 24 h PEx	[$^2H_5$] Phe	~30-36	3x10-12RM on squat, LP & KE	Mixed	24-27h	Young Old	0.055 0.055	-	0.11 0.065	-	-	No relationship with PEx MPS & muscle hypertrophy
Moore et al 2009	6M, 29±2	Effect of egg protein dosing on PEx MPS	[^{13}C] Leu	80-100	Bilateral: 4X8-10 LP, KE, KC	Mixed	1-4hr Post Ingestion	0	NONE	-	0.053	-	-	All measures were PEx
Burd et al. 2010	16M, 23±1	effect of KE RE with COX inhibitor or placebo	[$^2H_5$] Phe	100	unilateral high-intensity ECC KE	Mixed	24 hr post	Cox-2 placebo	0.056 0.074	-	0.108 0.091	-	-	COX inhibitor does not blunt PEx MPS

Author	Subjects	Study	Tracer	# reps	Exercise	Protein Fraction	FSR Bx Time PEx	Group	MPS (fasted, %/hr)			Net Bal	Age Dif	Other Notes
									Rest	Ex	PEx			
Kumar et al. 2009	Young: 25M, 24±6 Old: 25M, 70±5	Effect of age & work matched exercise with varying intensities on early PEx MPS timecourse	[13C] Leu	81	3x27= 20%1RM	Myo	0-4h	Young 20	0.039	-	0.06	-	-	subjects separated into different groups, Only when all the groups were combines, was old dif vs young
				42	3x14= 40%1RM			Young 40	-	-	0.068	-	-	
				27	3x9= 60%1RM			Young 60	-	-	0.095	-	-	
				24	3x8= 75%1RM			Young 75	-	-	0.105	-	-	
				18	6x3 90%1RM			Young 90	-	-	0.094	-	-	
					exercise with each leg			Old 20	0.043	-	0.041	-	YES	
					Unilateral KE/flex (1-2s)			Old 40	-	-	0.045	-	YES	
					2m rest			Old 60	-	-	0.067	-	YES	
								Old 75	-	-	0.065	-	YES	
								Old 90	-	-	0.064	-	YES	
	ALL intesities	60-90% collapsed data			averaged		(0-1,1-2,2-4)	Young	0.04	-	0.058,0.108,0.055	-	-	
					60–90% 1 RM			Old	-	-	0.045,0.075,0.048	-	YES	
Dreyer et al. 2010	9YM, 27±2 8YW, 26±3	Effect of sex on early PEx MPS	[$^2H_5$] Phe	100	bilateral cybex KE 10x10 @70%1RM, 3m rest	Mixed	rest, 0-2h	Men	0.057	-	0.085	-	-	Similar increases in PEx between men & women
								Women	0.06	-	0.091	-	-	
Doessing et al. 2010	sedentary, 10M, 30±2	Effect of exercise & recombinant human GH (rhGH) on PEx muscle & tendon PS in young	1-[13C] Pro, [15N] Pro	100	unlateral KE 10x10 @70%1RM	Myo/Col	24hr post exercise	Control	0.047	-	0.05/0.03	-	-	14 day administration of 33-50 microg kg(-1) day(-1), rest leg was control. Pattella tendon also
								rhGH	0.049	-	0.051/0.06	-	-	
Fry et al. 2010	?, 7OM, 70±2	Effect of low intnesity RE & BFR on PEx MPS in old men	1-[13C] Leu	75	20%1RM 1x30, 3x15, 30s rest, BFR 1x30 KE	Mixed	2.5-4.5 (rest) 4.5-4.75 (RE, BFR)	Control	0.054	-	0.052	-	-	BFR increases MPS in older men
								BFR	0.049	-	0.077	-	-	
Holm et al 2010	UT, 20M, 25±1	Effect of contraction intensity & feeding on MPS.	[^{13}C] Leu	LL: 36 HL: 80	Low-load leg @17% 1RM ((1 rep every 5th s for 3 m)); High-load leg @70% 1RM,	Myo Col Myo Col	0-4h, Pre -2:45-45, Post: 30m, 3h, 5:30h	LL	0.08	-	0.115,0.095	-	-	unilateral RE, Fed during infusion every 30min or not fed, intensities equalized for total lifted load. alternating legs during Ex
								LL	-	-	0.14,0.188	-	-	
								HL	0.08	-	0.086,0.14	-	-	
								HL	-	-	0.163,0.15	-	-	
Etheridge et al. 2011	Rec, 7M, 21±1	Effect of hypoxia on PEx MPS	2-[^{13}C] Leu	32-48	unilateral 6x8 KE 70% 1RM,	Myo	0-3.5h	Normoxia	0.033	-	0.104	-	-	Hypoxia blunts Pex, not basal MPS; Normoxia (22% insp O2), Hypoxia (12% insp O2 3.5h)
								Hypoxia	0.043	-	0.06	-	-	
Dideriksen et al. 2011	Rec, 15OM, 9OW, 68	Effect of whey vs casein (pre/post) ingest on PEx MPS	[^{13}C] Leu	80	5X8 on unilateral KE & bilateral LP at ~80% 1RM	Myo/Col	30-390 min post RE	Water Imed PEx	-	-	0.07	-	-	All measures were PEx
Fry et al. 2011	16MW, 27±2 16MW, 70±2	Effect of aging on MPS 24h time course	[$^{13}C_6$] Phe	100	10x10 KE, 70 % 1RM, 3 m rest	Mixed	rest, 0- 3, 3-6, 24-27	Young	0.051	-	0.065,0.078,0.079	-	YES	PEx MPS is attenuated with age over a 24h time course
								Old	0.05	-	0.06,0.063,0.062	-	YES	
Kumar et al. 2012	?, YM, 24±6 ?, 12OM, 70±5	Effect of age, vol & intensity on PEx MPS	[13C] Leu	42	3x8= 75%1RM	Myo	rest, 0-1,1-2,2-4h	Y 40 3set	-		↔	-	YES	PEx MPS is intensity and volume dependent & attenuated with age
				84	6x8= 75%1RM			Y40 6 set	-		↔	-	-	
				24	-			Y 75 3set	-	0.042	0.07,0.12,0.05	-	YES	
				48	-			Y 75 6set	0.04		0.04,0.08,0.05	-	YES	
				42	Unilateral KE			O 40 3set	0.04		↔	-	YES	
				84	2m rest			O40 6 set	-		0.08,0.09,↔	-	YES	
				24	3x14= 40%1RM			O 75 3set	0.04	0.04	0.02,0.06,0.06	-	YES	
				48	6x14= 40%1RM			O 75 6set	-		0.07,0.09,0.057	-	YES	
Camera et al 2012	TR, 8?, 23±3 TR, 8?, 23±4	Effect of glycogen depletion on PEx MPS	[$^{13}C_6$] Phe	50	warmup (2x5 @ 55% 1RM) & 8X5 LP 80% 1RM	Myo	1-4h PEx	Norm Gylcogen depleted	-	-	0.045	-	-	PEx MPS does is not hampered by low muscle glycogen
									-	-	0.049	-	-	
Gundermann et al. 2012	Rec, 6M, 24±2	Effect of reactive hyperemia during low intensity RE on PEx MPS	[$^{13}C_6$] Phe	75	1x30, 3x15 w/ BFR @ 20% 1RM 30 sec rest	Mixed	rest, 1-3h	BFR	0.056	-	0.078	-	-	reactive hyperemia not responsible for BFR induced increase in MPS
								SNP	0.057	-	0.045	-	-	
Res et al. 2012	Rec, 8M, 23±1	Effect of PEx overnight MPS	[$^2H_5$] Phe	128	(8x8 reps LP/KE, ~70% 1RM) (45 m)	Mixed	2330 to 0700 (8hr)	water PLA	-	-	0.048	-	-	All measures were PEx

Author	Subjects	Study	Tracer	# reps	Exercise	Protein Fraction	FSR Bx Time PEx	Group	MPS (fasted, %/hr)			Net Bal	Age Dif	Other Notes
									Rest	Ex	PEx			
Yang et al. 2012	?, 10OM, 71	Effect of whey protein dosing on resting & PEx MPS	[$^{13}C_6$] Phe	100	Unilateral KE	Myo	0-4h post ex	fasted	0.03	-	0.045	-	-	No vs. Fast non exercised
Gundermann eta l. 2014	Rec, 8M, ~25	Effect of Rapamycin on low intensity RE w/ BFR on PEx MPS timecourse	[$^{13}C_6$] Phe	75	1x30, 3x15 w/ BFR @ 20% 1RM 30 sec rest	Mixed	rrest, 0-3, 5-6 & 22-24h, MPB (rest, 6,24h)	BFR	~0.048	-	~0.07,0.05,0.08	↑ 24	-	FBR unchanged, but net bal improved 24hr post in CON
	Rec, 8M, ~25							BFR+Rap	~0.055	-	~0.057,0.05,0.07	↔	-	
Witard et al. 2014	RT, 12?, 22?	Effect of whey protein dosing on resting & PEx MPS	[$^{13}C_6$] Phe	80	unilateral EX (8 x 10 LP,KE; @ 80% 1RM)	Myo	0-4h post ex	0	0.032	-	0.052	-	-	3hr after breakfast
Effect of RET														
Yarasheski et al. 1993	2 M/4 F 4 OM/2 OW	Effect of 2-wk WB RET & Age on MPS & myofib proteolysis	[^{13}C] Leu	>48	WB, 3-4x4-8 reps per Ex at 75-90% 1RM	Mixed	4 h	Y	0.049	-	0.075	-	Basal, yes; PEx, No	Studied 3 h after last bout of Ex, 3d meat free diet, admit day b4 for overnight 12-14hr fast
								O	0.03	-	0.076	↔ 3MH		
Yarasheski et al. 1993	7 M RT, 23±2	Effect of 2-wk GH administration during RE training on MPS	1-[^{13}C] & 1,2-[$^{13}C_2$] Leu	?	~WB 5-10 lifts @ 75-90% 1RM, 3-6d/wk	Mixed	6 h, 2-8h	Initial	-	-	0.034	-	↔	GH has no effect on MPS in experanced weight lifters during Ex training
								GH			0.034	-	↔	
Welle et al. 1995	9 Y (22-31y) 5m,4W 9 O (62-72y) 5m,4W	Effect of12-wk RET, 3d/wk, & Age on MPS & myofib proteolysis	[^{13}C] Leu	>24	3x8 at 80% 3-RM; Ex on 1st & 3rd d as inpatients, & MyoMPS was on 4th d	Myo	6 h	Y	0.061	-	0.062	↑ 3MH	Basal, yes; PEx, No	24 h PEx, No change in normal activity & diet, except for RT, admit to CRC 3d b4, meat free diet
								O	0.041	-	0.045	↔ 3MH		
Hasten et al. 2000	4 M/3 F 3 OM/4 OW	Effect of 2-wk WB RET & Age on myosin heave chain MPS & myofib proteolyis	[^{13}C] Leu	>16	WB, 9 Ex, 2–3x8–12 at 60–90% 1RM	Mixed/MHC	12–13 h	Y	0.048/0.038	-	0.10/0.072	-	Basal, yes; PEx, No 4 Mixed, yes 4 MHC	16 h PEx, may be temporal effect, not training effect
								O	0.037/0.024	-	0.102/0.050	-		
Yarasheski et al. 1999	4 OM 8 OW stretching	Effect of12-wk RET, 3d/wk, & Age on MPS & myofib proteolysis	[^{13}C] Leu	>24	WB Physicial therapy, 8 Ex, 2–3x6–12 at 65–100% 1RM	Mixed	12 h	OM	105/0.056	-	170	-	-	~17 h PEx, MPS inversely correlated with TNFa, mg·kg–1·h–1 absolute rate, on 10 day diet
								OW	95/0.050	-	150	-	-	
								Con	103	-	100	-	-	
Balagopal et al. 2001	19 M/20 OM 12MA(~55y),14 O (65y)	Effect of 10-wk RET, 3d/wk, & advancing age on mixed & MHC MPS	[^{13}C] Leu	>24	WB, 7Ex, 3x8 at 50–80% 1RM	Mixed/MHC	5 h	EX	0.041/0.028	-	**0.066/0.042**	-	Basal, no; PEx, Mixed yes, MHC no	RET ↑mixed & MHC MPS in elderly. 4d PEx, given 5d diet, 2d as outpatients, admitted as inpatients on the evening of day 2, MPS on Day 6., 4-6d since last Ex bout?
								Con	0.039/0.035	-	0.043/0.039	-		
Phillips et al. 1999	3 M/3 F 3 M/3 F; RE Trained (>5y)	Cross-sectional comparision of RT & UT basal & Post-Ex MPS	$^{2}H^{5}$, 15^{N} Phe	80	8 x 10 KE 120% (unilateral) Ex & Rest leg	Mixed	2, 5, 5 40 min, 6h; 3–4 h	UT	**0.045**	-	0.067	< neg	-	RT slightly > basal MPS & no ↑ MPB PEx. Acute Ex only ↑UT MPB: rest, 0.074; PEx, 0.105.
								TR	**0.073**	↔	0.082	<neg	-	MPB: rest, 0.075; PEx, 0.086, overnight stay, refrained from leg RE 4d b4, 4d since last Ex bout
Kim et al. 2005	8 M	Effect of RT on basal & PEx MPS (PEx was same realitve intensity, thus a > intensity post)	[$^{13}C_6$] Phe	80	4 x 10 reps 80% LP, 4 x 10 reps 80% KE, 8-wk training	Mixed/Myo	4 h	UT	0.041/0.027	-	0.093/0.039	-	-	↑ basal Mixed FSR, ↔ MyoFSR post RET, ↑ MPS of non-myofibrillar proteins?, rest & 12h acute PEx, 72h since last Ex
								TR	0.061/0.030	-	0.075/0.043	-	-	

O, old; Y, young; MA, middle Aged, M, men; W, women; OW, overweight; Ex, exercise; vol, volume; phe, phenylalanine; leu, leucine; COX, cyclooxygenase; BB, biceps brachii; RM, repetition maximum; WB, whole body; LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; LC, leg curls; Si, Single leg; Ecc, ECCcontractions; Con, concentric contractions; PEx, post-exercose; MVC, maximal voluntary contractions; NSAID, nonsteroidal anti-inflammatory drug; MPS, muscle protein synthesis; MPB, muscle protein breakdown; FBR, Fractional breakdown rate; AA, amino acids; EAA, essential amino acids; imed, immediate; PEx, PEx; h, hour; min, minutes; sec, seconds; PRT, Progressive resistance training; Rec, recreationally active; RT, resistance trained; AE, aerobic exercise; RE, resistance exercise; ST, strength trained; ET, endurance trained; TR, Trained; UT, Untrained; LBM, Lean body mass; GH, Growth hormone; ACET, acetaminophen group; IBU, ibuprofen group or PL, placebo; BRF, Blood flow restriction; SNP, sodium nitoprusside; N/A, not available; W, watts; Rap, Rapamycin; Fail, exercise to failure; WM, work-matched; INF, infusion, Myo, myofibriillar protein fraction; Sarc, sarcoplasmic protein fraction; Col, collagen fraction.

Table 1.8. Summary of human skeletal muscle protein turnover responses after RE in the fed state

Author	Subjects, Status,N,Age	Study	Tracer	#rep	Exercise	Protein Fraction	FSR Bx Time PEx	Nutrition/Group	Leu g	Nutrition Type	MPS(fasted) Basal	MPS(fed) Ex	MPS(fed) PEx	MPS(fed) Basal	MPS(fed) PEx	Net Bal	Age Dif	Intervention Dif	Other Notes
Welle et al. 1998	UT,6O,66±1 UT,6O,71±2 UT,6O,68±1	Effect of Low, Norm, High PRO diet on PEx MPS	1-[^{13}C] Leu	50	4x10 on day 1&4, 5x10 on day 6, KE, 80%1RM	Myo	Day 7: ~16-23h	Low PRO Norm PRO High PRO	? ? ?	7% PRO (~30g PRO over 7h) 14% PRO (~59g PRO over 7h) 28% PRO(~118g PRO over 7h)	0.065 0.072 0.073	- - -	- - -	- - -	0.088 0.09 0.088	- - -	↓↔↑ N/A N/A	Ex ↑ FSR	Ex @1500h, PEx subjects went to GCRC and fed meal (10kcal/kg) b4 2100h, fed every 30 m ~60% total energy (Ensure)
Biolo et al. 1997	UT,6M,29±5	Effect of AA Inf & RE on MPS	[^{13}C$_6$] Phe	146	None LP, squats, LCs, KE 4-5X8-10 75%1RM	Mixed Mixed	During 3h INF	lots Travasol INF		3hr Travasol 3hr Travasol (1-4hr PE)	0.064 -	- -	0.1 -	0.144 -	- -	N/A N/A	↑ FSR ↑ FSR	Additional AA (from infused AA) ↑ MPS and Ex + AA causes further ↑
Tipton et al. 1999	UT,3M,3W,22±2	Effect of oral AA+CHOingestion on PEx MPS	[^{2}H$_5$] Phe	82	LP, squats, LCs, KE 4,5X8,10 75%	N/A N/A N/A	45m after Nutr (~4-5 h)	40g mix AA 40g EAA PLA	4.4 8.3 0	1L total, given as 100 ml every 18-20 ms (~30 m post exercise to ~4hr post exercise)	No No No	- - -	- - 53	- - -	93 80 -	10 30 -	N/A N/A N/A	↑ NB ↑ NB NB Negative	3-pool kinetics, AV=Bal. AA are necessary for PEx +NB. Non-essential AA are not necessary.
Rasmussen et al. 2000	Active,3M,3W,34±3	Effect of bolus timg of PEx EAA+ CHO on MPS	[^{2}H$_5$] Phe	144	10x8 @ 80% 1RM: LP; 8x8 for KE. 2-m rest	N/A N/A	1,3h	1h 3hr	1.2 x2 1.2 x2	(6 g EAA, 35 g sucrose) or PLA 1 h or 3 h	No No	- -	- -	- -	175 160	90 75	No No	↑ NB ↑ NB	PEx Timg (1 vs 3h) doesn't effect the MPS ↑
Borsheim et al. 2000	Rec,5M,3W,29y	EAA. PRO & CHO vs CHO on Net bal after RE	[^{2}H$_5$] Phe	80	10x8, KE, 80% 1RM	N/A N/A	-2, +4h	EAA+PRO +CHO CHO	~2 0	77g Malto, 18 WPC, 5g AA, 1h PEx 100g Malto, 1h PEx	No No	- -	- -	- -	↑1h ↔	↑ NB ↔	- -	↑ NB NB Negative	↑ NB 60-100, 150m in PAAC, ↔ 2-pool breakdown, NB w/ 2-pool model, MPB ↔
Borsheim et al. 2002	Rec,3M,3F,23±2	Effect of AA composition & CHO on PEx MPS	[^{2}H$_5$] Phe	180	10x10 LP, 8x8 of KE at 80% 1RM	N/A, AV-Bal	pre, 1,2 & 3h	EAA, given 2x	1.2	EAA	No	-	-	-	310	225	No	↑ NB, FSR, inward transport	compare to other studies,for AUC net uptake of phe for dif AA drinks. MAA, mixed amo acids; MPB ↔
Louis et al. 2003	PE students UT,7M,21±1	Effect of adding Creatine to CHO+PRO on PEx MPS	1-[^{13}C] Leu	200flex/ KE	20x10 Cybex, unilateral LC,KE 75%max effort	Myo Sarc	0-3h	CHO+PRO Cr+CHO+PRO CHO+PRO Cr+CHO+PRO	? ? ? ?	3h oral fed CHO (0.3g/kg Malto), PRO (~0.08 g/kg skimmed milk protein powder) +/- 7 g creatine monohydrate every 20 m	- - - -	- - - -	- - - -	0.057 0.062 0.068 0.07	0.164 0.119 0.218 0.22	Phe: 21 Phe: 10 Leu: 72 Leu: 55	- - - -	- - - -	2 visits unilateral ex versus control leg, FED every 20m post Ex CHO+PRO or CHO+PRO+Cr, MPB ↔
Miller et al. 2003	6M,4F	IndePExndent & Combined effects of CHO & AA on PEx MPS	[^{2}H$_5$] Phe	164	10x10 @ ~75% 1RM: LP; 8x8 for KE, h 2-m rest, ~40 m	N/A, AV-Bal	1,2 & 3h	CHO CHO+AA AA	_ 0.54 0.54	35g 1&2hr PE 35g + 6g AA 1&2hr PE 6g AA 1&2hr PE	- - -	- - -	- - -	- - -	↔↕ ↔↕ ↔↕	- - -	- - -	- - -	CHO+AA had > Phe uptake, mor changes in MPB (PEx0, ↔↓ CHO, ↔↑in AA and CHO+AA
Tipton et al. 2004, 2007, 2009	UT,9Y,28±2 UT,7Y,24±3 UT,7Y,23±1 UT,8Y,26±3	Effect of whey, casein or PLA on PEx Net bal Effect of 20g whey PRO b4 RE on Net Bal	NONE?	80 80	10x8 reps @80% 1RM w/ 2 m rest	N/A	-1,1,2,5h	Whey PEx Casein PLA Whey Pre Ex	2.3 1.7 0 ~1.5-2g	20g Whey, 1h PEx, 300ml 20 g Casein, 1h PEx, 300ml Water, 1h PEx, 300ml 20g Whey, imed PreEx, 300ml	No No No No	- - - -	- - - -	- - - -	- - - -	↑1st h ↑1st h ↔,neg ↑ in 1st h PEx	No No No No	Whey=Casein > PLA Pre=Post	AV-Bal, RE & addition of 20g Whey or Casein after RE ↑ NB, Ingestion of whey Pre Ex is same as PEx
Holm et al 2005	UT,5M,3F,30±3 UT,6M,1F,25±2 UT,8OW,56±1	Effect of whey+Leu or PLA on PEx Net bal Effect of PRO+CHO on PEx NB	[^{2}H$_5$] Phe	80 100	LP,mod LP, KE, 6x10RM, 4x10RM	net bal	0.5-4h	PLA Whey+Leu PRO+CHO or PLA	0 3.4+2 -	Water, 1h PEx, 300ml 16.6g+3.4g Leu, 1h PEx, 300ml 10g Soy+milk,+ 31g dextrose	No No -	- - -	- - ↔	- - -	- - ↔	↔,neg ↑ ↑all	No No -	20gWhey=16.6whey+3.4 leu > PLA Nutrients ↑ NB	total whey protein content ↓ & 3.4g of leu added to match AA content to 20g whey (2004 study), the response is similar Nutrients provide ↑ NB in older women
Moore et al. 2005	Rec,8M,22±1	Effect of Ecc/Con Ex & Myo, sarc & Col MPS in the fed state	1,2-[^{13}C] Leu	60ecc workmatchCON	dynamometer ext Ecc 6x10 @max, 2.5m rest, con=matched work to ecc leg	Myo Col	1-4.5, 1-8.5h	Ecc Con Ecc Con	lots	Myoplex every 30m, @ 0.1g/kg/h. At rest: ~ 36g PRO, 320kcal; Exercise: ~67g PRO 59kcals	- - - -	- - - -	- - - -	0.07 - 0.015 -	0.11,0.105 0.09,0.115 0.06,0.059 0.06,0.058	- - - -	- - - -	↑ECC=CON early ↑ECC>CON late ↑ECC=CON all	ECC prolonged myofib MPS. 2 visits : Rest Fed & Ex Fed, one leg ECC KE, one leg Con KE, dynamometer ext Ecc 6x10 @max, 2.5m rest, con=matched work to ecc leg
Koopman et al. 2005	UT,8M,22±1	Effect of CHO, CHO+PRO & CHO+PRO+Leu on PEx MPS	[^{13}C$_6$] Phe	128	8X8 LP & KE at 80% 1RM	Mixed	0-6h PIn	CHO CHO+PRO CHO+PRO +Leu	0 9 54	25g Malto & 25g glucose 33g WPH, 25g Malto&25g glucose 33g WPH, 16.6g Leu, 25g Malto & 25g glucose	No No No	- - -	- - -	NM - -	0.061 0.082 0.095	- - -	N/A N/A N/A	- ↑ ↑ FSR vs. CHO	intermittent for 6h post RE, every 30 mutes FSR: CHO+PRO = CHO+PRO+Leu. Adding Leu to 33g whey ↔ FSR. Insulin AUC ↑ for CHO+PRO+leu . CHO+PRO+leu ↑ WBPB but close to CHO+PRO
Cuthbertson et al. 2006	UT,8M,25±5	Effect of Ecc & Con contractions on PEx MPS in the fed state	[^{13}C] Leu [^{13}C] Val	manysteps	12m step up/down, carrying 25%BW length/short leg, 6m steps, 2x3ms steps, 2m rest	Myo Myo Sarc Sarc Col Col	Rest, 0-3, 0-6, 0-24h	Ecc Con Ecc Con Ecc Con	lots	2h prior to each Bx 45g EAA + 135g CHO (sucrose) to meet participants 24h energy needs	- - - - - -	- - - - - -	- - - - - -	0.042 0.048 0.061 - 0.016 -	0.051,0.133,0.132 0.048,0.118,0.139 0.06,0.146,0.125 0.066,0.14,0.117 0.048,0.051 0.032,0.058	- - - - - -	- - - - - -	↑ECC=CON @ 6&24h, ↔@3h ↑ECC>CON early ↑ECC=CON late	ECC and Con not matched for total work, yet identical increases in Myo and sarcoplasmic MPS, but not Col MPS
Elliot et al. 2006	UT,3M,5F,26±2 UT,6M,2F,28±3 UT,7M,1F,24±1 UT,8YM,20±1	Effect of milk form on PEx Net bal	NONE?	80	10x8 reps @80% 1RM w/ 2 m rest	N/A	-1,1,2,5h	FF milk Whole milk Isocal FFM	<1g <1g ~1-1.2g	FF-milk (237g) 8g PRO, 12 CHO .6 fat Whole-milk (237g) 8g PRO. 11g CHO 8g Fat, 627kcal isoFFM (393g) 14.5g PRO. 20g CHO 1g Fat, 626kcal	- - -	- - -	- - -	- - -	- - -	↑ 1st h, 120&150 m -	- - -	- - -	RE & 3 types of milk, whole milk exhibited ↑ uptake of AA
Koopman et al. 2006	UT,8OM,75±1 UT,8YM,20±1 UT,8OM,75±1	Effect of age & PRO+Leu on PEx MPS	[^{13}C$_6$] , [^{2}H$_5$] Tyr	120	6X10 on LP & KE at 40-75% 1RM	Mixed	0-6h PIn	CHO CHO CHO+PRO (~70g CHO+PRO (~70g	0 0 ~18 ~18	intermittent for 6h post RE, every 30m, 92g Malto & 92g glucose, 60g WPH, ~10g Leu	No No No No	- - - -	- - - -	- - - -	0.06 0.042 0.082 0.072	- - ↑ ↑	Yes Yes Yes Yes	- - ↑ > CHO (same age)	intermittent for 6h post RE, every 30 mutes. WBPB < in CHO+PRO+LEU vs. CHO in both young & old. NB only ↑in CHO+PRO+LEU. young > FSR vs. Old, change vs. CHO+PRO Old +young.
Tang et al. 2007	RT,8M,21±1	Effect of mimal oral Whey PRO to CHO on PEx MPS	[^{2}H$_5$] Phe	64-80	unilateral, 4x8-10, KE,LP, 80%1RM	Mixed	1-3h	Whey+CHO CHO	~1 0	10g WPI + 21g fructose, 227ml, Imed PEx 10g Malto + 21g fructose, 227ml, Imed PEx	- -	- -	- -	0.061 0.049	~0.12 ~0.08	- -	- -	- -	PEx pulse tracer injection. non ex leg control FED Oral

Author	Subjects, Status,N,Age	Study	Tracer	#rep	Exercise	Protein Fraction	FSR Bx Time PEx	Nutrition/Group	Leu g	Nutrition Type	MPS(fasted) Basal	MPS(fasted) Ex	MPS(fasted) PEx	MPS(fed) Basal	MPS(fed) PEx	Net Bal	Age Dif	Intervention Dif	Other Notes
Wilkinson et al. 2007	TR,8M,22±1	Effect of soy vs milk on PEx MPS	1-[^{13}C) Leu, [$^{2}H_{4}$] Phe	80	4x10 @80 1RM, 2m rest, LP,cur l& KE (single leg)	Mixed	0-3h	Milk	~1.5g	Soy w/ Malto, 500mL drink 745 kJ, 18.2g PRO, 1.5g fat, 23g CHO	no	-	-	-	0.100	-	-	-	RA-FV Bal, whole-body & muscle protein turnover. Milk prolongs MPS
								Soy milk	~1.8-2g	milk w/ lactose 500mL drink 745 kJ, 18.2g PRO, 1.5g fat, 23g CHO	no	-	-	-	0.070	-	-	-	
Beelen et al. 2008	UT,10M,20±1	Effect of CHO or CHO+PRO during AE+RE on MPS	[$^{13}C_{6}$] Phe [$^{2}H_{2}$] Tyr	RE90,AE40m	2h RE-like acitivity in combination with Intervals, 4x5m cycle@65%Wmax	Mixed	0-2h	CHO	lots	1.5 ml/kg every 15 m during Ex @ dose of 0.15 g/kg/h CHO (50% glucose& 50% maltodextrin), w/ or w/out 0.15 g/kg/h CPH	-	-	-	-	DuringEx,0.06	-	-	-	Added PRO increased MPS and WBPS during concurrent Ex vs CHO ingestion only. Nutrition was given in small pulses. 2 visits FED IM
								CHO+PRO			-	-	-	-	DuringEx,0.088	WB ↑	-	↑ > CHO	
Beelen et al. 2008	UT,20M,20±1	Effect of CHO or CHO+PRO during AE+RE on PEx MPS	[$^{13}C_{6}$] Phe [$^{2}H_{2}$] Tyr			Mixed	0-2h	2h W	none	water only	-	0.06	-	-	-	-	-		
								9h W			-	-	0.057	-	-	-	-		
							0-9h	2H CP	lots	1.5 ml/kg every 15 m during Ex @ dose 0.15 g/kg/h CHO (50% glucose& 50% malto) & 0.15 g/kg/h CPH, 2 bolus @ 30 & 90m PEx	-	-	-	-	DuringEx,0.083	-	-	↑ > CHO, during Ex but not recovery	2 visits FED IM, total 11h FSR and WBPB was identical.
								9H CP			-	-	-	-	0.056	-	-		
Dreyer et al. 2008	UT,8M,27±2	Effect of Leu-EAA on PEx MPS	[$^{2}H_{4}$] Phe	100	10x10 Bilateral KE 70%1RM, 3m rest	Mixed	1-2h PEx, 0-1h PIn	Leu-EAA+CHO	7	20g EAA +35g CHO	0.062	0.04	-	-	0.165	-	N/A	↑ FSR (double)	Nutrition has an additive effect on post-Ex FSR
	UT,8M,30±2						1-2h PE	none	0	none	-	-	0.09	-	-	-	N/A	↑ FSR	
Drummond et al. 2008	UT,7YM,30±2	Effect of age with Leu-EAA on PEx MPS	[$^{2}H_{4}$] Phe	80	8x10 bilateral KE 70%1RM, 3m rest	Mixed	1-3,3-6, 1-5	Young EAA	7	20g EAA, 1h PEx	0.04	0.03	-	-	0.11,0.1,0.11	-	Yes	↑ FSR early & late	MPS similarly between young & old men. overall mean 5h NS
	UT,6OM,72±2							Old EAA	7	20g EAA	0.041	0.04	-	-	0.05,0.16,0.12	-	Yes	↑ FSR late	
Koopman et al. 2008	UT,8M,73±1	Effect of Leu with lots of Pro on PEx MPS	[$^{13}C_{6}$] Phe [$^{2}H_{2}$] Tyr	120	6X10 on LP & KE 40-75% 1RM	Mixed	0-6h PIn	CHO+PRO	4.7	intermittent for 6h post RE, every 30 ms ~69g whey +/-~13g LEU	No	-	-	-	0.082	-	N/A	No	Probly more than enough Leu in PRO only group. ↑ LEU did appear to reduce WB leucine oxidation, & thus ↑ net balance slightly.
								CHO+PRO	17.5		No	-	-	-	0.081	↑↔	N/A	No	
Fujita et al. 2009	UT,7M,4F,27±2	Effect of EAA timg on time course of EX & early PEx MPS	[$^{2}H_{5}$] Phe	100	KE 10x10 @70%1RM	Mixed	Rest, dur Ex, 0-1, 1-2, 0-2h PE	Fast		Fasted	0.06	0.05	0.08,0.09,0.073	-	-	-	-	-	2 groups: EAA+CHO 1h before Ex & Control (Ex + no fed), fasted control data from Dreyer et al. 2006. **MPS 0.06%/h ↑ during fed EX.**
	UT,6M,5F,25±1							EAA + CHO	7	~20g EAA (0.35 g/kg FFM) ~25g Sucrose (0.5 g/kg FFM)	-	-	-	0.12	0.12,0.089,0.098	-	-	-	
Moore et al. 2009	Rec,7M,26±3	Effect of whey protein on resting & PEx MPS	[$^{13}C_{6}$] Phe	80-100	5X8-10 LP, KE	Myo, Sarc	1-3, 3-5h PIn	25 g Whey EX	3	Bolus Imed post RE	0.025,0.052(sarc)	-	-	-	0.066,0.07(0.084,0.077)	-	N/A	YES, 5hr	Ex prolongs feeding induced MPS, Fed breakfast?
					None - Non Exercise Leg	Myo, Sarc		25 g Whey Rest				-	-	-	0.051,0.049(0.086,0.074)	-	N/A	-	
Moore et al. 2009	TR,6M,29±2	Effect of egg protein dosing on PEx MPS & album protein synthesis (APS)	[^{13}C] Leu	80-100	Bilateral: 4X8-10 LP, KE, LC	Mixed	1-4h PIn	0	0	Bolus Imed post RE	OnlyPEx	-	0.053	-	-	-	-	-	Ingesting 20g of Whey protein following RE appears to elicit a maximal FSR response in young adults. Above 20g there is ↑ leucine oxidation & no further ↑ in FSR.
								5g	0.4			-	-	-	0.075	-		Yes, vs. 0	
								10g	0.8			-	-	-	0.08	-	N/A	Yes, vs. 0	
								20g	1.6			-	-	-	0.11	-		Yes, vs. 0, 5, 10	
								40g	3.2			-	-	-	0.115	-		Yes, vs. 0, 5, 10	
Tang et al. 2009	RT,6M,23±4	FSR to (whey & soy) & (casein) proteins at rest & after RE	[$^{13}C_{6}$] Phe	80-100	unilateral 4x10-12RM Leg KE & LP	Mixed	no background, 3h	Whey	2.3	WPH 21.4g, Bolus Imed PEx	no	-	-	0.091	0.15	-		-	FSR to (whey & soy) & (casein) proteins at rest & after RE, compare ingestion of isolated protein sources after RE on MPS
	RT,6M,23±4							Casein	1.8	Micelular Caein 21.4g, Bolus Imed PEx	no	-	-	0.047	0.072	-	N/A	-	
	RT,6M,23±4							Soy	1.8	Soy Isolate 21.4g, Bolus Imed PEx	no	-	-	0.078	0.125	-		-	
West et al. 2009	?,8M,20±1	Fed breakfast?, RE during Low or High hormone conditions on PEx MPS	[$^{13}C_{6}$] Phe	40	elbow flexor Ex 4x10, ~95% of 10RM for LH, and HH was followed by 5x10 ~90% of 10RM LP, 3x12 KE and KC	Mixed	4h, mixed plasma protein Bk	LH	~2.5-3g	25g whey protein post arm Ex	0.06	-	-	-	**0.08**	-	-		no diference in MPS ↑ between LH and HH. fed, Biceps Bracii Muscle
						Myo		HH			0.06	-	-	-	**0.081**	-	-		
								LH			0.04	-	-	-	**0.071**	-	-	↑ HH = LH	
								HH			0.04	-	-	-	**0.064**	-	-		
Burd et al. 2010	RT,8M,24±5	Fed breakfast?, effect of RE volume on Myo FSR & time couse	[$^{13}C_{6}$] Phe	14	unilateral 70% 1RM to fatigue, rest leg control, 1 or 3 sets (2m rest)	Myo	rest, 5h fed, 24h fast and 29h fed	1 set	~2-2.5g	20g whey protein	0.03	-	-	-	0.065,0.035	-	-		multiple set > ↑ in MPS vs. single set
				24				3 set			-	-	-	-	0.078,0.06	-	-	↑ > 1 set	
Burd et al. 2010	15M,Rec,21±1	Effect of Ex intensity/vol on PEx MPS time course	[$^{13}C_{6}$] Phe	5	KE, 4sets 90%1RM to fail (90FAIL), 30%1RM (30WM to 90%), 30%1RM to fail (30FAIL)	Mixed	4, 24h	90FAIL	?	breakfast, 2h prior to arrival, Ensure plus; 61% CHO, 15% PRO, & 24% fat) ~15% of caloric need	0.048	-	-	0.049	0.16,0.08	-	-	↑>30WM 4h, not 24h	Ex intensity, rep to failure is a greater determant of PEx MPS than EX volume. 241 % increase in 90fail, NS bs fail groups 241 % ↑ in 90fail, NS bs fail groups
				14				30FAIL			0.048	-	-	-	0.14,0.095	-	-		
				21				30WM			0.047	-	-	-	0.085,0.075	-	-	↔	
				5				90FAIL			0.047	-	-	0.025	0.08,0.055	-	-	↑30WM 4h	
				14		Myo		30FAIL			0.046	-	-	-	0.095,0.08	-	-	↑> 90F, 30WM 24h	
				21				30WM			0.046	-	-	-	0.06,0.05	-	-	↔	
				5				90FAIL			0.025	-	-	0.05	0.085,0.05	-	-	↑30WM 4h	
				14		Sarc		30FAIL			0.025	-	-	-	0.075,0.075	-	-	> 90F 24h	
				21				30WM			0.025	-	-	-	0.06,0.06	-	-	↔	
Holm et al. 2010	UT,20M,25±1	Effect of feedeing & contraction intensity on Myo & Col MPS	[^{13}C] Leu	LL:36	Low-load leg @17% 1RM ((1 rep every 5th s for 3 m)); High-load leg @70% 1RM, intensities equalized for load SiKE RE	Myo	0-4h, Pre -2:45-45, Post: 30m, 3h, 5:30h	LL	?	Fed during INF every 30m or not fed (water), a multinutrient supplement [17% PRO (soy & milk), 52 E% CHO & 31 E% fat + a variety of merals & vitams	0.08	-	0.115,0.095	0.18	0.139,0.17	-	-	↑>fast late, fast ↑ HL early	LL ↑ Myo MPS early, but HL ↑ Myo MPS late, feeding equalizes and prolongs the response, no effect of feeding on ↑ of Col MPS
								HL			-	-	0.086,0.14	-	0.15,0.21	-	-	↑>fast late, Fast > LL late	
				HL:80		Col		LL			0.08	-	0.14,0.188	0.06	0.1,0.124	-	-		
								HL			-	-	0.163,0.15	-	0.123,0.126	-	-	↑, no effect feeding	
Mikkelsen et al. 2010	TR,8M,23±1	Effect of local NSAID leg INF during heavy novel RE on MPS next day	1-2[$^{13}C_{2}$] Leu	200	200 maximal ECC contractions, on leg each	Myo/Col	24-28h	PL	~2g	18~23g PRO & 26~34g CHO w/in an 1h PEx,	-	-	-	-	0.11/0.06	-	-	-	2h PEx a sandwitch was given
								NSAID			-	-	-	-	0.14/0.11	-	-	-	
Pennings et al. 2010	Active,12M,21±1	Effect of age & Ex w/ Mic Casein on on resting & PEx MPS	[$^{13}C_{6}$] , [$^{2}H_{5}$] Tyr	120	Cycling, LP + KE 6x10 each	Mixed	0-6 h PIn	Y Casein	1.7	20g Bolus of 250 mL	OnlyPEx	-	-	0.061	0.072	-	No	Exercise greater than rest, no diff by age	4 groups of subjects Young and old with Ex and rested control
	Active,12M,73±1							O Casein				-	-	0.057	0.073	-	No		

Author	Subjects, Status,N,Age	Study	Tracer	#rep	Exercise	Protein Fraction	FSR Bx Time PEx	Nutrition/Group	Leu g	Nutrition Type	MPS(fasted)			MPS(fed)		Net Bal	Age Dif	Intervention Dif	Other Notes
											Basal	Ex	PEx	Basal	PEx				
Symons et al. 2011	7Y,29±3	Effect of age w/ beef dosing on PEx MPS	[$^{13}C_6$] Phe	48	6X8 KE at ~80% 1RM	Mixed	Meal to 3h PEx. 5h total. Includes Ex	Young Beef	~6	340g Beef Patty Ingestion 1 hr Pre Exercise	0.073	-	-	-	0.156	-	No	No	Old = Young PEx MPS response w/ pre -ex 90g PRO . Ex ↑ the effects 90g of protein on MPS (see Symons 2009)
	7Y,67±2							Old Beef	~6		0.075	-	-	-	0.152	-	No	No	
Dideriksen et al. 2011	Active,4M,2W,71±3	Effect of whey vs casein on PEx MPS	L-[1-^{13}C]leu	80	5X8 on unilateral KE & bilateral LP at ~80% 1RM	Myofib, Collagen	30-390 m post RE	Casein Pre	8.77	100g Cas, Bolus Imed pre RE, 30m	OnlyPEx	-	-	-	0.1	-	N/A	↑ > Control	Milk ingestion before & after RE may be good strategy. No differences if ingested pre or post RE
	Active,3M,3W,70±2							Casein Post	8.77	100g Cas, Bolus Imed PEx		-	-	-	0.09	-	N/A	Trend > Cntl	
	Active,4M,2W,64±1							Whey Post	11.77	100g Whey, Bolus Imed PEx		-	-	-	0.09	-	N/A	Trend > Cntl	
	Active,4M,2W,68±2							PLA Post	0	Bolus Imed PEx		-	0.07	-	-	-	N/A	-	
West et al. 2011	Rec,8M,22±1	Effect of whey ingestion pattern on PEx MPS	[$^{13}C_6$] Phe	80-100	8x 8-10reps @ 10RM w/ 2 m rest	Myo	1,3,5	BOLUS	3.5	Bolus 25g Whey Imed PEx	0.02	-	-	-	0.041,0.06	-	N/A	-	Test divergent AA in blood, alter ingestion patterns of whey on MPS & anabolic signaling after RE
								PULSE	3.5	Pulse: 10 x 2.5g, every 20m	-	-	-	-	0.03,0.045	-	N/A	-	
Reitelseder et al. 2011	Active,9M,28±2	Effect of whey vs casein on Early, Late & Entire PEx MPS	L-[1-^{13}C]leu	80	10x8reps @ 80% 1RM unilateral KE 3m rest btw sets 2 biopsies in one leg	Myo	1-3.5,3.5-6, 1-6h	Casein	1.53	Cas, 17.5, 0.30 g/kg LBM, Bolus Imed pre RE	~0.056	-	-	-	0.098,0.105,0.10	-	N/A	-	intrinsically labeled whey or caseinate after RE
						Myo		Whey	2.06	WPI, 17.5, 0.30 g/kg LBM, Bolus Imed pre RE	-	-	-	-	0.1230.098,0.10	-	N/A	-	
	Active,8M,26±2					Myo		PLA	N/A	N/A	-	-	0.072,0.075,0.073	-	-	-	N/A	-	
Burd et al. 2012	TR,8M,?24	Effect of time under tension on time course of subfraction PEx MPS	[$^{13}C_6$] Phe	~25	Slow (6s con/ecc) 1 leg	Myo, Mito, Sarc	0-6, 24-30h	SLOW	~2-2.5g	20g whey protein	0.021	-	-	-	0.024,0.053	-	-	↑ > cntrl	Mito ↑ 0-6 & 24-30 for Slow but only 24-30 for CTL, Sarc only ↑ for Slow
				~25	Cntl (1s con/ecc) other leg			Cntrl			-	-	-	-	0.026,0.03	-	-	-	
Burd et al. 2012	Active,14OM,72±1	Effect of whey vs casein on resting & PEx MPS	[$^{13}C_6$] Phe	~30-50	Unilateral KE 3x10RM, none - Non Exercise Leg	Myo	Start tracers PEx. Only bx 4h post RE	20g Casein	1.6	Bolus of 400 mL Imed post RE	~0.03	-	-	~0.025	~0.035	-	N/A	↑ > non-Ex	Dif w/ casein & whey due to leucine content? Ex + PRO ingestion > PRO only
								20g Whey	2.8		-	-	-	~0.042	~0.055	-	N/A	↑ > non-Ex & EXvCas	
Burke et al. 2012	RT,12M,27±1	Effect of pre-Ex whey protein+Leu on PEx MPS	[$^{13}C_6$] Phe	~64-80	8x 8-10reps @ 10RM w/ 2 m rest	Mixed	0-5	Bolus, 25g whey PRO+5g leu	8	B4 EX, (1x25-g dose)	None	-	-	-	0.085	-	N/A	-	If dif feeding patterns of a whey mixture b4 RE affect postEx intracellular signaling & MPS
								Pulse, 25g whey PRO+5g leu	8	B4 EX, (15x2-g every 15m)	-	-	-	-	0.095	-	N/A	-	
								PLA	_		-	-	-	-	0.037	-	N/A	-	
Camera et al. 2012	TR,8M,23±3	Effect of glycogen depletion & PEx PRO sup on PEx MPS	[$^{13}C_6$] Phe	50	warmup (2x5 @ 55% 1RM) & 8X5 LP 80% 1RM	Myo	0-4h	Norm	~2-2.5g	20g Whey + 40g maltadKErin	-	-	0.045	-	0.07	-	N/A	none	No effect of glycogen depetion on PEx ingestion of Pro to ↑ MPS
	TR,8M,23±4							Gylcogen depleted leg			-	-	0.049	-	0.068	-	N/A	none	
Donges et al. 2012	Sed,8M,53±2	Effect of isolated bouts of (RE), (AE), or AE+RE on PEx MPS in MA men	[$^{13}C_6$] Phe	64 lots	8x8 KE@ 70% 1RM peak power	Myo/Mito	fast, (1, 4h 20g PRO)	RE	~2-2.5g	20g WPI	0.032	-	-	-	0.06/0.065	-	-	↑ > AE	All modes ↑ mito MPS, on modes with RE ↑ Myo MPS
				32+lots	50% of each trial			AE			-	-	-	-	0.03/0.03	-	-	-	
								AE+RE			-	-	-	-	0.07/0.06	-	-	↑ > AE	
Churchward-Venne et al. 2012	Rec,8M,22±1	Effect of Leu +/- Pro/EAA on resting & PEx MPS	[$^{13}C_6$] Phe	~80-100	Unilateral 4X10 Leg KE & LP, ~95% 10RM, None - Non Exercise Leg	Myo	1-3, 3-5 h post RE	Whey	3	25 g WPI, Bolus Imed post RE	~0.03	-	-	0.061,~0.05	0.064,0.088	-	N/A	Longer ↑ in FSR	Whey maintaim ↑ FSR late PEx. less whey w/ extra Leucine or EAA w/out leucine only ↑ FSR ealry PEx. Ex + PRO feeding = PRO feeding in magnitude. May need all AA to prolong MPS. One leg Ex, other Non-Ex
	Rec,8M,22±1							low Whey+Leu	3	6.25g WPI, Bolus Imed post RE	-	-	-	0.068,~0.049	0.068,0.048	-	N/A	-	
	Rec,8M,23±1							low Whey+EAA NO Leu	0.75	6.25g WPI, Bolus Imed post RE	-	-	-	0.063,~0.050	0.069,0.050	-	N/A	-	
Gaiser et al. 2012	Rec,12M,22y	Effect of Ex+feeding vs feeding only on 24h MPS	D_20	~50-80	5xfail @85% 1RM	Mixed/Myo	24H (16h PE)	One leg Con, 1 leg EX	?	normal day	-	-	-	0.76/0.94	0.69/0.75	-	-	-	Use D20 with high level of precusror
Res et al. 2012	Rec,8M,23±1	Effect of PRO on PEx overnight MPS	[$^2H_5$] Phe	128	(8x8 reps LP/KE, ~70% 1RM) (45 m)	Mixed	2330 to 0700 (8h)	PRO (40g casein)	3-4g	Bolus 450 mL 2.5h post RE	-	-	0.048	-	-	61	N/A	↑ FSR	Casein but not PLA ingestion the night following RE ↑ MPS during sleep.
	Rec,7M,22±1							PLA (water)	0 _		-	-	-	-	0.059	-16	N/A	-	
Yang et al. 2012	Active,10M,71±5	Effect of whey or soy protein dosing on resting & PEx MPS	[^{13}C] Leu , [$^{13}C_6$] Phe	~30-50	Unilateral KE 3x10RM, none - Non Exercise Leg	Myo	?,Started tracers PEx. Final bx 4h post RE	20g Whey	2	Bolus of 400 mL Imed post RE	-	-	-	0.026	0.03	-	N/A	= Fast non-Ex	20g of Whey PEx ↑ MPS, 40g to maximize PEx MPS (4 h) At rest older adults need 20g to ↑ MPS & > amounts had no more effect, Soy had a dimished effect, but was hydrsolated, not typical soy protein
	Active,10M,72±5							20g Soy	1.6		-	-	-	0.042	0.055	-	N/A	= Fast non-Ex	
	Active,10M,70±4							40g Whey	4		-	-	-	0.028	0.04	-	N/A	= Fast non-Ex	
	Active,10M,72±6							40g Soy	3.2		-	-	-	0.055	0.08	-	N/A	-	
	Active,10M,70±5								0 _		-	-	-	0.032	0.055	-	N/A	↑ > all	
	Active,10M,71±5										0.03	-	0.045	-	-	-		= Fast non-Ex	
Yang et al. 2012	Active,7M,70±3	Effect of whey protein dosing on resting & PEx MPS	[^{13}C] Leu , [$^{13}C_6$] Phe	~30-50	Unilateral KE 3x10RM, none - Non Exercise Leg	Myo		10g Whey	1	Bolus of 400 mL Imed post RE	-	-	-	0.040	0.048	-	N/A	= Fast non-Ex	20g of Whey PEx ↑ MPS, 40g to maximize PEx MPS (4 h) At rest older adults need 20g to ↑ MPS & > amounts had no more effect
	Active,9M,70±4							20g Whey	2		-	-	-	0.047	0.061	-		↑ > Fast-Ex	
	Active,10M,70±4							40g Whey	4		-	-	-	0.051	0.083	-		↑ > all	
West et al. 2012	Rec,8M,20±1	Effect of gender on MPS to RE+PRO	[$^{13}C_6$] Phe	~64-100	8x 8-10reps @ 10RM w/ 2 m rest	Myo	1,3,5, 24-26h	Men 25g whey	3.5	25g whey PRO 12.8g EAAs, 3.5g leu, no cho or fat	0.021	-	-	-	0.057,0.071,0.06	-	N/A	-	The effect of sex on MPS & anabolic signaling after RE in the fed state
	Rec,8W,22±2							Women 25g whey	3.5		0.020	-	-	-	0.054,0.0680.06	-	N/A	-	
Bechshoeft et al. 2013	Sed10M,23±5	Effect of light load Ex on prolonging PEx MPS	L-[1-13C]leu	360	10x36 @16%1RM (10sets of 3 m), unilateral	Myo	30-630, 30-180, 180,330, 330-480, 480-630	Ex or feeding	7.1	Oral PRO every hr, initial whey bolus followed by casein, for 10 hr, 64.9 g PRO total	-	-	-	0.059,0.052,0.041	0.064,0.053,0.057,0.062	-	-	FSR only dif at 480-630m	Light loading prolongss MPS 8-10 hr PEx
Reidy et al. 2013	Rec,8M,1F,25±1	Effect of whey vs Blend on PEx MPS	[$^{13}C_6$] Phe	~80	8x10reps @ 70%1RM w/ 3 m rest	Mixed	1,3,5h	Whey (17.5g PRO)	1.90	Bolus of 300 mL 1hr post RE	0.056	-	-	-	0.078,0.074,0.077	-	N/A	-	A protein blend prolongs MPS, matching proteins by leucine content may negate early PEx differences in MPS
	Rec,9M,1F,23±1							Blend (19g PRO)	1.80		0.055	-	-	-	0.088,0.087,0.087	-	N/A	-	
Reitelseder et al. 2013	Active,8M,24±2	Effect of whey vs casein on PEx Net bal & Leu oxidation	L-[1-^{13}C]leu	80	10x8reps @ 80% 1RM unilateral KE 3m rest btw sets 2 biopsies in one leg		1-3.5,3.5-6	Casein	1.53	Cas, 17.5, 0.30 g/kg LBM, Bolus Imed pre RE	-	-	-	-	↔	↑90m	N/A	↔	use 2-pool method w/ ultrasound, mor changes in MPS or MPB (time effect, ↔↓) with method; oxidation ↑; intrinsically labeled whey or caseinate after RE
	Active,6M,26±3							Whey	2.06	WPI, 17.5, 0.30 g/kg LBM, Bolus Imed pre RE	-	-	-	-	↑↔		N/A	↔	
	Active,7M,24±2							PLA	N/A	N/A	-	-	↔	-	-	↔	N/A	↔	

Author	Subjects, Status,N,Age	Study	Tracer	#rep	Exercise	Protein Fraction	FSR Bx Time PEx	Nutrition/Group	Leu g	Nutrition Type	MPS(fasted) Basal	MPS(fasted) Ex	MPS(fasted) PEx	MPS(fed) Basal	MPS(fed) PEx	Net Bal	Age Dif	Intervention Dif	Other Notes
Robinson et al. 2013	UT,35M,59±2	Effect of ground beef dosing on resting & PEx MPS	[$^{13}C_6$] Phe	~30-40	Unilateral 3X10-12 Leg KE ~ 80% 1RM	Myo	0-4h	0g	0	Bolus Imed post RE of (15%fat) groundbeef	~0.024	-	-	0.023	0.03	-	N/A	Oxidation was 170 > 113> the other	only 170g beef (36g pro) stimulated MPS in middle aged men
								57g(2oz;12gPRO)	0.96		-	-	-	0.023	0.032	-	N/A		
								113g(4oz;24gPRO)	1.92		-	-	-	0.03	0.04	-	N/A		
								170g(6oz;36gPRO)	2.88		-	-	-	0.046	0.062	-	N/A		
Wilkinson et al. 2013	Rec,8M,22±4	Effect of D20 tracer to assess MPS over 8d of EX+nutrition or control	D_2O	160	4x8 reps, 80%1RM Unilateral RET	Myo, Sarc, Col	0-2,2-4,4-8 days	Exercise Training leg (5 sessions+ 20g Whey)	~2g	20g WPI, Muscletech	-	-	-	-	0.082,0.082,0.075	-	N/A	-	Use D20. Ex + Pro better MPS over 8 days than nothing.
					None			CTRL Leg			0.06,0.06,0.056	-	-	-	-	-	N/A	-	
Areta et al. 2014	RT,8M,25±2	Effect of whey protein dosing & timg on PEx MPS	[$^{13}C_6$] Phe	~80	2 warm-up sets & 4x10reps @ 80%1RM w/ 3 m rest	Myo	0, 1,4,6, 12h	Bolus 40g 2x	3-4g	2 Boluses of 500 mL	~0.03	-	-	-	0.055	-	N/A	-	20g ingested every 3h over a 12h period keeps MPS ↑ . May need cycling of feeding simitulation on MPS
	RT,7M,25±1							Med bolus 20g 4x	~2g	4 Boluses of 250 mL	-	-	-	-	0.079	-	N/A	best	
	RT,8M,25±2							Pulse 10g 4x	<1g	8 Boluses of 125 mL	-	-	-	-	0.057	-	N/A	delayed & sluggish	
Areta et al. 2014	8M,8F,27±4	Effect of short term energy decift & whey protein dosing on PEx MPS	[$^{13}C_6$] Phe	48	2 warm-up sets & 6x8reps @ 80%1RM w/ 3 m rest	Myo	rest, 0-4	Energy Balance	_	45 kcal/kg/ffm	0.026	-	-	-	-	-		-	energy decifit blunts MPS, 15 and 30g ↑ PEx MPS above PLA, 30g is max. Higher doses (>20) may be more effective in energy decifit.
								Energy Decifit - PLA	_	30 kcal/kg/ffm - water 500ml	0.019	-	0.024	-	-	-	N/A		
								Energy Decifit - 15g	~1.5g	15g whey - 500ml	-	-	-	-	0.030	-			
								Energy Decifit - 30g	~3g	30g whey - 500ml	-	-	-	-	0.038	-		best	
Camera et al. 2014	TR,8M,8F,19±1	Effect of PRO on Post-concurrent exercise MPS	[$^{13}C_6$] Phe	40+AE	(8x5 reps KE, 80% 1RM) & (30 m, 63% peak power output)	Myo	rest, 0-4	PRO or PLA	~2.5-3g	25g whey or flavored water	0.030	-	0.052	-	0.072	-	N/A	-	Most of the effect of PRO was driven by 2 non-reponders in PLA
Churchward-Venne et al. 2014	Rec,8M,21±1	Effect of Leu/BCAA dosing on resting & PEx MPS	[$^{13}C_6$] Phe	~80-100	Unilateral 8X10-12 Leg KE ~ 80% 1RM, None - Non Exercise Leg	Myo	0-1.5, 1.5-4.5h post RE	25 g Whey Protein (WP)	3	Bolus Imed post RE	-	-	-	~0.05,0.063	~0.052,0.065	-	N/A	-	Whey and/or AA was coingested with fat & CHO to mimic meal situations. 5g Leu with mimal whey = 25g whey. Extra Val and iLeu may hinder prolonging MPS.
	Rec,8M,20±1							6.15 g WP +Gyl+Ala	0.75	Bolus Imed post RE	-	-	-	0.063,0.050	0.069,0.050	-	N/A	-	
	Rec,8M,21±1							6.15g WP+LoLeu +Gyl+Ala	3	Bolus Imed post RE	-	-	-	0.052,0.042	0.062,0.038	-	N/A	Longer ↑ in FSR	
	Rec,8M,20±1							6.15g WP+HiLeu +Gyl+Ala	5	Bolus Imed post RE	-	-	-	~0.057,0.059	~0.054,0.063	-	N/A	-	
	Rec,8M,21±1							6.15g WP+BCAA +Gyl+Ala	5	Bolus Imed post RE	-	-	-	0.048,0.052	0.057,0.048	-	N/A	-	
Churchward-Venne et al. 2014	7OM,72±2	Effect of Whey, Whey+Citrulline, Whey+NEAA on resting & PEx MPS	[$^{13}C_6$] Phe	~60-72	Unilateral 6X10-12 KE ~ 80% 1RM, None - Non Exercise Leg	Myo	0-2.5, 2.5-5, 0-5 post RE	45 g Whey	5.4	Bolus Imed post RE	0.018	-	-	~0.04,0.027(0.036)	~0.054,0.049(0.05)	-		-	48g of whey maximizes and prolongs PEx MPS in older men. No effect from Citrulline to enhance AA delivery with mimal AA and whey PRO.
	7OM,74±1							6.15 g Whey, + Cit +Gyl+Ala	0.75		0.016	-	-	~0.021,0.023(0.021)	~0.03,0.029 (0.031)	-	N/A	-	
	7OM,72±2							6.15g Whey+LoLeu +Gyl+Ala	3		0.017	-	-	~0.023,0.02(0.02)	~0.028,0.027(0.03)	-		Longer ↑ in FSR	
Dickinson et al. 2014	Active,7OM,74±2	Effect of added Leucine to EEA on PEx MPS	[$^{13}C_6$] Phe	80	8x10reps @ 65%1RM w/ 3 m rest	Myo	rest, 2-5 & 24h	CTRL (10g EAA w/ 1.8g Leu)	1.85	Bolus of 350 mL 1hr post RE	0.050	-	-	-	0.095,0.07	-	N/A	same day, same response	Similar at 2-5h PE, greater mPS 24h PEx
	Active,8OM,71±3							EAA+LEU (10g EAA w/ 3.5g Leu)	3.50		0.051	-	-	-	0.09,0.10	-	N/A	Prolonged to nKE day	
Mitchell et al. 2014	Active,23M,24±1	If PEx+PRO MPS in untrained subjects is predicitve of RET induced hypertrophy	[$^{13}C_6$] Phe		4x8 LP,KE, LC, CP	Myo	Rest, 1-3,3-6,1-6	30g milk PRO	~3g	milk PRO imed post &/or w/ breakfast	~0.033	-	-	-	~0.06,0.05	-	N/A	-	No relationship with PE MPS & muscle hypertrophy
Parr et al. 2014	TR,8M,21±5	Effect of alcohol with PRO or CHO on PEx MPS	[$^{13}C_6$] Phe	~40+	(8x5 reps KE, 80% 1RM)& (30 m, 63% peak power output (PPO)) & high intensity interval (10x30 s, 110% PPO)	Myo	2-8h PExercise	PRO (25g whey 2x)	1.4	Bolus 500 mL imed & 4h post RE	0.025	-	-	-	0.052	-	N/A	↑ FSR	Ethanol ingestion (1.5 g/kg BM) of alcohol) attenuates PEx MPS ↑
								ALC-PRO,25g whey,2x	2.8		-	-	-	-	0.039	-	N/A	no ↑ in FSR	
								ALC-CHO,25g malto,2x	2.8		-	-	-	-	0.032	-	N/A	no ↑ in FSR	
Rahbek et al. 2014	Rec,24M,24±1	Effect of Whey/CHO sup & contraction mode on PEx MPS	[$^{13}C_6$] Phe		CON: 6x10reps Max	Myo	1-3h, 3-5h PE	Whey+ CHO	~1.5-1.9	Bolus 500ml ~18g PRO+~18g CHO	No	-	-	-	0.106,0.106	-	-	-	No resting comparison, Whey values had higher means, not significant, subset of the training study
					ECC: 6x10reps Max							-	-	-	0.106,0.09	-	-	-	
					CON: 6x10reps Max							-	-	-	0.08,0.10	-	-	-	
					ECC: 6x10reps Max			CHO	_	Bolus 500ml ~36g CHO		-	-	-	0.095,0.09	-	-	-	
Reidy et al. 2014	Rec,8Y,24±1	Effect of whey vs Blend on PEx MyoMPS & Net Bal	[$^{13}C_6$] Phe	~80	8x10reps @ 70%1RM w/ 3 m rest	Myo	3,5h	Whey (17.3g PRO)	1.90	Bolus of 300 mL 1hr post RE	0.041	-	-	-	0.093	↑1h	N/A	↔	FSR from 3-5h equal, NB from 1-2h elevated the same, but prolonged 2-3h in Blend
	Rec,8Y,22±1							Blend (20g PRO)	1.90		0.350	-	-	-	0.081	↑1&2h	N/A	-	
	RT,12M,22±3							0	_	none	0.032	-	0.052	-	-	-	-	-	
Witard et al. 2014	RT,12M,20±1	Effect of whey protein dosing on resting & PEx MPS	[$^{13}C_6$] Phe	~160	unilateral EX (8 × 10 LP & KE; @ 80% 1RM), 3hr after breakfast	Myo	0-4h post ex	10g whey	0.67	Bolus of ? mL Imed post RE	-	-	-	0.04	0.059	-	-	-	For a more applicable situation subjects where fed breakfast. Dose effect similar to Moore 2009, 20g is maximal, Fed breakfast
	RT,12M,22±3							20g whey	1.34		-	-	-	0.05	0.069	-	-	-	
	RT,12M,20±1							40g whey	2.68		-	-	-	0.049	0.071	-	-	-	
Witard et al. 2014	Rec,5M,3F,30±3	Effect of PEx timg of EAA+CHO on PEx Net bal	[$^{13}C_6$] Phe	~30-50	8 × 10 KE; @ 80% 1RM),	none	1,2,3,7h	EAA+CHO / PLA	2.7g	50g sucrose + 15g EAA 1h PEx	-	-	-	-	0.11,0.0.086	-	-	-	FSR not different between trials, net Phe exchange only higher in sep during 1st h PIn, Fed breakfast
								CHO/ EAA		50g sucrose 1h Pex+15g EAA 2h	-	-	-	-	0.109,0.089	↑1h	-	-	

Author	Subjects, Status,N,Age	Study	Tracer	#rep	Exercise	Protein Fraction	FSR Bx Time PEx	Nutrition/Group	Leu g	Nutrition Type	MPS(fasted)			MPS(fed)		Net Bal	Age Dif	Intervention Dif	Other Notes
											Basal	Ex	PEx	Basal	PEx				
Effect of RET											-	-	-	-	-	-	-	-	
Yarasheski et al. 1992	9MPL+EX 7MGH+Ex	Effect of 12-wk, 5d/wk RE training in young men with GH on MPS	1-[13C] Leu	lots	WB, 4x4-8, 75-90% 1RM	Mixed	6 h	PI + Ex GH	?	1/12 daily intake/30 m	- -	- -	- -	0.048 0.048	**0.066** **0.07**	- -	- -	↑ = ↑	~18-20h PEx, bx5-17h, 10 day diet , FFM ↑ in GH (4.5kg) > PL (1.6kg), ~50%↑ strength in both groups
Phillips et al. 2002	UT,19(11M),24±3	Effect of RET on resting & PEx MPS & MPB in the fed state	$^2H^5$, 15^N Phe	80	8-wk WB, bilateral, split-routine RET (1 h/d, 6 d/wk); Acute: 2x10 U-LP, 6x10 KE, 2m rest @ 80% pre-TR 1RM	Mixed	6-7h	UT TR	?	Fed IM 3847 ± 1029 kJ during infusion	- -	- -	- -	~0.065 **~0.082**	**~0.083** ~0.1	↔, ↑PEx ↔	- -	↑ Basal > UT,	Pooled CrM +CHO and PRO+CHO intervention groups. UT: no Ex 3d prior; T: 72 h following last workout, overnight-fasted state (10 h), UT MPB: rest 0.047, PEx 0.057; TR: rest 0.066, PEx 0.070.
Tang et al. 2008	Rec,10M21±2	Effect of RE in the fed state on MPS before & fter RET	$^2H^5$ Phe, AKIc [$^{13}C_6$] Phe		6 x 10 reps 80% KE, 8-wk unilaterial training (KE only)	Mixed	3h rest, 4h PEx, 3h @ 28h	UT TR	?	Fed Boost IM, ~7 g·PRO/h, every 30 m (0.1 g PRO/kg/h).	- -	- -	- -	0.045 0.048	**4hr:0.09024hr:0.074** 4hr:0.12324hr:0.062	4hr:↑ 24hr:↑ 4hr:↑ 24hr:↔	- -	PEx:↑ both; Basal UT=TR, PEx: ↑ 4> UT;28h < UT	Basal not high, despite feeding and training, Dietary intake controled 48h pre, Rest: 4d, 2 RE bouts1 wk later Ex trial 4 h after RE, 24 h later
Wilkinson et al. 2008	UT,10M21±1	Effect or RET or AET on basal & acute PEx Myo & Mito MPS	(D3-α-KIC	50 lots	5 x 10 reps 80% KE, 10-wk training 45 m 75% O2max, 10-wk cycling	Myo/Mito	4 h	UT TR UT TR	?	Fed IM 1.1g·PRO/kg	- - - -	- - - -	- - - -	0.054/0.080 **0.08**/0.075 0.051/0.074 0.054/0.072	**0.12/0.15** **0.12**/0.052 0.051/**0.18** 0.075/**0.15**	- - - -	- - - -	↑ ↑Myo > ↔Mito ↔ ↑↔	Mito FSR ↑ only in AE post TR, only RE ↑ MyoFSR, asked to refrain from EX 2 d prior, post: 9/10 4d, 1 was 2d
Villareal et al. 2011	5M&4W,65-80, Obese&OW	Effect of strength, endurance, flexibility, & balance training on basal & fed MPS in obese older adults	[5,5,5-$^2H_3$] L-Leu	–	multicomponent exercise training program, 12-wk	Mixed	at rest, 3.5h, feeding 3h	UT TR	?	Ensure: energy 15% PRO, 55% CHO, & 30% fat) IM every 10m for 150m, primg dose 23 mg PRO·kg/FFM followed by 175 mgPRO/kg/FFM during 2.5 h feeding of 726 mgCHO/kg/FFM & 176 mg fat/kg/FFM.	0.053 **0.073**	- -	- -	**0.075** 0.098	- -	- -	- -	↑ fed TR >UT	Time since last EX session?, ↑ feeding most in UT, TR > UT, fed dif less
Smith et al. 2012	7M65-80, Obese&OW 7W65-80. Obese&OW	Effect of a multicomponent exercise training program on basal & fed MPS by sex in obese older adults	[5,5,5-$^2H_3$] L-Leu	–	multicomponent exercise training program, 12-wk	Mixed	at rest, 3h, feeding 3h	M -UT W - UT M - TR W - TR			0.039 0.064 0.082 0.081	- - - -	- - - -	**0.069** 0.065 **0.113** 0.098	- - - -	— - - -	- - - -	TR >UT TR >UT TR >UT TR >UT	~15-20h Pex, ↑ feeding most in UT and in men > women, TR > UT, fed dif less
Lambert et al. 2015	25Y,16M,40±4, 9W,38±4	Effect of RET, RE+AE l& ET or RE+AE aquatic ET on acute 24h Myo MPS pre & Post TR	D_2O	lots	11-wk training; Acute: WB, 4x12 @ 50-60% 1RM, 90s rest, 250 kcals of AE	Myo (UT/TR)	0-24h	RT RT-LTM RT-ATM	?	5 meals of Boost: gave (total) 8,037 kJ, 52% CHO, 20% PRO, & 28% fat @30m 3, 5.5, 8.5, & 11h PEx	- - -	- - -	- - -	- - -	8.84/10.21 10.44/10.18 14.52/12.48	- - -	- - -	- - -	same absolute intensity post, Use large bolus of D2O, values are % per day

PEX, Post-exercise; PIn, post ingestion; NR, not reported; PRO, protein, PLA, placebo; CHO, Carbohydrate; Malto, Maltodextrin; L, low, H, high; FFM, Fat-free mass; CTRL, Control; O, old; Y, young; M, men; W, women; OW, overweight; MHC, myosin heavy chain; WB, Whole Body; RE, resistance Exercise; RT, resistance trained;, UT, untrained; Rec, recreationally active; Sed, sendentary; AE, aerobic exercise; RE, resistance exercise; ST, strength trained; ET, endurance trained; TR, Trained; 3MH, 3-Methylhistidine; AA, amino acids; EAA, essential amino acids; WPI, Whey Protein Isolate; WPH, Whey Protein Hydyosolate; WPC, Whey Protein Concentrate; ARG, arginine; Ala, alaninine; Gyl, glycine;, ALC, alcohol; D20, deterium; AUC, Area under the curve; FFM, fat-free mass; WBPB, Whole Body Protein Balance; Malto, Maltodextrin; IM, intermittent; Ex, exercise; vol, volume; phe, phenylalanine; leu, leucine; BB, biceps brachii; RM, repetition maximum; WB, whole body; LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; LC, leg curls; Si, Single leg; Ecc, eccentric contractions; Con, concentric contractions; MVC, maximal voluntary contractions; MPS, muscle protein synthesis; MPB, muscle protein breakdown; FBR, Fractional breakdown rate; imed, immediate; PEx, post-exercise; h, hour; min, minutes; sec, seconds; LBM, Lean body mass; BRF, Blood flow restriction; N/A, not available; W, watts; Rap, Rapamycin; Fail, exercise to failure; WM, work-matched; INF, infusion, Myo, myofibriillar protein fraction; Sarc, sarcoplasmic protein fraction; Col, collagen fraction, Net Bal, Net Balance; U unilateral

Table 1.9. Percent change of human skeletal muscle protein turnover responses after RE by incorporation period and protein fraction

Myofibrillar	Hours post RE																													
	Ex	1	2	3	4	5	0-2	0-3	0-4	0-5	0-6	0-12	0-24	1-3	1-4	1-5	1-6	1-8	1-9	2-4	2-5	2-8	3-5	3-6	4-6	6-8	8-10	6-12	sleep	24	24 re-fed	Ave
Ex-Fast vs rest									**43%**					**23%**								**28%**	**44%**									**38%**
min									15%					7%								28%	13%									7%
max									73%					38%								28%	75%									75%
Fed vs rest							**136%**	**71%**	**45%**	**126%**				**130%**		**91%**	**0%**		**8%**		**196%**		**53%**							**80%**		**92%**
min							-25%	31%	-7%	18%				103%		85%	0%		8%		133%		18%							64%		-25%
max							250%	122%	112%	217%				180%		96%	0%		8%		250%		67%							100%		250%
Ex-Fed vs rest							**227%**	**90%**	**119%**	**156%**	**11%**	**105%**		**123%**	**94%**	**134%**	**68%**				**156%**	**82%**	**144%**	**107%**	**103%**			**105%**		**91%**	**163%**	**129%**
min							189%	7%	30%	76%	7%	83%		50%	-12%	88%	68%				76%	56%	59%	52%	60%			73%		40%	17%	-12%
max							283%	200%	272%	244%	14%	147%		220%	150%	196%	68%				261%	108%	240%	162%	153%			141%		200%	340%	340%
EX-Fed vs PLA/ CHO									**52%**		**33%**			**42%**		**12%**	**36%**					**42%**	**36%**							**59%**	**45%**
min									7%		29%			12%		6%	35%					22%	0%							11%	0%
max									167%		43%			74%		18%	37%					63%	89%							89%	167%
Ex-Fed vs Fed							**9%**	**23%**	**52%**	**20%**				**7%**		**30%**		**38%**			**-5%**		**41%**			**4%**	**51%**			**27%**	**79%**	**33%**
min							-5%	-7%	20%	0%				0%		-1%		0%			-23%		-2%			4%	51%			20%	5%	-23%
max							19%	43%	127%	50%				14%		57%		64%			7%		105%			4%	51%			35%	168%	168%

Mixed	Hours post RE																													
	Ex	1	2	3	4	5	0-2	0-3	0-4	0-5	0-6	0-12	0-24	1-3	1-4	1-5	1-6	1-8	1-9	2-4	2-5	2-8	3-5	3-6	4-6	6-8	8-10	6-12	sleep	24	24 re-fed	
Ex-Fast vs rest	**-16%**	**42%**	**58%**					**24%**			**64%**				**144%**									**39%**						**39%**		**53%**
min	-28%	42%	58%					20%			50%				144%									26%						24%		20%
max	-5%	42%	58%					27%			79%				144%									53%						55%		144%
Fed vs rest		**100%**													**56%**																	**50%**
min		100%													56%																	-15%
max		100%													56%																	170%
Ex-Fed vs rest	**-22%**		**175%**				**110%**	**166%**	**108%**					**74%**	**125%**	**116%**							**45%**	**220%**						**72%**		**108%**
min	-22%		175%				31%	81%	103%					22%	125%	38%							32%	150%						60%		22%
max	-22%		175%				173%	227%	114%					175%	125%	193%							58%	290%						94%		290%
EX-Fed vs PLA/ CHO	**9%**		**74%**						**143%**	**54%**				**26%**	**70%**	**74%**								**91%**					**26%**			**75%**
min	9%		74%						130%	37%				-17%	31%	54%								28%					23%			-17%
max	9%		74%						157%	71%				69%	117%	95%								154%					29%			157%
Ex-Fed vs Fed		**38%**						**59%**			**21%**			**80%**	**100%**					**27%**												**56%**
min		38%						53%			18%			63%	44%					22%												18%
max		38%						65%			28%			97%	156%					32%												156%

Mitochondrial	Hours post RE																													
	Ex	1	2	3	4	5	0-2	0-3	0-4	0-5	0-6	0-12	0-24	1-3	1-4	1-5	1-6	1-8	1-9	2-4	2-5	2-8	3-5	3-6	4-6	6-8	8-10	6-12	sleep	24	24 re-fed	
Ex-Fast vs rest																																
min																																
max																																
Fed vs rest																																
min																																
max																																
Ex-Fed vs rest											**44%**				**5%**															**112%**	**47%**
min											**15%**				**-39%**															**92%**	**-39%**
max											**73%**				**33%**															**131%**	**131%**
EX-Fed vs PLA/ CHO																																
min																																
max																																
Ex-Fed vs Fed									**28%**																							**28%**
min									**-31%**																							**-31%**
max									**88%**																							**88%**

2/3-pool	Hours post RE																													
	Ex	1	2	3	4	5	0-2	0-3	0-4	0-5	0-6	0-12	0-24	1-3	1-4	1-5	1-6	1-8	1-9	2-4	2-5	2-8	3-5	3-6	4-6	6-8	8-10	6-12	sleep	24	24 re-fed	
Ex-Fast vs rest	**-9%**	**4%**	**38%**	**82%**	**-2%**	**51%**	**10%**	**49%**	**31%**																							**29%**
min	-56%	-22%	3%	50%	-6%	51%	-10%	41%	31%																							-22%
max	22%	63%	90%	130%	0%	51%	37%	58%	31%																							130%
Fed vs rest															**81%**																	**81%**
min															81%																	81%
max															81%																	81%
Ex-Fed vs rest	**233%**	**185%**	**180%**	**117%**	**150%**	**147%**	**160%**	**161%**						**206%**	**208%**																	**170%**
min	233%	67%	0%	17%	-16%	129%	39%	108%						83%	207%																	-16%
max	233%	300%	483%	190%	467%	166%	308%	213%						313%	209%																	483%
EX-Fed vs PLA/ CHO		**102%**	**217%**	**24%**	**-21%**	**63%**	**92%**	**65%**						**143%**	**90%**																	**94%**
min		57%	51%	-30%	-33%	51%	65%	32%						143%	34%																	-33%
max		164%	600%	74%	-9%	75%	112%	99%						143%	147%																	600%
Ex-Fed vs Fed															**69%**																	**69%**
min															69%																	69%
max															69%																	69%

Sarcoplasmic	Hours post RE																													
	Ex	1	2	3	4	5	0-2	0-3	0-4	0-5	0-6	0-12	0-24	1-3	1-4	1-5	1-6	1-8	1-9	2-4	2-5	2-8	3-5	3-6	4-6	6-8	8-10	6-12	sleep	24	24 re-fed	
Ex-Fast vs rest																																
min																																
max																																
Fed vs rest														**170%**		**35%**							**15%**									**73%**
min														170%		35%							15%									15%
max														170%		35%							15%									170%
Ex-Fed vs rest								**3%**	**58%**					**88%**		**48%**							**19%**	**134%**						**32%**	**56%**	**54%**
min								-2%	30%					88%		48%							19%	130%						6%	8%	-2%
max								8%	81%					88%		48%							19%	139%						61%	107%	139%
EX-Fed vs PLA/ CHO																																
min																																
max																																
Ex-Fed vs Fed														**-9%**		**10%**							**35%**									**12%**
min														-9%		10%							35%									-9%
max														-9%		10%							35%									35%

ALL	Hours post RE																													
	Ex	1	2	3	4	5	0-2	0-3	0-4	0-5	0-6	0-12	0-24	1-3	1-4	1-5	1-6	1-8	1-9	2-4	2-5	2-8	3-5	3-6	4-6	6-8	8-10	6-12	sleep	24	24 re-fed	ave
Ex-Fast vs rest	**-12%**	**12%**	**42%**	**82%**	**-2%**	**51%**	**10%**	**54%**	**41%**		**64%**			**23%**	**144%**							**28%**	**44%**	**39%**						**39%**		**52%**
min	-56%	-22%	3%	50%	-6%	51%	-10%	20%	15%		50%			7%	144%							28%	13%	26%						24%		40%
max	22%	63%	90%	130%	0%	51%	37%	104%	73%		79%			38%	144%							28%	75%	53%						55%		
Fed vs rest							**100%**	**58%**	**45%**	**126%**				**138%**	**69%**	**80%**			**8%**		**196%**		**48%**	**111%**						**80%**		**86%**
min							-25%	-15%	-7%	18%				103%	56%	35%			8%		133%		15%	88%						64%		**68%**
max							250%	122%	112%	217%				180%	81%	96%			8%		250%		67%	135%						100%		
Ex-Fed vs rest	**106%**	**185%**	**180%**	**117%**	**150%**		**193%**	**88%**	**117%**	**148%**	**27%**	**105%**		**124%**	**93%**	**122%**	**68%**				**156%**	**82%**	**126%**	**157%**	**103%**			**105%**		**73%**	**131%**	**123%**
min	-22%	67%	0%	17%	-16%		39%	-2%	30%	76%	7%	83%		22%	-39%	38%	68%				76%	56%	19%	52%	60%			73%		6%	8%	**105%**
max	233%	300%	483%	190%	467%		308%	213%	272%	244%	73%	147%		313%	209%	196%	68%				261%	108%	240%	290%	153%			141%		200%	340%	
EX-Fed vs PLA/ CHO	**455%**	**102%**	**189%**	**24%**	**-21%**	**63%**	**92%**	**65%**	**52%**	**143%**	**45%**			**50%**	**76%**	**43%**	**36%**					**42%**	**36%**	**91%**					**26%**		**59%**	**64%**
min	9%	57%	51%	-30%	-33%	51%	65%	32%	7%	130%	29%			-17%	31%	6%	35%					22%	0%	28%					23%		11%	66%
max	900%	164%	600%	74%	-9%	75%	112%	99%	167%	157%	71%			143%	147%	95%	37%					63%	89%	154%					29%		89%	
Ex-Fed vs Fed		**38%**					**9%**	**39%**	**50%**	**20%**	**21%**			**26%**	**92%**	**88%**		**139%**		**27%**	**-5%**		**40%**	**0%**		**4%**	**51%**			**27%**	**66%**	**47%**
min		38%					-5%	-7%	-31%	0%	18%			-9%	44%	-1%		0%		22%	-23%		-2%	0%		4%	51%			20%	5%	82%
max		38%					19%	65%	127%	50%	28%			97%	156%	300%		293%		32%	7%		105%	0%		4%	51%			35%	168%	
Ex-Fast vs Fed		**-29%**		**27%**					**-3%**																							
min		-29%		27%					-45%																							
max		-29%		27%					30%																							

METHODOLOGICAL APPROACHES TO MPS AND MPB INFLUENCING THE ACUTE RESPONSE

Most studies examining the PRO/AA induced stimulation of human muscle protein synthesis have utilized mixed and myofibrillar protein synthesis in the *vastus lateralis* using the direct incorporation approach. However, many early studies have used arterial-femoral balance techniques across a limb with and without biopsies and tracers to calculate or estimate muscle protein turnover. There has been some disagreement between these methods and interpretation that has resulted in some confusion for the lay population. In order to provide some clarification we present a variety of explanations for these discrepancies.

Temporal differences in mixed or sub-fraction protein synthesis are often assessed during different and extended time periods of recovery, representing an average incorporation during that time frame, whereas the arterial-venous balance (A-V Bal) approaches are taken at a specific time and can theoretically, be taken over a prolonged period, but as more collections are taken are the likelihood of disturbing the system increases, thus decreasing the validity of the assessment. A-V Bal assesses flux of AA in all the muscles of the limb irrespective of the potential protein(s) being synthesized, which experience different or in some instances no activation with exercise, whereas the precursor product assessment of mixed-muscle, sarcoplasmic, mitochondrial or myofibrillar protein synthesis is only specific to the activity of that specific protein fraction in the *vastus lateralis*.

The only studies that detect an increase in protein synthesis using 3-pool (artery, vein and muscle) modeling several hours following RE in the fasted state used a complete leg work-out of several exercises, but only in the later 2-3h recovery period [69, 274,

275]. Whereas those studies examining RE in the fasted state using a partial leg work-out of leg extension only were not able to demonstrate an increase in protein synthesis using 3-pool modeling [110, 261, 276] or did not report it [29, 232, 254, 261, 277, 278].

An early study in protein metabolism has demonstrated decreased protein synthesis and breakdown with a slight improvement in net balance in the non-exercised muscle 3 hours post-exercise [279]. This occurred although there was an increase in whole body protein synthesis [279], which highlights the compartmentalization that occurs in protein metabolism following an exercise stimulus. It is reasonable to assume unless all the muscles of the leg are similarly exercised they will provide divergent influence on parameters assessed via the A-V Bal technique and the intracellular "status " of the exercised vs non-exercised muscle is likely to be different, especially in the fasted state. In other words, the non-exercised muscle could be diluting the 2 and 3-pool kinetic parameters by functioning in the separate manner as the exercised muscle. This could explain why so many of the studies mentioned above do not support what the direct incorporation approach has constantly demonstrated, that muscle protein synthesis in increased in the hours following RE in the fasted state [29, 69, 90, 101, 267, 274, 280]. It would be interesting to test this theory with assessment of perfusion and/or biopsies on the exercised and non-exercised muscle from the same limb.

Only those studies that detect an increase in protein synthesis following RE using 2-pool or 3-pool modeling have some form of amino acid provision [110, 224, 246, 252, 255, 257, 258, 260, 281] and even these responses were as transient (or even more) than would be expected from amino acid provision alone [38, 229, 231, 282-286]. This suggests that amino acid provision temporarily equalizes the "status" of all the muscles

across the leg (exercised and non-exercise, especially if it was a recently exercised limb with elevated blood flow.

The increase in protein synthesis via 2 or 3-pool kinetics is directly proportional to the increase in amino concentration in the blood/muscle. Those studies giving a large bolus of AA via intravenous infusion [253] or orally ingested amino acids [252, 255, 257, 258, 260] reach a larger increase in amino acid concentrations and thus can safely calculate the kinetic parameters with minimal influence from the intrinsically variability of blood flow or other variables. Of those studies using AV-Bal and muscle biopsies to examine intact/dietary protein ingestion following exercise [232, 246, 247, 254, 277, 278, 281] none have reported 3-pool modeling and only those studies using ultrasound to assess blood flow (gives lower, less variable values) have reported 2-pool kinetics [246, 281, 287]. Similar to orally ingested amino acids, whey protein ingestion following exercise stimulates a transient (< 60 min) increase in estimates of protein synthesis (Rd) [281], milk stimulates a very brief (30 min) increase in Rd [246] and the increase in phenylalanine net balance from whey [232, 277, 281] or milk [247] ingestion in close proximity to exercise is just as brief. However, milk [246, 247] or the slowly digested casein [281] can prolong net balance up to 2 hours post-ingestion, which we have similarly demonstrated with the a soy-dairy protein blend [189]. Several of these studies [189, 246, 281] reveal a much longer stimulation of muscle protein synthesis using biopsy samples from the *vastus lateralis* and the direct- incorporation approach [65, 237, 246]. Other reasons for these discrepancies may stem from the fact that the direct-incorporation approach has 3 parameters (time, precursor and product TTR) with less

intrinsic variability whereas the 2-3 pool models have a few more parameters, AA concentrations and especially blood flow which have a higher intrinsic variability.

As mentioned previously, several studies have presented amino acid net balance results with examination of PRO/AA interventions in close proximity of exercise. However, few people understand the limitations of this method. Blood flow, a possible dilution of non-exercised muscle and other tissues present a confounding influence; in addition, other factors such as changes in free AA intracellular pool size may make conclusions difficult. An increased net balance could be due to a transient increase of inward AA transport and or reduced muscle protein breakdown and not necessarily a difference in in MPS. This factor is likely modulated, masked and further complicated by transient changes in muscle swelling [288, 289] that do occur following RE. Changes in net balance data should be interpreted with caution and assessment of other variables to give evidence that the "anabolic response" is not just a transient change in pool size are warranted. Given the evidences of the following 1) potential dilution factor of non-exercised muscle 2) transient effect from amino acids causing a change in AA pool size 3) transient changes in muscle swelling; and 4) a high influence from blood flow 2 and 3-pool methods should be interpreted with caution and applied in research studies designed to minimize variance from these factors so that the effect of PRO/AA feeding may be better understood and the unique kinetic assessments from these models may be correctly applied to further advance the field.

PROTEIN DOSE

Due to the implications of finding an effect, an exorbitant amount of attention has been placed on interventions to enhance the acute response of MPS in the early recovery period post-RE. In 2009, Moore et al. described, in six subjects, a dose effect of post-RE MPS with egg protein ingestion [233]. They discovered that MPS was maximized with 20g of Pro [233] which corresponds to ~8-9g of EAA and about 1.8g of leucine. Follow-up research with whey protein has demonstrated similar findings, in elderly men [238, 245], when subjects were fed breakfast [242], in energy deficit [51] or with beef ingestion [248]. These and other studies helped shape the general consensus that 20-30g (containing ~8-15g EAA) is likely to maximize the post-exercise MPS response, at least in young men. This is a general finding, and may not may not always apply, as there are several modifiers to the effect amino acid sensitivity in skeletal muscle. Certain individuals with a larger lean mass or body mass may benefit from a larger post-exercise PRO dose [51]. Also, previous physical activity may lower the dose while catabolic conditions of energy deficit [51] or various health concerns (inflammation, sickness, aging) may necessitate a higher dose [238, 245, 248, 290]. We are not aware of any evidence regarding an interaction with post-exercise pharmacokinetics and PRO/AA nutrition - an area of future investigation.

PROTEIN TYPE/SOURCE

Besides factors intrinsic to the individual, the type/source of protein/AA ingested has been thought to modulate the post-exercise MPS response. Potential differences could be due to the overall protein quality (i.e., amino acid composition) of the protein source and the extracellular AA appearance reflected by it its digestion rate (i.e., fast,

intermediate, or slow). It is clear that crystalline AA have a potent effect on post-exercise MPS [40, 41, 54, 252, 253, 255, 258]. Also, intact protein ingestion in the form of soy, casein, whey, egg or beef increases post-exercise MPS [43, 65, 190, 218, 232, 233, 238, 241, 245, 246, 249, 291]. Due to several methodological differences between investigators, there is some disagreement about whether different protein sources produce superior effects on MPS.

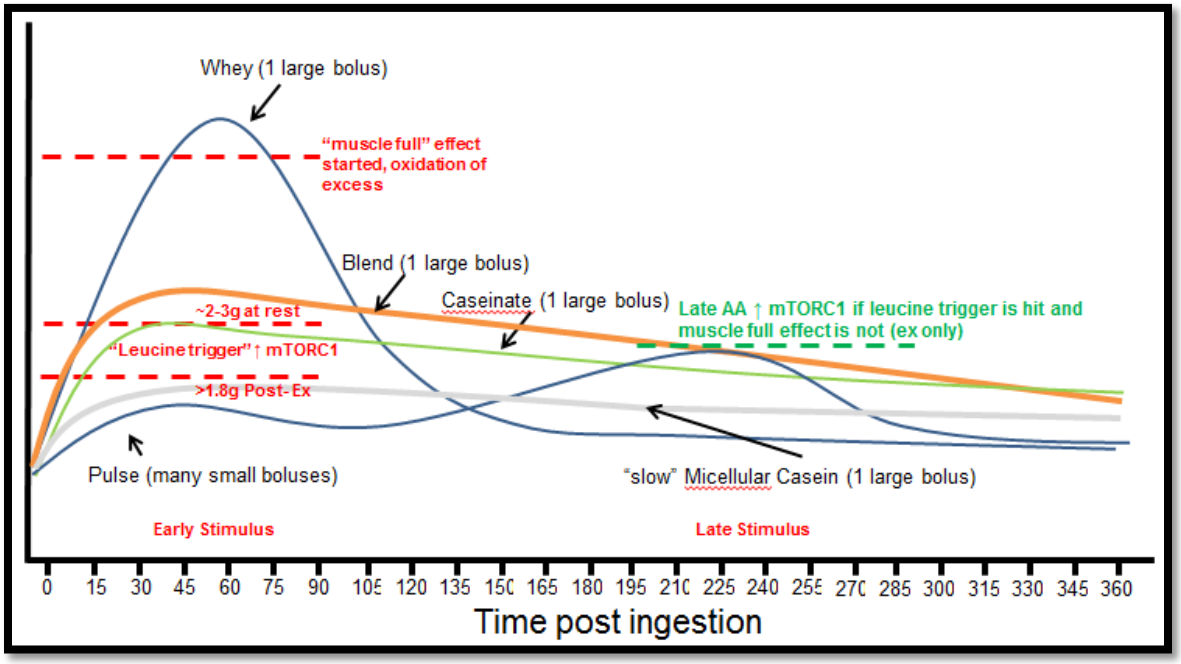
One reason for discrepancies between effects of protein supplement type on the post-exercise MPS response is that matching PRO by total protein content results in an imbalance of total leucine content across the protein interventions. In studies with this imbalance there are some differences in acute post-RE MPS between protein supplement types [239, 292]. It is abundantly clear that leucine stimulates MPS [293-299]. It seems that the potent stimulatory effect of the higher leucine content of a supplement will impact the MPS response and mTORC1 signaling more than a minor change (3-10g) of total protein. Also, the difference in total protein ingested is mostly composed of non-essential AA which do not stimulate muscle protein anabolism [252, 300]. Although energy status may be important in some cases [51], but not others [52] a 12-40kcal kcal difference in total energy from the supplement is extremely unlikely to influence the MPS response. Others have demonstrated that adding 120 kcals in the form of carbohydrate does not further stimulate muscle protein anabolism when sufficient EAA are provided [301]. Two recent studies have elegantly demonstrated that the leucine content in a supplement is a primary stimulator of MPS, especially when the total protein or content of other amino acids is low [217, 302]. Studies comparing the ingestion of

isolated protein supplements following RE that found differences in post-exercise MPS are shown in **Table 1.10**.

Table 1.10. Summary of the effect of leucine content in an ingested protein supplement on MPS in the vastus lateralis following acute resistance exercise in humans.

MPS response	Leucine Content (grams)	Post-Ex FSR Period	Ref
Whey > Soy > Casein	**2.3,1.8,1.8**	0-3h	[1]
Whey > Soy	**2.0, 1.6 (20g Pro)**	0-4h	[2]
Whey > Casein	**2.8, 1.6**	0-4h	[3]
Whey > Casein 1-3.5h (trend) **Whey = Casein 0-6h,**	**2.1, 1.5**	1-3.5h, 3.5-6h, 1-6h	[4]
Whey Bolus > Whey Pulse	3.5 bolus, 3.5 spread out	1-3, 3-5h	[5]
Whey Pulse = Whey Bolus	7-8, 7-8 (both + 5g free Leu)	1-5h	[6]

Figure 1.4. Theoretical construct for the effect of resistance exercise protein supplement type on blood amino acids and resultant anabolic stimulation



It appears that the digestion rate and AA composition of a protein are two factors that should be considered together as they may not act independently. Protein appears to

be is most effective when given as a bolus (with an adequate amount of leucine) in close proximity to exercise [234] to maximize the feeding effect since even a pulse ingestion [34, 43, 234, 265] poorly mimics the blood AA release from a bolus of slower digesting protein [65, 225, 291]. Further support to the stimulatory effect of leucine is demonstrated by evidence showing that added free-leucine to a whey pulse is just as effective as a whey bolus [43] when given before exercise. Thus, PRO/AA ingestion in close proximity (days) to exercise may lower the leucine threshold by exercise-induced facilitation of AA flux. Examining the literature (**Table 1.8**) suggests that a greater leucine stimulus may be needed in the rested vs exercised condition to prolong/enhance the MPS response. We would estimate that a PRO/AA source containing ~1.8-2g would be the sufficient to activate a post-exercise "leucine trigger" due to the exercise-induced AA flux and that the rested condition may require 2-3g or more, especially in some catabolic conditions or aging. Leucine plays a key role in the post-exercise MPS response, at least when total PRO intake is lower. However, if the PRO/AA dose given contains sufficient leucine it seems clear that it does not matter what protein source is used, providing it is a higher quality source, digestible and containing all the essential amino acids. This hypothesis has been tested and proven by chronic exercise and supplement studies discussed in later sections.

As mentioned, differences in protein types on MPS may be partly a factor of methodological differences. Examination of the literature suggests that the intrinsic properties of the ingested protein type/source are reflected in the physiologic MPS response (**Figure 1.4, 1.5 & 1.6 & Table 1.10**). A fast, rapidly digested source causes a rapid and maximal increase in MPS [232, 234, 241, 245, 303, 304], whereas a slowly

digested source is more likely to cause a delayed, more prolonged response [65, 291], at least in the exercised condition. Because of a higher BCAA content, [292], and rapid increases blood amino acid concentrations following whey protein has been considered superior to other isolated protein sources [234, 238, 245, 305, 306]. However, our scientific interpretations of these findings are shaped by the limits of our observations. Most of the studies examining various protein types/sources utilize a window of 3-4 hours post-exercise (**Table 1.8**). A study extending the post-exercise window to 1-6h comparing post-exercise ingestion of whey vs casein and found no difference in the MPS, but they trended to see differences in early and late periods [65]. We have demonstrated a similar pattern with a protein blend of multiple amino acid release profiles [225]. This evidence suggests the limits in our observation may be skewing the interpretation. It seems clear that when examining the evidence from many acute studies (**Figure 1.5 & 1.6**), there is no difference in protein source on the magnitude or duration of the MPS response when examined over a longer post-exercise incorporation window (past 4h post-exercise). **Table 1.11** also demonstrates that the FSR window of assessment is important in capturing the MPS response to protein ingestion with regards to leucine intake. This hypothesis has been tested and gives credible evidence in chronic exercise and supplement studies discussed in later sections.

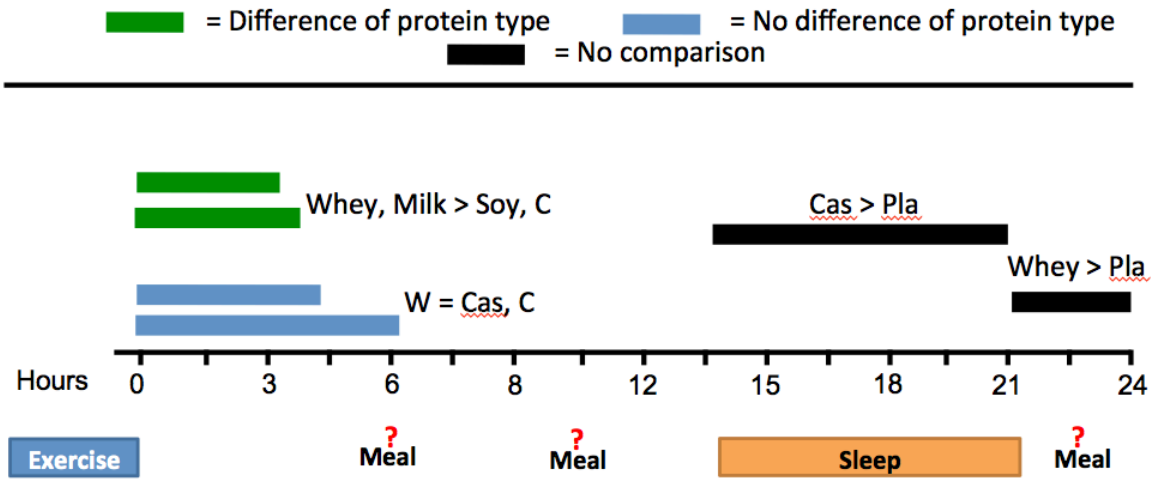
Figure 1.5. Increases in *vastus lateralis* FSR during various recovery periods in response to resistance exercise with protein amino acid supplementation

	Early Period	Late Period	Entire Period	Later Period	Very Late Period
Hours	**0,1-3,4**	**3-5,6**	**0-5,6**	**6-12**	**24-27,29**
Leu-EAA [1]	Y↑↑, O↑	Y↑, O↑↑	↑↑ (O&Y)		↑ O
Whey	↑↑ [2-6] ↑ mf [7, 8]	↑mf [6-8]	↑↑mf [9] ↑mf [6, 8, 10]	↑↑mf [15] (20gx4)	↑mf [9, 11]
Caseinate [6]	↑↑mf	↑↑mf	↑↑mf		
Micelluar Casein [2, 5]	↔↑		↑ [14, 16]		
"Whey pulse"	↔ mf [8]	↑mf [8]	↑mf [8], ↑↑ [12]		
Soy [2, 4, 13]	↑				
Milk [13]	↑↑				
Blend [17]					

1. Drummond J Appl Physiol 2008. **2.** Tang J Appl Physiol 2009. **3.** Tang Appl Physiol Nutr Metab 2007. **4.** Yang Nutr Metab (Lond) 2012. **5.** Burd Br J Nutr 2012. **6.**Reitelseder Am J Physiol Endocrinol Metab 2011. **7.** Moore J Physiol 2009. **8.** West Am J Clin Nutr 2011. **9.** Burd PLoS One 2010. **10.** Dideriksen Scand J Med Sci Sports 2011. **11.** Burd J Nutr. 2011. **12.** Burke Med Sci Sports Exerc 2012. **13.** Wilkinson Am J Clin Nutr. 2007. **14.** Pennings Am J Clin Nutr 2011. **15.** Areta, J Phys 2014. **16.** Koopman B J Nutr 2008. **17.** Koopman AJPE 2005 **18.** Reidy J Nutr. 2013/JAP 2014.

mf, myofibrillar fraction; leu-EAA, leucine enriched essential amino acid.

Figure 1.6. Anabolic effect (MPS) of protein type + resistance exercise: window of assessment



1. Drummond J Appl Physiol 2008. **2.** Tang J Appl Physiol 2009. **3.** Tang Appl Physiol Nutr Metab 2007. **4.** Yang Nutr Metab (Lond) 2012. **5.** Burd Br J Nutr 2012. **6.**Reitelseder Am J Physiol Endocrinol Metab 2011. **7.** Moore J Physiol 2009. **8.** West Am J Clin Nutr 2011. **9.** Burd PLoS One 2010. **10.** Dideriksen Scand J Med Sci Sports 2011. **11.** Burd J Nutr. 2011. **12.** Burke Med Sci Sports Exerc 2012. **13.** Wilkinson Am J Clin Nutr. 2007. **14.** Pennings Am J Clin Nutr 2011. **15.** Areta, J Phys 2014. **16.** Koopman B J Nutr 2008. **17.** Koopman AJPE 2005 **18.** Reidy J Nutr. 2013/JAP 2014.

Cas, casein; W, whey; Pla, placebo.

AGING & SEX EFFECTS AND RESISTANCE EXERCISE

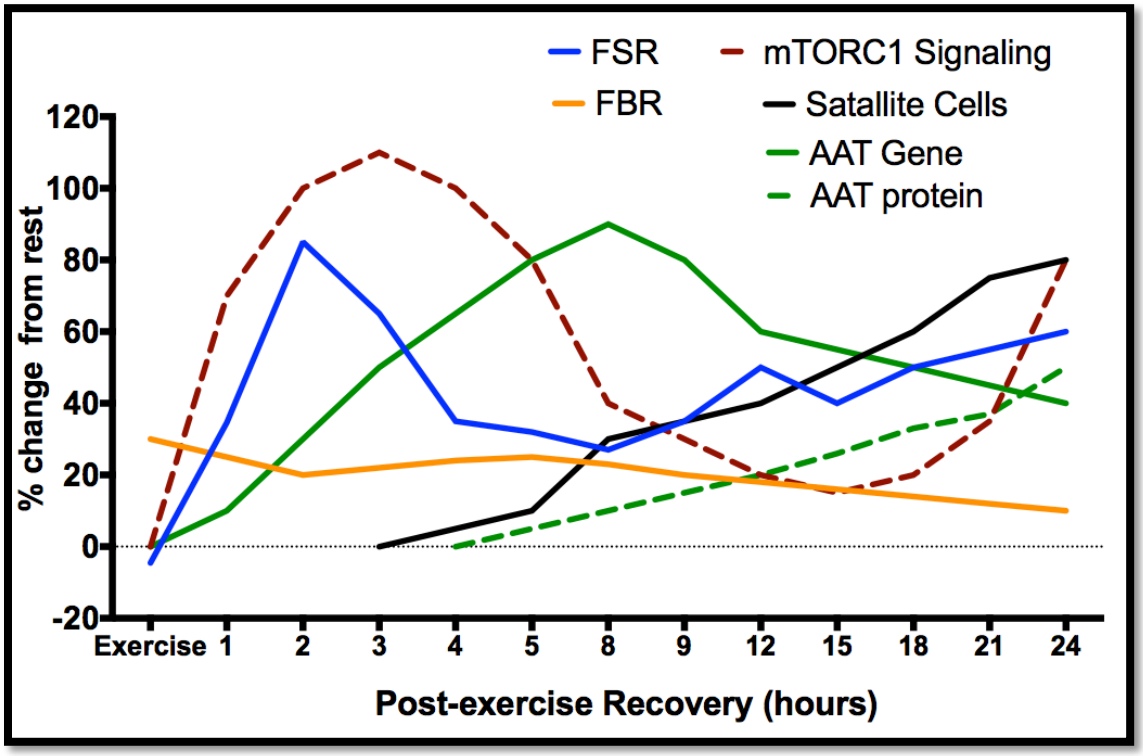
In the fasted [9, 220] and whey protein fed condition [223] following RE, MPS and mTORC1 signaling are not different between men and women. However, evidence has shown disparities between older and younger cohorts of adults through a blunting of the MPS response, mTORC1 signaling [9, 31, 91] and dysregulation of amino acid transporters [140] following RE (**see Figures 1.3 & 1.5**). A potential explanation for the altered transcriptional [115, 307] and translational blunting following RE [115, 116, 140] is thought to be the increased chronic inflammation seen with aging (see earlier section). Because of the progressive decline of muscle mass with aging, this evidence is disturbing and interventions such as large boluses of amino acids [41] whey protein [235, 238, 239] beef [248] or other high quality protein sources hold promise as a means to restore the MPS response. Although 20-30g of PRO has been implicated in maximizing the MPS response in young adults, the current evidence suggests that a higher dose of ~30-40g may be more effective in older adults. In further examination of the anabolic resistance in older adults, researchers have not been able to identify a disparity in FBR between old and young adults [10] and this outcome changes less than FSR [267] in response to RE, interventions have targeted FSR.

A factor overlooked in most studies considering the impact of PRO/AA nutrition on post-exercise MPS responses is the gut physiology and adaptation. Splanchnic uptake extracts half or more of the AA released during digestion during the first pass splanchnic extraction [308-311]. Because if the higher turnover of proteins in those tissues, the splanchnic region is a primary site of AA flux and supply (of certain AA) to other tissues under various conditions [312-314]. This response likely is dependent on frequency/size

of the ingested bolus, aging and probably the AA composition of the ingested protein source [314-316]. We know very little regarding the interplay of this process with regards to modulating post-exercise MPS, especially in regards to chronic exposure to the stimulus.

Most studies investigate the acute response of post-exercise MPS report data in the format of means and then direct generalized conclusions toward the population. Unfortunately, we have very little published information on individual variability in an acute MPS response to nutrition. Our own experiences demonstrate that there is significant variability in the magnitude and duration of post-exercise MPS between individuals (unpublished observations). Also, a few recent publications [44, 243] have demonstrated diverse individual responses. It is evident that a portion of the population (suggested to be ~25%) does not respond to PRO/AA supplements [44, 317]. Future research should be designed to elucidate more precise estimates of the prevalence of and the mechanisms underpinning this phenomenon such that a quarter of the population is not neglected with continued generalization of findings.

Figure 1.7. Percent change approximation in human skeletal mixed-muscle FSR and FBR, mTORC1 signaling, satellite cells and amino acid transporter (AAT) gene expression and protein over the course of a 24h recovery period following a bout of moderate-hard intensity resistance exercise.



FSR, fractional synthesis rate; FBR, fractional breakdown rate; AAT, amino acid transporter; mTORC1, mammalian target of rapamycin complex 1.

Association with Acute Molecular Events and the Physiological Response to Resistance Exercise

Animal, cell and other basic science models have clearly delineated a necessary role for mTORC1 and other signaling pathways in controlling muscle protein synthesis. For obvious reasons, cause and effect studies are difficult to perform in human models however; pharmaceutical approaches have provided a means to gain some insight into the cause and effect of these mechanisms in human physiology. Our laboratory has been able to use the drug rapamycin as a means to gain insight regarding the cause and effect of RE and EAA on MPS in human skeletal muscle. Even with a minimal dose of

the drug, we were able to block the contraction [90, 104] and EAA [231] induced stimulation of mTORC1 signaling and MPS, in human muscle, clearly indicating an increase in mTORC1 signaling is necessary for increasing MPS as a result of these anabolic stimuli. However, as discussed above, although many reports provide concomitant activity, several reports do not demonstrate concomitant increases in mTORC1 signaling and MPS. Several researchers have voiced dissension and frustration regarding "discordance" between mTORC1 signaling and MPS [82, 243]. Although, much of this inconsistency could be explained by differences in analytical methods, antibody batch effects, or timing of the assessment relative to the assessment of MPS it is not surprising to see a perfect time course between mTORC1 signaling and MPS. In fact, a discordance should be expected given the molecular roles of signal transduction and biological complexity of the system. It would seem presumptuous to assume that a few static one second "snapshots" of mTORC1 signaling would be representative of the MPS response over a several hour (2-6h or 7200-21600sec) post-exercise recovery period. Nonetheless, even with a signal encompassing less than 0.001% of the MPS time period, several investigations have reported correlations between mTORC1 signaling and MPS following AE [318, 319] and RE in the fasted [9, 32, 91] and fed [33] conditions. In the fasted state, this association is only present on young adults [9, 91] and not older adults, reflective of the "anabolic resistance" seen with aging and suggestive of dysregulation of mTORC1. The paradigm thus far has been to examine the association between changes in a single marker (i.e. S6K1 or mTOR) and MPS. It may be a more appropriate reflection of the biological system to use statistical modeling to test the interaction of several of signal transduction molecules on MPS.

We have also demonstrated that rapamycin administration does not influence resting post-absorptive protein synthesis indicating that other mechanisms besides mTORC1 signaling can be involved. Collectively, our data suggest that increases in mTORC1 activity is akin to an "anabolic switch" to turn on MPS in response to a stimulus. It seems very likely that up to a certain point this "switch" may serve as an on/off or on/low/high function in a permissive, but necessary role to increase MPS rather than a sensitive "dimmer" switch fine-tuning the MPS response. Indeed, as support of this concept, we have shown that additional activation of mTORC1 signaling by adding leucine to a maximal dose of EAA does not further enhance MPS [230]. However, if the dose is not optimal, slight modifications to amplify mTORC1 signaling (i.e. increasing leucine) and thus MPS, may be successful, if conditions are appropriate [111, 113]. The evidence from explorations into human skeletal muscle signal transduction demonstrate that an increase in mTORC1 activity and translation initiation occurs following exercise corresponding with increases in MPS, however, although exercise prolongs the MPS response, this effect wanes (**Figure 1.5 & Table 1.9**) suggesting that other factors (energy, available substrate, substrate composition, substrate flux, cell swelling and AA sensing) may be involved and take precedent over mTORC1 signaling after the initial stimulus fades.

Table 1.11. Summary of studies demonstrating an association with intracellular signaling and muscle protein synthesis in the vastus lateralis following acute resistance exercise

Reference	Feeding	Groups	Time course (PEx)	mTOR (Ser2448)	S6K1 (Thr389)	4E-BP1 (Thr37/46)	Hypoxia
		Overal Pattern		Yes, in young	Yes, in young	Yes, in young	yes
Burd et al. (2010)	Breakfast	4x5 to fail 90% 1RM 4x~14 to WM 30% 1RM 4x~28 to fail 30% 1RM	rest, 4, 24h		MPS 24h PE (r2 =0.13, P= 0.055)	MPS 24h PE r2 =0.14, P =0.049)	
Burd et al. (2010)	20g Whey	1 set LE to fatique, 70% 1RM TR 3 sets LE to fatique, 70% 1RM TR	5F, 29F h		MPS r = 0.34, P=0.033		
Etheridge et al. (2011)		Unilateral; 6x8; Young 70% 1RM, Normoxia Unilateral; 6x8; Young 70% 1RM, Hypoxia	0,3.5h (NR)		MPS (r2= 0.23, P > 0.05)		MPS 2.5 h after REand mean hypoxic SpO2 (r2 0.49, P < 0.05)
Fry et al. (2011)	Fasted	10x10 Young, 70 % 1RM, 3 min rest ~8-10x10 Old, 70 % 1RM, 3 min rest	24h PE	Yes, r2 = 0.39 no,r2 = 0.01 P = 0.71	yes, r2 = 0.29 P = 0.04 no r2 = 0.01 P = 0.69		
Kumar et al. (2009)		LE, 60-90% 1RM, young LE, 60-90% 1RM, Old	rest, 10min, 1,2 & 4h		yes, r2 = 0.31 P = 0.049 no r2 = 0.01 P = 0.7		
D'Souza et al. (2014)	10g Whey 20g Whey 30g Whey 40g Whey	3x8-10 Squat, LP, KE, ~80% of 1RM, Untrained OM	Rest, 2 & 4h		Intracellular leucine, r2 =0.32 P = 0.03		

#x# = sets x reps; LE, leg extension; TR, trained; LP, leg press; KE, knee extension; MPS, Muscle protein synthesis; PE or PEx, post-exercise; Ex, exercise; PRO, protein; net bal, net-balance; EAA essential amino acids; CHO, carbohydrate WM, work-matched; RM, repetition maximum.

CHRONIC PHENOTYPICAL ADAPTATION TO RESISTANCE EXERCISE WITH AND WITHOUT PROTEIN AND/OR AMINO ACID FEEDING

Many molecular and metabolic investigations have demonstrated the effectiveness of protein or amino acid supplementation following an acute RE session in the enhancement of MPS and signal transduction (see above). There is a clear benefit of resistance exercise-training (RET) to increase muscle size and strength in the young [320, 321] and older [322, 323] adults. However, there is lack of clarity regarding whether chronic protein supplementation during RET enhances muscle growth as compared to RET without protein supplementation. Although, many studies have shown no effect of added protein/AA supplementation [324-339], other studies with a high quality protein supplement during RET occasionally demonstrate improved muscle mass and, more infrequently, strength as compared to no protein supplementation [78, 329, 340-345]. The reasons for the confusion in the literature have been suggested to stem from differences in study design, choice and measurement of outcomes, target populations, exercise protocols and the timing, and the type and amount of the protein supplement or placebo given. Also quality control (internal validity) of some studies may provide an added layer of variability to these complex clinical trials [346]. It is likely that other unknown variables are involved as well. We have tabulated all available literature in for younger (**Table 1.13**) and older (**Table 1.14**) adults examining the role of protein/amino acid supplementation/intake on RET improvements in muscle size, lean mass and strength.

In the past few years alone there have been many systematic reviews, meta-analysis and even more opinion papers regarding the effects of protein supplementation on exercise adaptations of muscle mass, body composition, strength, power and exercise performance [292, 305, 324, 336, 347-355]. Given the heterogeneity of long-term

exercise training studies, these reviews have been commendable undertakings, necessary to provide evidence-based application for the sports nutrition practitioner. Nonetheless, further expansion and assessment of the literature on this topic is still needed, as no clear consensus has been found regarding the effects of protein supplementation to augment exercise adaptation/performance. Each meta-analysis or systematic review on the topic has taken a slightly different approach and often many studies were excluded to enable meta-analysis. We have compiled all the relevant literature to demonstrate the heterogeneity in the field and to encourage a complete and critical review. The data from the meta-analyses are compiled in **Table 1.12.**

One of these examinations, a recent meta-analysis was designed to answer this question with the studies available at the time [324]. After exclusion of a several studies to reduce the heterogeneity, they pooled 22 studies with 46 groups examining the effect of protein supplementation during RET in old and young RE trained and untrained subjects. Each study utilized a variety of variables such as lean body mass (LBM), fat-free mass (FFM), myofiber cross-section (fCSA), and muscle strength to investigate this question. The pooled results for the meta-analysis showed gains in FFM, Type I & II muscle fiber CSA (fCSA) and 1-RM leg-press with protein supplementation vs. no protein supplementation following prolonged (>6 wk) RET [324] (**Table 1.12**) The effects were evident in both young and older adults, but of greater magnitude in the young and limited in older adults. With added protein supplementation during RT, compared to placebo, the young gained approximately an additional 1 kg of FFM, with additional increases of 20% in leg press strength and 45 and 54% of Type I and II CSA, respectively. However, the percent changes for fiber CSA seem rather robust, as

increases in fCSA fall in the range of 10-30% following RET with increases of fCSA of about 500-1500 μm^2. They suggested that protein supplementation provided an additional 212 μm^2 and 291μm^2 gain in MHC I and II fibers, respectively. This amount is well within the error of the technique, thus it is no wonder that individual studies have had provided sparse positive results regarding the effectiveness of protein supplementation to enhance fCSA during RET.

Interestingly, the younger subjects who had previous resistance exercise-training experience demonstrated a greater benefit, on FFM gains, compared to untrained subjects. The authors suggested this finding reflected an improved sensitivity of nutritional support to help overcome a plateau in adaptation to RE [356]. This is an interesting hypothesis considering most of the acute investigations of MPS have suggested that RT individuals have a shortened and reduced sensitivity to post-exercise PRO/AA compared to resting conditions (see earlier sections). A more probable explanation is that the RET trained participants were given much more protein (median: 84g/d, mean 74g/d) compared to the untrained participants (median: 38g/d, mean 32g/d).

A recent systematic review has suggested that as resistance-training duration progresses and the intensity/volume is increased an effect of PRO/AA is more likely [353] to occur. The longest-running RET and protein supplement study in young adults, to date, evaluated participants at 12,24 and 36 weeks of a periodized resistance exercise training program [333]. In contrast to the some suggestions from some of these commentaries, they demonstrated that lean mass plateaued at 12 weeks with PRO and further supplementation throughout a progressively difficult RET program had no additional effect. A similar pattern was shown when using ultrasound to assess muscle

thickness at 10.5 and 21wks of progressive RET and protein supplementation [53]. Collectively, these data suggest a slowing or "hypertrophic plateau" at ~8-14 weeks of RET, which coincides with the recently described time course of muscle hypertrophy [357]. A more in-depth statistical approach examining the effect of prior training was taken by Schoenfeld and colleges [349]. They could not demonstrate that previous training status was an important predictor of lean mass or strength changes with RET.

Table 1.12. Meta-Analyses of chronic effect of resistance exercise training with PRO/AA nutrition on muscle size & strength

Author, Year	Subjects	Groups	Protein/Other	Mass /CSA	RET	Δ Size, CSA	Δ FFM or LM	Δ % FAT or body fat	Δ Strength 1RM	PRO intake g/kg/d	PRO g/d
Finger 2014 PMID: 25355074, report SDM	462 older adults	PRO	PRO (0.45 g/kg/bw) (range: 0.3-0.8 k/g/bw)	DEXA, MRI, CT, fCSA,	Varied, < 6wk	0.14 (-0.05,0.32)	0.23kg (0.05,0.42)	-	0.13 (-0.6,0.32)	0.46 change	Median:20g, Mean26g
Cermak 2013 PMID: 23134885	213 older adults	PRO (N=42-106), PLA (N=39-109)	PRO (42 ± 30 g (range: 6–106 g) on Ex days	DEXA, few fCSA	Varied, < 6wk	T1:-17(-324,291) T2:-132(-410,147)	0.48kg (0.10,0.85)	-0.11kg (-0.5,0.29)	13.1kg (0.32,25.9)	-	Median:20g, Mean27g
Cermak 2013 PMID: 23134885	444 young adults, PLA (N=51-188)	PRO all (N=67-264)	More PRO (42 ± 30 g (range: 6–106 g) on training days	DEXA, some fCSA	Varied, < 6wk	T1:241(131,350) T2:477(333,620)	0.81kg (0.53,1.1)		14.4kg (5.2,23.6)	-	Median:40g, Mean47g
		PRO TR (N=7-47)				-	0.98kg (0.45,1.5)	-0.11kg (-0.5,0.29)	-	-	Median:84g, Mean72g
		PRO UT (N=5-85)				-	0.75kg (0.42,1.1)		-	-	Median:38g, Mean32g
Miller 2014 PMID: 24724774	626 young and old, RET subgroup = 258	Whey, diet replacement	35-88g/d	DEXA,	no-EX + EX, < 4wk	-	−0.66 (−2.91,1.59)	−0.60 (-4.08,2.88)	-	0.23-1.2	35-88g/d
		Whey, supplement				-	0.28kg (−2.79,3.35)	-0.21kg (−2.16,1.75)	-		
		Whey vs other sources				-	0.37kg (−1.47,2.21)	0.14kg (−2.05,1.76)	-		
		Whey+RET			Varied Ex	-	2.24kg (0.66,3.81)	-	-		
Schenfield 2013 PMID: 24299050, report effect size	Strenght: 484 young & old; Lean mass: 525 young & old	overal Effect size	all pooled	DEXA, fCSA	Varied, < 6wk	-	0.47 (0.31,0.63)	-	1.39 (0.88,1.90)	-	-
		PRO basic model	(treatment or control) as a predictor.			-	0.24 (0.04,0.44)	-	0.38kg (-0.34,1.10)	-	-
		PRO All covariates	Group, PRO matched, training status, blinding, gender, age, body mass, training duration			-	0.16 (-0.07,0.38)	-	0.28kg (-0.52,1.07)	-	-
		PRO Reduced Model	protein intake, study duration & blinding			-	0.14 (-0.07,0.35)	-	0.39kg (-0.34,1.11)	-	-
		FFM or CSA				0.14 (-0.17,0.46)	0.08 (-0.07,0.24)	-	-	-	-
		Total Pro intake only Model	protein intake			-	0.14 (-0.07,0.35)	-	-	-	-

PRO, Protein group; PLA, placebo; TR, trained; UT, untrained; RET, resistance exercise training; bw, body weight; MRI, magnetic resonance imaging; CT, computed tomography; EX, exercise; DEXA, dual-xray absorbmerty; fCSA, myofiber cross-sectional area; FFM, fat-free mass; wk, week; kg, kilogram; g, grams.

It would appear that there is a "protein paradox" in the literature. General physical activity [358, 359] and resistance training [360-362] in particular improves efficiency of protein turnover so theoretically those more trained wouldn't need more protein, yet many have posited that resistance trained participants benefit the most [324, 347, 352, 353, 356]. It is possible that those who are higher responders to RET and nutritional intervention are more likely to continue a successful pursuit, thus a higher proportion of these high responders are being enrolled in studies using RT participants.

This hypothesis warrants further investigation. Some have suggested that because trained individuals have a more transient MPS response, protein timing may be important, however, the two studies investigating this hypothesis have yielded equivocal results [363, 364].

Another meta-analysis set out to examine if protein timing in close proximity to the exercise bout was an important factor that mediated these exercise adaptations [349]. In modeling without covariates, they demonstrated a modest effect of protein supplements on muscle hypertrophy, but no effect on strength. When including other variables, such as total protein intake, the effect of protein supplements was not observed [349] and they discovered that total protein intake was the best predictor of improvements in muscle mass in their model. None of their statistical models demonstrated a PRO/AA effect on strength. This finding is contrary to the commonly preached message that protein supplements should be ingested within close proximity of RE, within the legendary "anabolic window" [347, 365-367]. Unfortunately, these less than convincing reports have used evidence from trials of carbohydrate supplementation for endurance exercise performance, where an anabolic window truly exists, to make the case for protein. Nonetheless, it can be considered a pragmatic strategy to ingest both macronutrients at the same time. In fact, only a small handful of investigations suggest a benefit from protein timing [363, 368], whereas a host of both acute and chronic investigations clearly indicate timing may be an inconsequential argument [348, 349], as exercise sensitizes the muscle to protein/AA up to 24h post-exercise [250, 369, 370]. The finding of a greater effect from total protein intake and not protein timing, in relation to the exercise bout, during resistance exercise-training should not come as a surprise. As

further support for the role of total protein intake others have reported [348] that "successful" protein supplementation studies implement a significant change (~66% increase) in the subjects' supplemental protein intake and also a significant difference of ~60% greater protein intake in the PRO supplemented group compared to the control group [348]. This is an important factor, and possibly more relevant than exercise related supplement timing is the amount or distribution of protein intake across the day.

A surprising finding of the Cermak et al. meta-analysis was that the protein supplementation with RET provided an effect even though the young subjects were typically well above (by 0.4g/kg/day) the recommendations for adequate protein intake (RDA) before commencing the intervention [324]. Indeed, consuming minimal protein, 0.5g/kg/d, has been shown to attenuate RET outcomes in young adults [371]. Some have reasoned that higher levels of protein intake, not supplementation in proximity to exercise, are more likely to affect the responses to RET [348, 349]. Yet, evidence suggests that if a certain level of protein intake is met, any further changes in protein intake have less bearing on the adaptation [324, 328, 365, 372, 373] (**Table 1.13**). This fits with a recent paradigm that distribution, amount and spread of protein throughout the day many have greater efficiency and relevance on the protein metabolic response [259, 363, 366, 374], particularly with the slowly absorbed intact proteins humans typically ingest during a complete meal (containing all the macronutrients). The authors suggested that future investigations expand the literature on protein supplementation during RET by investigating protein timing, source and exercise intensity [324, 365].

Protein Type

In regards to protein type, whey protein, in its various forms, has been the most frequently studied in its ability to augment muscle mass during RET. The amount of evidence comprising whey protein as a supplement prompted another meta-analysis examining the changes in body composition with supplementation of specifically this protein [355]. They concluded that whey protein demonstrates significant increases of lean body mass (~2.2kg) when taken during RET. Further, they found no effect in regards to whey protein form (isolate vs. concentrate) or when whey was compared to other protein sources [355]. The author noted that these analyses were conducted in only a handful of studies and as such, are susceptible to greater bias from outlying studies in their analysis. Thus further examination of protein type is warranted.

To examine chronic supplementation during RET by protein type we have compiled a section of **Table 1.13** to only include studies [333, 338, 339, 342-344, 375-379] that have a direct comparison of 2 or more different protein sources/types/forms on lean mass and strength. Only two of these studies actually demonstrated an improved strength outcome when comparing protein forms [343, 377], in this case, whey vs. casein, but these studies provide conflicting results, leaving me to conclude that no particular protein source type or form investigated to date provides an greater enhancement of strength over another high quality source. Also, four studies compared whey or milk to soy protein [333, 342, 344, 378] and 2 studies demonstrated that milk/whey was superior to soy [333, 344] for enhancing lean mass gains whereas two others did not [342, 378]. It should be noted that in the studies where the dairy proteins were more beneficial, a lower protein dose (~20g or less) was given, such that the leucine content for soy was likely less than optimal (< 1.8g). However, in the studies [342, 378] where equivalence was

found between the proteins sources a higher protein dose was given (>28g). As such, the leucine dose likely "triggered" a maximal response in both treatments [380]. This finding is further supported by Joy et al. [375] and Babault et al. [338] who found that protein quality "disparities" between whey and rice protein or whey and pea protein can be overcome by providing a higher dose during RET. Indeed, another study comparing whey to a mix of whey, casein and BCAA found similar results [379]. This data brings into fresh relief the idea suggested earlier in examination of the acute response, that protein type is likely irrelevant, if a high quality protein is ingested at a dose sufficient to reach the leucine threshold for that protein.

Exercise Type/Intensity/Volume

The most recent systematic review on this topic by Pasiakos et al. [353], examined younger adults and determined that protein supplements have an effect on the possible enhancement of muscle mass and performance, but they suggested that this effect appeared most apparent in trained individuals who undergo PRT of sufficient volume and intensity and consume an adequate protein intake. This finding suggesting a need for higher intensity exercise is puzzling when examining the evidence. Due to the logistic issues of a massive study design there is less evidence examining the role of protein supplementation and exercise intensity/volume/mode on muscle mass and strength gains with RET. However, some inferences can be made. Recently, a complex study by Rahbek et al. [381] and Farup et al. [210] examined the effect of Whey+CHO vs. CHO supplementation during unilateral concentric (CON) or eccentric (ECC) exercise training or the knee extensors. At the whole muscle level, assessed via MRI, they found an effect of protein supplementation independent of contraction mode [381],

on muscle size, however the absolute post-training muscle size was not different between treatment groups. However, when examining the myofiber CSA, via immunohistochemistry, they found a greater effect of protein supplementation vs CHO and that CON exhibited a greater response than ECC with protein supplementation [382]. These data suggest that protein supplementation enhances hypertrophy independent of contraction mode, as assessed with MRI but not myofiber CSA suggestion that this enhancement may be anatomically specific. Their whole muscle findings are in line with previous work from the McMaster laboratory demonstrating no difference in muscle size following RET with varying exercise intensities/volumes when protein was supplied during training to maximize MPS [167, 383, 384].

Two studies have shown that whey protein supplementation with low (1 or 2 sets) vs higher volume (2-3 sets) training results in similar responses in muscle size and strength with [385] or with-out whey protein supplementation [386]. Although clear evidence [387] suggests that greater volumes of training elicit more of an effect, these two studies [385, 386]suggest that protein supplementation during RET may negate such differences, at least in the conditions examined.

In evaluation of the literature regarding changes in lean mass with RET and PRO/AA supplementation few studies demonstrate differences between the changes in PRO and placebo (PLA) groups, several demonstrate a trend while some show significant increases with outcomes in the PRO group, but not in the PLA group, while the majority of the evidence demonstrates identical increases in PLA and PRO (**Table 1.13**). The effect on strength is even more elusive, and only present when whole body RET, not training of isolated limbs, is conducted. The PRO effect on a regional assessment of a

muscle mass is also more apparent with whole body RET, but is not consistently demonstrated. The lack of a clear pattern defining the effect of PRO/AA to enhance adaptation to RET suggests individual variation or selection bias and future investigations should seek to examine this further.

Table 1.13. Chronic effect of resistance exercise training with protein and/or amino acid nutrition on muscle size and strength in young adults

Author, Year	Subjects	Groups	Feeding: Protein/AA/Other	Mass	RET Stimulus & #setsx#reps	Duration	Change		Strength		PRO, intake		
							CSA	FFM/LM	Measure	Δ%	g sup	g/kg/d	Δ
Compare PRO vs PLA only													
Torun 1977 crossover	8 YM, UT	Higher PRO	1g/k/bw/day egg & Milk	K counting	75 min isometric Ex	6x/wk, 4-6wk	-	↑	ISO	-	-	1	0.5
					none			↑				1	0.5
		Lower Pro	0.5g/k/bw/day egg & Milk		75 min isometric Ex		-	↓		-	-	0.5	-
					none			↓				0.5	
Fern 1991	12M UT	PRO	2g/k/bw/day	HW, Anth	WB, 7Ex,	3x/wk, 4wk	-	↑	-	-	-	2	0.7
		PLA	acaloric wheat bran				-	↑	-	-	-	~1.3	-
Willoughby 2007	19 M UT	PRO + AA	20g (14g WH & Cas, 6g AA) 1 h pre/post Ex	HW, Anth	WB, Split PRT, 3x6–8 at 85–90% of 1RM	3x-5x/wk, 10wk	↑	↑	BP & LP 1RM	**BP, LP: ↑**	40	2.81	0.66
		CHO	20g dextrose 1 h pre/post Ex 40g/day				↑	↑		BP, LP: ↑	0	2.24	0.18
Josse 2010	20W UT	Milk	500 mL, 18g PRO,24g CHO, 2x, imed & 1h post	DXA	PRT, rotating WB, Split, 2,3,4x12,10,8,6 @ 80% 1RM	5x/wk, 12wk	-	↑	1RM for each EX"	↑~↑ w/ **chest flys**	~20	1.25	0.25
		CHO	500ml isokcals maltodextrin, 9% soln				-	↑		↔	0	1.01449	↔
Walker 2010	30M RT	PRO	19.7g Whey + 6.2g leu	DXA	Required EX for air force, 2h/wk	~2x/wk, 8wk	-	↔↑	1-RM	↔	~26	measured, not reported	
		PLA	28g CHO				-	↔		↔	0		
Burke 2001	36M RT	Whey	WH (1.2 g/kg/day)	DXA	PRT, WB, split,Heavy, high volume, 4x6-12	4x/wk, 1st 6wk w/ sup, last 6wk w/sup	-	↑	Bench, Squat 1RM, KE peak torque	Sq,BP↑, KEPT ↑	~26x4	2.2	1.2
		WCr	WH & CrM (0.1 g/kg/day)				-	↑↑		SQ ↑,BP↑↑,KEPT ↑↑	~25x4	3.3	1.2
		PLA	(1.2 g/kg/day maltodextrin)				-	↑↔		Sq,BP↑, KEPT↔	0	1.2	↔
Antonio 2001	21W UT	EAA	18.3g EAA,3.5g of Leu	DXA	PRT, Split,WB, (3x6-8,10-12, WB) & AET (20 min @ ~70% of Hrmax)	3x/wk, 6 wk	-	↔	-	↔, better TTE	18.3	1.24638	0.26087
		PLA	cellulose				-	↔	-	↔	18.3	0.89189	↔
Ispoglou 2011	26M UT	L-Leu	4 g/d of L-leucine	DXA	8 Ex machines	2x/wk, 12wk	-	↑	5-RM	**40.80%**	4	0.9	↔
		Lac	5 g/d of lactose				-	↑		30.10%	0	0.88	↔
Farnfield (2012)	16YM, UT	PRO	Whey:27g AA,as 3.6 Leu	-	WB, PRT 2-3x? 80% 1RM	3x/wk, 12wk	-	-	1RM, CON, ECC	↑, ECC	27	1.9	?
		PLA	PLA				-	-		↑	0	1.6	?
Rankin 2004	19M UT (18-25)	Choc Milk	CHO 5kcals/kg 1.25 g/kg (Gatorade), LF-chocolate milk 5 kcal/kg, 0.92 g/kg carb, 0.21 g/kg PRO, 0.06 g/kg fat & vitamins	DXA, Anth	PRT, 3x12 to 3x3 from 55-97% 1RM, 7 Ex	3x/wk, 10wk	-	↑↔	1RM for 7 Ex	all ↑ 44%	~16	1.3	↔
		CHO-electro					-	↑			0	1.2	↔
Chromiak 2004	41M UT	PRO + AA	WH (13 g), AA (0.53g of leu), CrM, & CHO	HW, AnTH	PRT, WB, 3-4x8-10	4x/wk, 10wk	-	↑↔	1RM BP, LP, endurance	↑	13+	NM	
		CHO	CHO				-	↑		↑	-		
Kreider 1996	28M, RT	PRO+CHO	60g PRO, 290g CHO, 1400Kcal/d	DXA	maintain & & record training	4wk	-	↑↔	-	-	30x2	2.17	0.3
		PRO+CHO++	67g PRO, 64g CHO, 1400Kcal/d & other stuff…				-	↑		-	~33x2	1.87	0.38
		CHO	129g /3				-	↑↔		-	0	1.43	0.06
Rozeneck 2002	73M, REC gym	CHO/PRO	(2010 kcal) 356 g CHO, 106g PRO /2	HW	PRT, split WB, , 70%1RM	4x/wk, 8wk	-	↑↑	1RM, 3 Ex	↑	106	~3	1.4
		CHO	(2010 kcal) 460 g CHO, 24g PRO /2				-	↑↑		↑	24	1.7	0.3
		PLA	none given				-	↑		↑	0	1.4	-0.1
Ratamess 2003	17M RT	EAA	~36g AA (0.4g/kg/d)	DXA	4wk RET , WB, wk overreaching	3x/wk, 6wk	-	↔	1RM squat & BP	↑	~36	measured, not reported	
		PLA	powered cellulose				-	↔			-		
Mullins 2005	24W UT	HP	WH 96g	DXA	1h, 3x6-10, 13 Ex, 75-85% MVC	3x wk, 8 wk	-	↑	1RM	↑	~96	2.4	1.5
		Control	isoenergetic CHO				-	↑		↑	0	1	↔
Thomas 2011	29YW UT	Yogurt	20g CHO+5g Pro 2x Pre/post - hypocal diet	DXA, Anth	PRT	16wk	-	↑	1RM	↑	20	1.07	0.14
		CHO	25g CHO 2x Pre/post - hypocal diet				-	↑		↑	0	0.97	0.05

Author, Year	Subjects	Groups	Feeding: Protein/AA/Other	Mass	RET Stimulus & #setsx#reps	Duration	Change		Strength		PRO, intake		
							CSA	FFM/LM	Measure	Δ%	g sup	g/kg/d	Δ
Compare PRO vs PLA only													
	Regional Assement of Muscle Mass or CSA												
Cribb 2007	33M Rec BB	CrM CHO	CrM w/ glucose 1.5 gm/kg bw/d, dose of CrM (0.1 g/kg bw/d)	DXA, fCSA	WB, RE, 70-95% 1RM.	3x/wk, 11wk	1↑,2↑	3.7 kg	1RM Squat, BP & Pulldown (abs & rel to BW)	↑ all	0	1.5	↔
		CrM Whey	(50% WHI; 50% glucose) 1.5 gm/kg bw/d				1↑,2↑	3.4 kg		↑ all	90	3.1	1.1
		Whey PRO	WHI, 1.5 gm/kg bw/d				1↑,2↑	2.3 kg		↑ all	103	3.4	1.3
		CHO	1.5 gm/kg bw/d				1↔,2↑	0.7 kg		↑ all	0	1.6	↔
Hulmi 2009	31M UT	Pro	15g of WHI, 2x	CSA, MRI, Anth	PRT, WB, leg dominant, 5x10	2x per wk, 21 wk	↑	-	Maximal ISO, bilateral KE	↑	15x2	1.48	~0.4
		PLA	non-energetic placebo				↑	-		↑	0	1.41	↔
		Control	nothing		no RE, habitual activity		↔	-		↔	↔	↔	↔
Hulmi 2009 (subgroup from previous)	29M UT	Pro	15g of WHI, 2x	CSA	PRT, WB, leg dominant, 5x10	2x per wk, 21 wk	↑↑	-	-	-	15x2	1.48	~0.4
		PLA	non-energetic placebo				↑↑	-		-	0	1.41	↔
		Control	nothing		no RE, habitual activity		↔	-		-	↔	↔	↔
Vieillevoye 2010	29M UT	EAA + CHO	30g mix powder, 15g AA & 15g saccharose	US, Anth	Split PRT: 3 lower, 2 upper body exercises. 70– 85% 1RM	4x/wk, 12wk	↑	↑	1RM	↑	15	1.5	0.18
		PLA	30g saccharose				↔	↑		↑	0	1.3	↔
Bird 2006	32M UT	EAA	6g EAA	DXA, fCSA	WB, 3x8-10@ 75% 1RM	2x/wk, 12wk	↑	↑	1RM, ISO, Knee ext & flexors	↑↑LP, ↑ ISO	6	measured, not reported	
		EAA + CHO	6% CHO solution + 6g EAA				↑↑	↑↑		↑ 1RM LP. ISO	6		
		PLA	water				↑	↑		↑ 1RM LP. ISO	-		
		CHO	6% CHO solution				↑	↑		↑ 1RM LP. ISO	-		
Olsen 2006	32M UT	CrM-TR	6g CrM + 14g CHO/d, 80g CHO (w/ EX)	DXA, mean fCSA	PRT, 3 leg Ex, 3–5x6–12 (6–12 RM) to 8-10 1RM & 6-8 1RM	3x wk, 16wk	↑	-	Max isometric, KE	15%, MVC	-	NM	
		PRO-TR	14g CHO/d, 20g PRO + 80g CHO (w/ EX)				↑	-		18%	20		
		Con-TR	14g CHO/d), 80g CHO (w/ EX)				↑↔	-		22%	-		
		CON	No sup, no training				↔	-		↔	-		
Lemon 1990	12YM UT, crossover	PRO	2.62 g/k/bw/day PRO	MRI, density, Muscle N,	WB, 6d-slpilt, 4x<10 75-80% 1RM	6x/wk, 4wk	↔↑	↔	MVC, PTT, 1RM	↑	-	2.62	1.18
		PLA	CHO placebo, isocaloric				↔↑	↔		↑	-	1.35	-0.09
Wiedeman 1990	21 YM UT	PRO	2.94 g/k/bw/day	MRI, anth	Squat, KE, KC 3x8-12RM	3x/wk, 13wk	↑	↑	1RM	↑	-	2.94	-
		PLA	CHO placebo				↑	↑		↑	-	1.3	-
Cribb 2007	31M RT BB	CrM PRO-CHO	CrM w/ (50% WHI; 50% glucose) 1.5 gm/kg bw/d, CrM (0.1 g/kg bw/d)	DXA, fCSA	WB, RE, 70-95% 1RM.	3x/wk, 10wk	1↑,2↑	7 kg	1RM Squat, BP & Pulldown (abs & rel to BW)	↑↑ (~20-25kg)	52/3	2.5	0.7
		PRO-CHO	(50% WHI; 50% glucose) 1.5 gm/kg bw/d				1,2↑↔	4 kg		↑ 12	48/3	2.6	0.6
		PRO	(WHI) 1.5 gm/kg bw/d				1,2↑↔	4.9 kg		↑ 12	103/3	3.8	1.5
Weisgarber 2 012	17 UT	PRO	Whey: 0.3 g/kg protein, During (Pre/Post)	US, DXA	PRT, 9 WB, 3x6-10	4x/wk, 8wk	↑	↔	1-RM	↑	~26	1.5	↔
		CHO	0.2 g/kg maltodextrin + 0.1 g/kg sucrose				↑	↔		↑	0	1.3	-0.16
Mitchell 2015	16 Rec	Milk	500ml chocolate milk (~14g Pro, 5g fat, 54h Cho)	fCSA	WB, RE, 2d lower body, 1d upper, 75-85% 1RM.	3x/wk, 12wk	↑	-	1-RM, Iso	↑	14	-	-
		CHO	500ml placebo (~0.4g Pro, 5g fat, 66g Cho)				↑	-		↑	0.4	-	-

Author, Year	Subjects	Groups	Feeding: Protein/AA/Other	Mass	RET Stimulus & #setsx#reps	Duration	Change		Strength		PRO, intake		
							CSA	FFM/LM	Measure	Δ%	g sup	g/kg/d	Δ
Compare PRO vs PLA only			**Trained Isolated Limb(s) Only**										
Holm 2005	26MF ACL injured	Nutrient	10g PRO (skim milk & soybean), 7g CHO, 3.3g fat	MRI	3 leg Ex, 3x15, 3x12, 3x8 to 5x8 @ 20-8RM	3x/wk, 12wk	↑ ↑, ↑	-	Isometric	↑ 13.3%, KN angle	10	1.16	0.06
		Control	isocaloric, 17g CHO, 3.3g fat				↑	-		↔ 10.5%	-	1.23	↔
		PLA	1.4g CHO & 1g fat				↑,↔	-		↔ 11.7%	-	1.15	↔
Farup 2014	22 Rec	Con	WH 19.5g +CHO (19.5g glucose) (half pre/post)	fCSA	Same as Farup 2013 (6-12x6-15)	2-3x/wk, 12wk (33 sessions)	1↑,2↑↑	-	Con, Ecc, Iso Dynomometer	↑	~20	NM	
		Ecc					1↑, 2↔	-		↑			
		Con	CHO (39g glucose) (half pre/post)				1↑, 2↔	-		↑	-		
		Ecc					1↑, 2↔	-		↑	-		
Farup 2013	22 Rec	Con	WH 19.5g +CHO (19.5g glucose) (half pre/post)	MRI	PRT, 1 leg Con, 1 leg Ecc: Isotonic KE, ; 6-12x6-15RM	2-3x/wk, 12wk (33 sessions)	↑↑	-	MVC	12.4	~20	NM	
		Ecc					↑↑	-					
		Con	CHO (39g glucose) (half pre/post)				↑	-		19	-		
		Ecc					↑	-			-		
Coburn 2006	33M UT	Supp	20g WH + 6.2g Leu in 8oz H20	CSA, MRI	unilateral LE, nondominant limb, 3-5x6, 80% 1RM	3x per wk, 8 wks	↑TR, ↑UT (prox)	↔	1RM	↑↑ TR (30.3%), ↑ UT (14.5)	20+6.2	NM	
		PLA	26.2g maltodextrin, 12oz H2O				↑ TR , ↔ UT	↔		↑ TR (22.4%), ↔ UT (2.8%)	-		
Andersen 2005	22M Sed	Control	Nothing	fCSA	No training	3/wk, 14wks	↔	↔	Torque, Squat & Countermovent jump	↔ (3.6-4.6%)	-	NM	
		PRO	(16.6g WH; 2.8g Cas; 2.8g of egg white; & 2.8g of l-glu, pre/post		3-4x Leg Ex: inclined LP, isolated KE & LCs, PRT 4-15 RM		↑	-		PRO ↑ all	25 mix		
		CHO	25g maltodextrin, pre/post				↔	-		CHO ↑ CMJ	-		
Williams 2001	19 M UT	AA + CHO	AA+Glu(11% Leu), 0.8g glucose/kg & 0.2g AA/kg	Anth	KE, 4x10, one leg each day	5x/wk, 10wk	-	↔	BP & LP 1RM	~↑↑	~13.8	NM	
		PLA (Milk)	0.5g dried milk powder				-	↔		↑	0		
Erskine 2012	33M UT	PRO	WH: 20g 2x (pre/post)	US, MRI	PRT, eblow flexor & extensor only	3x/wk, 12wk	↑	-	1RM, MVC	41.8, 12%	40	1.56	0.21
		PLA	6.8g lactose				↑	-		41.4,14.5%	0	1.35	↔
Mielke 2009	39 M UT	PRO 1 set	20g Whey & 6.2g Leucine, pre &post	HW	KE, BP 1x6 40%, 1x-6-8 @80% 1RM	3x/wk, 8wk	-	↔	1RM BP & KE, endurance	↑	26.2	-	-
		CHO 1 set	20g Maltodextrin, pre &post				-	↔		↑	-	-	-
		EX only 2 sets	no supplement		KE, BP 2x6 40%, 1x-6-8 @80% 1RM		-	↔		↑	-	-	-
Compare PRO Sources/Amounts													
Candow 2006	27MF UT	Whey+CHO	1.2 g/kg WH + 0.3 g/kg sucrose	DXA	4-5x6-12, 60-90%, 1RM,WB, 4-d split	4x wk, 6 wks	-	↑ (4.7%)	Bench, Squat 1RM	↑15-30%	~28x2	3.1	1.5
		Soy	1.2 g/kg Soy + 0.3 g/kg sucrose				-	↑ (3.1%)		↑15-30%	~28x2	3	1.2
		PLA	1.2 g/kg maltodextrin + 0.3 g/kg sucrose				-	↔ (0.5%)		↑5%	0	1.7	↔
Volek 2013	63 MF UT	Whey	21.6g 2.21g leucine + 22.5g CHO	DXA	PRT, 3-5x3-15 (~30-90% 1RM, light Med, High & Power days), flexible & nonlinear	3x/wk, 12,24,36	-	↑	1RM for BP & Squat	↑	21.6g	1.39	0.12
		Soy	20g 1.34 Leu + 24.5g CHO				-	↑		↑	20g	1.35	0.09
		CHO	45g CHO				-	↑		↑	-	1.06	-0.14
Cribb 2006	13M RT BB	WI	90g WHH, 3g CHO, 1.5g fat/100g 1.5 gm/kg bw/d	DXA	WB, RE, 70-95% 1RM.	3x/wk, 10wk	-	5.0	1RM Squat, BP & Pulldown (abs & rel to BW)	↑↑ (~20-30kg)	30x3	2.1	0.32
		Cas	90g PRO, 3g CHO, 1.5g fat/100g, 1.5 gm/kg bw/d				-	1.3		↑		2.1	0.24
Brown 2004	18 M&W	Whey+CHO	33g bar	HW	3x4-6, WB, 14 Ex, unsupervised	3/wk, 9 wk	-	↑	-	-	-	-	-
		Soy+CHO	33g bar				-	↑	-	-	-	-	-
		CHO	-				-	↔	-	-	-	-	-
Colker 2000	16M RT	PRO	30g WPC, 10g WPI	Anth	warm-up, BP, LP, RE, 70-95% 1RM. & normal routine 8-12 reps	3x/wk, 10wk	-	↔	1RM BP, LP	↑	40	2.1	~0.5
		PRO+BCAA	30g WPC, 10g WPI, 5g L-glut, 1.5g leu, 0.75g Val & iLeu				-	↑		↑		2.1	~0.5
Demling 2000	38M OW	Whey	WH ~70-75g	Anth	PRT, Main Muscle	4x/wk, 12wk	-	↑	8-10RM; BP & Squat	↑	37gx2	1.5	0.8
		Casein	Casein Hydrolysate				-	↑		↑		1.5	0.8
		diet only	-				-	↑↔		nc	-	0.8	0.1
Kerksick 2006	36M RT	PRO Mix	40g WH, 8g Cas	DXA	PRT, 3x6-10 (80% 1RM), 4-d split routine	4x wk, 10wk	-	1.9	1RM on BP & LP	↑	48g	2.2	0
		PRO+AA	40g WH, 5g Glu, 3g BCAA				-	↔		↑	48g	2.1	0
		CHO	48g CHO				-	↔		↑	-	1.4	0
Kerksick 2007	49M RT	PRO	43.5g Cas, 31.5g WH, 16g CHO	DXA	WB, PRT, Split, 3x6,8,10	4x wk, 10wk	-	↔	1RM, BP & LP	↑	75	2.2	?
		PRO/COL	7g Cas, 7g WH, 16g CHO, 60g colostrum				-	↔		↑	74	1.9	?
		PRO/Cr	43.5g Cas, 31.5g WH, 16g CHO + CrM				-	↔		↑	60	2	?
		COL/Cr	CrM, 16g CHO, 60g colostrum				-	↑		↑	-	2.3	?

Author, Year	Subjects	Groups	Feeding: Protein/AA/Other	Mass	RET Stimulus & #setsx#reps	Duration	Change		Strength		PRO, intake		
							CSA	FFM/LM	Measure	Δ%	g sup	g/kg/d	Δ
Compare PRO Sources/Amounts cont.				-	-	-	-	-	-	-	-	-	-
Ratamess 2007	33 RT M	High PRO	Stratified by diet intake, told to maintain	DXA	WB, split PRT, 3-4x4–10	4x/wk, 10wk	-	↔	1RM squat & BP	↑	211	High: >1.9	
		Med PRO					-	↔			142	Med: 1.2-1.9	
		Low PRO					-	↔			97	Low: < 1.2	
DeNysschen 2009	28M overweight, ~38y	Whey	Whey protein	Anth	PRT, Major muscle groups, 2-4x8-12 @ ?	3x/wk, 12wk	-	↑	1RM	↑	26.6	1.1	0.2
		Soy	Soy protein				-	↑		↑	25.8	1.2	0.18
		CHO	isocaloric CHO				-	↑		↑	0.6	1	↔
White 2009	35F UT	Yogurt	3 servings of yogurt w/ Vitamin D per day	DXA	PRT	3x-5x/wk, 8wk	-	↑	-	-	5	1.1	0.2
		PRO	maintain baseline low-dairy-calcium diet, isocaloric product w/out calcium or vitamin D				-	↑		-	5	1.1	0.1
		CHO	isocaloric product		-		-	↑	-	-	-	0.9	↔
Hambre 2012	24M	High energy Fast Food	Fast food: 51g fat,41g PRO,182g CHO,1370 kcal	DXA	1h, 3-5x8-10, unsupervised	3x/wk, 12wk	-	↑	MVC	↑	41	1.5	0.2
		PRO	33g Whey				-	↑		↑	33	1.8	0.5
Wilborn 2013	16F RT	Whey	WH	DXA	PRT+anaerobic, 1-3x12-15, 80% 1RM, 4-d split routine	4x/wk, 8wk	-	↑	& Power, Agility	↑	30gx4	2.1	0.23
		Casein	Cas				-	↑		↑		2.1	0.13
Antonio, 2014	36MW RT	High Protein	4.4g Pro/kg bw/d	BodPod	St&ard Habits, Logged	8wk	-	↔	-	-	~150	4.4	2.6
		Control	Standard Pro intake				-	↔		-	↔	1.8	non
Regional Assement of Muscle Mass or CSA													
Hartman 2007	56M UT	Milk (FF)	500mL, 17.5g PRO,25.7g CHO,0.4g fat	DXA, fCSA	rotating WB, Split, 2,3,4x12,10,8,6 @ 80%	5 d/wk, 12 wk	1↑,2↑	↑	1RM, for each EX	↑ ~62-102%	18x2	1.8	0.4
		Soy	500mL soy PRO, isoenergetic, isonitrogenous				1↑,2↑	↑		↑ ~42-98%	-	1.6	0.4
		Control	isoenergetic, 500mL CHO maltodextrin, 9% soln				1↔,2↑	↑		↑ ~51-87%	-	1.6	0.2
Herda 2013	106M UT	BioWhey	20g WHC + 7g Leu, 30m Pre/post	pQCT, HW	1-3x6, BP, LP 80% 1RM	3d/wk, 8wk	↑	↑	1RM, Endur	↑	54	1.78	0.18
		Whey	20g WHC, 30m Pre/post		3-5x6, BP, LP 80% 1RM		↑	↑		↑	54	1.79	0.45
		PLA	27g Maltodextrin, 30m Pre/post		3-5x6, BP, LP 80% 1RM		↑	↑		↑	40	1.96	0.63
		CON	none		3-5x6, BP, LP 80% 1RM		↑	↑		↑	54	1.26	-0.03
					3-5x6, BP, LP 80% 1RM		↑	↑		↑	0	1.28	-0.06
Joy 2013	24M RT	Whey	48g	DXA, US	PRT, 3x2-12 (~50-97% 1RM), flexible & nonlinear	3x/wk, 12wk	↑	↑	1RM	↑	48g	NM	
		Rice	48g				↑	↑		↑			
Babault (2014)	68M, Rec	Soluble PRO	10gx3, Milk Pro Isolate +11g CHO Morn, pre/post	US, DXA	3-5x8-15,; KE, KC, LP,	3x/wk, 10wk	↑	↔	Vert Jump, 1RM KE, Power, End	↑	30	Not measured	
		Micellar PRO	10gx3, Casein + 11g CHO Morn, pre/post				↑	↔		↑	30		
		PLA	30gx3 CHO Morn, pre/post				↑	↔		↑	0		
Babault (2015)	161M, Rec	Pea (n=47)	25gx2, Pea Pro Isolate Morning/post	US, Antro	2-5x5-15,; Biceps, triceps, BC, LP, BP	3x/wk, 12wk	~↑s	↔	Vert Jump, 1RM KE, Power, End	↑	50	Not measured	
		Whey (n=46)	25gx2,Whey Pro Morning/post				~↑	↔		↑	50		
		PLA (n=44)	25gx2 Maltodextrin CHO , Morning/post				↑	↔		↑	0		
Compare Timing													
Burk 2009 Crossover	13M UT	TFR	35g of PRO, morn & afternoon, b4 EX	DXA	WB, large muscle, 6x10, 75-80% 1RM.	4x wk, 8wk	-	↔	1RM, Squat & Bench	Squat & BP: ↑	35x2	2.3	0.9
		TDR	35g of PRO, 1 morn & 1 evening, 5h post				-	↑			35x2	2.3	0.9
Hoffman 2006	33M RT	PRO Mix	WH, Cas, collagen mix , pre/Pex	DXA	PRT, 3-4x6-10 (80% 1RM), 4-d split routine	4x wk, 10wk	-	↑	1RM on BP & LP	↑	48g	-	0
		CHO	48g CHO				-	↑		↑	-	-	0
Cribb 2006	23M RT BB	Pre-Post	(per 100 g), 40g WH 43g (glucose), 0.5g fat, & 7g CrMpre/post Ex	DXA	WB, RE, 70-95% 1RM.	4x/wk, 10wk	↑	↑	1RM Squat, BP & deadlift	all ↑, ↑ BP & squat > MOR-EVE	40x2	2.9	1
		Mor-EVE	(per 100g), 40g WH 43g (glucose), 0.5g fat, & 7g CrM morning & evening				↑↔	↔		all ↑		3.1	1

Arrows denote direction of change. ↑, significantly increased; ↓, significantly decreased; ↔, no change; ↔↑, trend to increase; ↔↓, tend to decrease; ↔. Red color arrows represent a group diference. Blue arrows represent an effect of feeding . RM, repetition maximum; LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Ecc, eccentric contractions; Con, concentric contractions; O, old; Y, young; M, men; W, women; OW, overweight; h, hour; m, minutes; sec, seconds; WB, whole body; PRT, Progressive resistance training; TR, trained, RT, resistance trained, ; RE, resistance exercise; ST, strength trained; ET, endurance trained.LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Si, Single leg; Rec, recreationally active; TR, Trained; MP, Military Press; UT, Untrained; BCAA, Branch Chain Amino Acids; HMB, A-hydroxy-A-methylbutyrate; CrM, Creatine Monohydrate; EAA, Essential Amino Acids; CHO, Carbohydrate; PRO, Protein; PLA, placebo; WH, Whey; WHI, Whey Protein Isolate; WHC, Whey Protein Concentrate; Cas, Casein; FFM, fat-free milk; lac, lactose; Con, control; HP, high protein; Lei, leucine; AA, amino acids; MCV, maximal voluntary contraction; Glu, glutamine; LBM, Lean body mass; pi, post-ingestion; DXA, dual-xray absorbmerty; HW, hydrostatic weighing; MRI, magnetic resonance imaging, CT, computed tomography; US, ultrasound; AnTH, anthropometry, AET, aerobic training; ISO, isokinetic;KEPT, knee extension peak torque;BW, body weight; wk, week; TTE, treadmill time to exhaustion; K, potassium; fCSA, myofiber cross-sectional area, soln, solution;

Older Adults

There is some debate as to whether younger adults can benefit with greater muscle size and strength from PRO/AA supplementation during RET, but there is even less clarity as to the role of protein supplementation during RET in older adults [324, 351]. Although many opinion papers and reviews enthusiastically and frequently discuss the potential for RET and PRO/AA in older adults, there exist few systematic reviews or meta-analyses describing evidence for the phenotypical result of PRO supplementation during RET in older adults [324, 336]. The first review examining older adults [324] found fewer studies in older populations than young; however, pooled improvements of 0.5 kg of FFM and 33% increase in leg strength were evident. However, due to the limited number of studies reporting the outcome; no increase was seen in myofiber CSA. In order to conduct the meta-analysis, the authors limited in their selection of available research due to their strict criteria to minimize heterogeneity in their analysis. Also, within the past year several new studies had contributed to the literature. To our knowledge, we have tabulated all of the current literature examining protein supplementation during RET in older adults. In supplemental **Table 1.14** we have tabulated according to if an effect of PRO/AA-containing supplements on muscle mass strength and most importantly, functional testing, was found (N=6 clinical trials; [388-397]) or absent (N=23 clinical trials; [56, 337, 362, 368, 398-420]). This overwhelming pattern is supported by a recent meta-analysis [336], which only was able to include nine of these studies into their report. As presented by Cermack et al., who only included six of these studies, older adults demonstrate less of an effect from PRO supplements on

muscle mass and strength than younger adults. The recent meta-analysis from Finger et al. gives evidence that only lean mass and not muscle mass or strength is improved by addition PRO/AA supplementation [336], recapitulating our findings to a degree (**Table 1.14**).

In those studies that did determine an effect of protein-containing supplements, we sought to discover the reason for that effect. Interestingly, only one of these studies was a true protein supplement study, in that the only nutritional component modified was protein [396]. We determined that either improved diet quality [388, 391, 393, 397] or an improved protein distribution/spread [388, 396] seem the most plausible explanation for an effect in the PRO/AA containing nutrient-supplemented RET groups. However, Holm et al. found that calcium and vitamin D content but not the energy and protein intake between the supplemented and control groups was different over the course of the study [393]. Regarding the diet quality, the study by Daly et al. had older participants incorporate ~45g of protein (split between lunch and dinner) in the form of lean red meat in their diet during 16 wk of RET, which resulted in a reduced % of energy from CHO and a higher zinc intake [388]. Interestingly, because this was strictly a meal-replacement study, the difference in protein intake between the meat+RET and RET only groups was only ~15g, which further supports our suggestion that the improvements were due to diet quality or distribution of protein rather than the total amount of protein [388]. Two other studies that demonstrated a clear effect from inclusion of protein-containing nutrients during RET gave minimal doses of protein (10 or 13g) per day, but likely benefited from the other nutrients (energy, calcium, vitamin D) supplied [391, 393]. Although a host of acute studies suggest that reaching a higher protein dose is critical for

stimulation of MPS in older adults [238, 245, 248, 306, 421], these data suggest that a lower protein dose during RET may be effective if diet quality is improved.

For the past five years it has been hypothesized [374] that evenly distributing protein at each meal throughout the day may be a more effective strategy to maximize protein retention. A clinical trial has recently provided support for this hypothesis [422], at least in young adults. In fact, 2 of the studies that did demonstrate an effect of PRO + RET utilized some form of this strategy [388, 396]. Tieland et al. gave participants a 15g PRO serving after breakfast and lunch, which served to ensure that a protein dose of 30g or more at every meal was achieved [396]. Daly et al. used meal-replacement to distribute ~22.5g of protein (from red meat) into lunch and dinner during RET [388]. The authors did not provide information regarding intake at breakfast and the overall spread of protein throughout the day, but this strategy was highly effective and ensured some spread in the protein intake.

Cermak et al. mentioned that although some minor changes (e.g. FFM in old) were not evident in many individual single studies, the pooled estimates revealed an effect. The absence of an effect could be attributable to the heterogeneity of individual responses to RE such as body type [423] and other factors [317, 372, 424, 425]. This finding would suggest the necessity to increase sample size to find statistical effects among these variable responses in these types of studies. This approach has been frequently attempted. However, even many of the studies that did not demonstrate an effect used very large sample sizes [398, 400, 402, 406, 407] suggesting other factors are likely involved, such as the individual variability suggested previously. These findings are puzzling since, as mentioned in previous sections, older adults display an early

"anabolic resistance" in close proximity to application of both exercise and PRO/AA examined independently. However, when contraction and sufficient nutrients are combined this anabolic resistance diminishes (**Table 1.8).** Thus it would seem intuitive that supplementing protein during RET be a more effective strategy to enhance muscle size and strength. However, when considering the other factors in these clinical trials, such as differences in protein intake with supplementation or between supplement groups, diet quality, protein dosing or distribution of protein at each meal the current findings are not surprising.

As previously proposed, a significant change or spread in PRO intake (with supplementation between groups) is an important factor mediating the long term effect of PRO supplementation during RET [348]. We observed minimal, if any, change in protein intake in the studies examining older adults (~20g/d). The most PRO intake increased in these studies was ~0.2g/kg/d or 20g extra per day on average, but several studies marked even less of a change (**Table 1.14**). This is in sharp contrast to studies in young adults, where protein intake increased the most and had more spread between groups (**Table 1.13**). Thus this may present one variable indicating why older adults are less responsive to PRO/AA with RET. Protein supplement RET interventions may more effective if a higher dose of protein is given. However, as mentioned above, this factor may not be as important as previously thought. One study examining older adults with moderate renal insufficiency demonstrated that RET with a low-protein diet (0.6g/kg/d) improves the efficiency of protein metabolism and does not impede RET induced improvements of muscle size and strength [362].

As mentioned previously, and suggested by countless acute investigations, increasing the protein dose at each consumption has been a highly suggested therapeutic strategy for the development of maintenance of muscle health in older adults [238, 245, 248, 306, 421, 426]. Besides, one study in middle-aged overweight/obese adults [404] the strategy of testing dosing of protein has not yet been applied in chronic exercise training studies in older adults (**Table 1.14**). A study by Weinheimer et al. is clearly the largest clinical trial to date examining the effects of protein supplementation during resistance-type exercise training [404]. Using a sample size of over 300 overweight/obese participants they meticulously determined that twice daily whey protein supplementation had no effect on RET adaptations at any dosage (10, 20 or 30g) [404]. Although, it could be said that this middle-aged population already had a higher amount of lean mass to maintain their higher body weight, there were no protein supplementation effects on regional redistribution of lean mass. The findings from this large clinical trial aside, one could argue that provision of a sub-optimal protein dose may be a plausible factor why 22/28 clinical trials did not see an effect of protein to enhance RET induced improvements in muscle size and strength. Only 3 [400, 401, 409, 410, 416] of the 22 studies were likely to achieve a maximal dose of protein (~30g or more) and most of the other studies gave 25g or less at each serving, which may be one reason for the lack of an effect from PRO/AA, in older adults, in these studies. The few exceptions [400, 401, 409, 410, 416] to this pattern may have several possible reasons explaining why the higher dose did not have an effect. One investigation [409, 410] gave participants 35g of whey protein immediately following RE, yet their participants, similar to the whey dosing study [404] already had a rather high FFM (65-70kg) before starting

the study. The concept that participants with higher lean mass have less to gain has been perpetuated as a reason for the lack of a PRO/AA effect in these chronic exercise studies; however, there has not been evidence to demonstrate such a phenomenon. In fact, solid evidence suggests the opposite effect, that those with greater FFM at the start of RET demonstrate the most change in FFM [423], at least in young adults. However, the overall protein intake in the PRO group 1.04g/kg/d was not different from the placebo group (0.95g/kg/d) suggesting that a lack of a change in the protein intake may partially explain the lack of a supplementation effect. A similar situation was observed with another study from the same investigators by Carter et al. [416]. A recent clinical trial investigated the effect of 40g of protein (20g after breakfast and also dinner) during RET [400]. This strategy would theoretically maximize the dose when combined with each meal, however they found that habitual protein intake actually decreased resulting in a difference of only 18g of protein more in the PRO group compared to placebo. Although they were only able to detect a trend for an increase in muscle mass (MRI) or lean mass between groups, they did demonstrate better improvements in lower limb power in the whey protein group. Shahar et al. recruited sarcopenic older adults to undergo a factorial designed study examining the effect of protein supplementation and therapeutic resistance exercise [401]. They gave men 20g per day and women 40g per day and found that this supplementation was only effective at enhancing upper body strength and reducing body fat. This data suggests potential for increasing the protein dose and future RET investigations are needed to follow-up on the acute protein dosing studies in older adults.

Table 1.14. Chronic effect of RET with protein and/or amino acid nutrition on muscle size and strength in older adults

Author, Year	Subjects	Groups	Protein/Other	PRO g/d	Mass/ CSA	RET Stimulus (#sets x # reps)	Dura-tion	Function test	Δ Size, CSA	Δ FFM/LM	Δ Leg LM	Δ % FAT	Δ Strength 1RM	PRO intake g/kg/d	Δ
Studies with a PRO Effect N=~5.5															
Daly 2014 PMID: 24477043	100W 60-90 y, 15 retirement villages	Meat	lean red meat (80-g servings, ~45g PRO)) 6 d/wk	45	CT, DXA	PRT & balance-agility training	2d/wk, 16wk	↑	↑	↑	↑	↓↓	↑	1.3	0.21
		CRT	1 serving pasta or rice/d (w25–35 g CHOs)					↑	↔	↔	↔	↓	↔	1.1	0.01
Tieland M 2012 Van de rest 2013 PMID: 24374288 PMID: 22770932	(127) 78 ± 1y, frail elderly	PRO	2x15g daily	30	DXA	warm-up cycle, 4x legs, 3x other (8-15 reps); 50-75% 1RM	2x/wk, 24wk	↑	-	↑	↑	↑	↑	1.3	0.3
		PLA	(>1.2 g PRO per kg per d), 7.1 g lactose, & 0.4 g calcium					↑		↔	↔	↓	↑	1	0
Kukuljan 2009, 2011 Peake 2011 PMID:18958384, PMID:19850735, PMID: 21455612, PMID: 21209030	180 MF (50-79)	PRT+SUP	400 ml day of milk containing 1,000 mg calcium plus 800 IU vitamin D(3)		DEXA, CT	PRT w/ wt-bearing impact Ex, 2-8x8–20, 50-85% 1RM. last 6 mo high-speed	3x/wk, 17 or 72wk?	↑	↑↑	↑	-	↔	↑	1.26	0.21, 0.06
		PRT	none	13				↑	↑	↑	-	↔		1.32	0
		Sup	400 ml day of milk containing 1,000 mg calcium plus 800 IU vitamin D(3)			none		↑	↔	↔	-	↑	-	1.23	0.16, 0
		Control	none			none		↑	↓	↔	-	↔	-	1.33	0
Holm et al, 2008 PMID: 18467544	60W Sed, ~55y	Nutrient	10g WH, 31g CHO, 1g fat, 5.0g vitamin D, 250mg calcium	10	MRI, DXA	36 sessions, 3x15, 3x12, 3x8 to 5x8 @ 20-8RM	2x/wk, 21wk		↑ 6	↑	-	↔	↑ 9-14%	1.05	0.09
		Control	6g CHO & 12mg calcium						↑ 4-5	↔	-	↔	↔↑ 8%	1	↔
Meredith et al. 1992 PMID: 1740600	12M UT (61-72)	Sup	200 kcal, 8.3g PRO, 22 CHO, 9 fat, vitamins and minerals	8.3	CT, HW, Anth, creatine	PRT, 3x8, KE, KC, ~80% 1RM	3x/wk, 12wk	-	↑↑	↔	-	↑	↑	118g/d	↑ 26g
		Control	none					-	↑	↔	-	↔	↑	72g/d	↓23g
Campbell 1999 PMID: 10584048	(19M) UT (51-69y)	Meat	omnivorous (meat-containing) diet	91	fCSA, HW, creatine	WBR, 3x , 80% 1RM, nonsequential days/wk	2d/wk, 12wk	-	↑	↑	-	↓	↑	1	↔0.09
		LOV	lactoovovegetarian (LOV) (meat-free) self selected diet	71					↑	↓	-	↑	↑	0.78	↓0.29
Studies with NO PRO Effect N=~9								-	-	-	-	-	-	-	-
Mitchell 2015 PMID: 25610954	16 Rec, 74 ± 5 y	Milk	500ml chocolate milk (~14g Pro, 5g fat, 54h Cho)	14	fCSA	WB, RE, 2d lower body, 1d upper, 75-85% 1RM.	3x/wk, 12wk	-	↑	-	-	-	↑	-	-
		CHO	500ml placebo (~0.4g Pro, 5g fat, 66g Cho)	0.4				-	↑	-	-	-	↑	-	-
Leenders M 2013 PMID: 22968306	(60) 70 ± 1 y M&W same	PRO	15g daily at breakfast	15	CT, DXA, biopsy	5m cycle, 4 sets legs, 3 sets other; Wk 1-4 60% to 75-80% 1RM	2x/wk, 24wk	↑	↑	↑	↑	↔	↑	1.2	0.21
		PLA	(>1.2 g PRO per kg per d)							↑			↑	1.2	NC
Kawada S 2013 PMID: 23681049	29	6gEAA	6g EAA		?	?	2x/wk, 24wk	↑↑		-	-	-			-
		3gEAA	3g EAA					↑↑	↑	-	-	-	?	?	-
		PLA	none					↑		-	-	-			-
Chalé A 2013 PMID: 23114462	(80) mobility-limited 70-85y	Whey	WPC 2x/d,20g PRO,25 g malto,1g fat	40	CT, DXA	PRT to 80% 1RM, from 2x10 to 3x12, 1- to 2-m rest	3d/wk, 24wk	↑	↑↑ non-sig	↑↑ non-sig	-	↔	↑	1.141	0.23
		PLA	isocaloric control, 45 g maltodextrin						↑	↑↔	-	↔		0.893	-0.04
Shahar 2013 PMID: 24143082	65 elderly w/ sarcopenia	PRO + EX	soy PRO drink (20-40g day)	20-40	DXA, BIA	~60m activity facilitated group Ex (Therabands) a relaxation program 1 time every 2 wks	2d/wk, 12wk	↑↑		↔	-	↔		1.5 g?	?
		PLA + EX		20-40				↑	many fluxuations	↑	-	↓↓	↔	?	?
		PRO	placebo drink					↑		↓	-	↔		1.5 g?	?
		PLA						↔		↓	-	↓		?	?
Arnarson 2013 PMID: 23317926	(161) 65-91y	PRO	20g Whey PRO	20	DXA	WB PRT, 10 Ex, machines 3x 6-8 reps @ 75-85% 1 RM	3d/wk, 12wk	↑	-	↑	-	-	↑	1.06	0.06
		PLA	isocaloric					↑	-	↑	-	-	↑	0.89	-0.03
Molsted S 2013 PMID: 22959782	29 patients undergoing dialysis	PRO+CHO	9.4g Whey PRO, 25g CHO, 12.5g fat	9.4	Biopsy	high-load PRT, outside of dialysis, 3 leg Exs; 3-4 set for 6-15 reps	3d/wk, 16wk	↑	↑	-	-	-	-	1.3	↔
		CHO	54.5 mL 2.4g CHO, 27.3g fat						↑	-	-	-	↑	1.3	
Farnfield 2012 PMID: 22148961	18OM	PRO	Whey:27g AA,as 3.6 Leu	27	-	WB, PRT 2-3x? 80% 1RM	3x/wk, 12wk	-	-	-	-	-	↑	1.55	?
		PLA	PLA	0				-	-	-	-	-	↑	1.2	?
Weinheimer 2012 PMID: 22718030	220~48, overweight obese	60g Whey	200kcalx2, 30gx2	60	DEXA, cir	RE 2d/wk, 3x8-12 @60-80% 1RM; AE 1d/wk, 50-70% max HR unsupervised	3d/wk, 36wk	-	-	↑	↑	↓	↑	1.68	0.67
		40g Whey	200kcalx2, 20gx2	40				-	-	↑	↑	↓	↑	1.44	0.36
		20g Whey	200kcalx2, 10gx2	20				-	-	↑	↑	↓	↑	1.15	0.16
		PLA	200kcalx2 maltodextrin	0				-	-	↑	↑	↓	↑	0.94	-0.11

Author, Year	Subjects	Groups	Protein/Other	PRO g/d	Mass/ CSA	RET Stimulus (#sets x # reps)	Dura-tion	Function test	Δ Size, CSA	Δ FFM/LM	Δ Leg LM	Δ % FAT	Δ Strength 1RM	PRO intake g/kg/d	Δ
Studies with NO PRO Effect N=~15								-	-	-	-	-	-	-	-
Kim 2012 PMID: 22142410	155 W (sarco, >76y)	EX + AA	3g EAA+Leu, 2x/d	0.15g AA/kg	BIA	60m Ex Therapy , LOW intensity strengthening, balance & gait training. Progress from seated to standing ~ 8 reps	2d/wk, 12wk	↑	-	↑	↑↔	-	↑	-	-
		EX	None					↑	-	↑	↑↔	-	↑	-	-
		AA	3g EAA+Leu, 2x/	0.15g AA/kg				-	-	↑	↑↑↑	-	↔	-	-
		health edu	None					-	-	↔	↔	-	↓	-	-
Deibert 2011 PMID: 22066824	40M (50-65)	PRO+RET	Soy-yogurt- honey nutrient drink	26.7	Anth	Lifestyle education, no RET	2d/wk, 12wk	↑	-	↑	-	↓	↑	-	-
		RET	none	0				↑	-	↔	-	↔	↑	-	-
		PRO	Soy-yogurt- honey nutrient drink	26.7		Lifestyle education, no RET		↔	-	↔	-	↔	↔	-	-
		Control	none	0				↔	-	↔	-	↔	↔	-	-
Carlsson 2011 PMID: 21808934	177 (69-99y) "disabled"	EX+PRO	PRO drink, 14.8g PRO & ~200 kcal	~15	BIS	"high-intensity" functional Ex program	2x/wk, 24wk	↑	-	↓↔	-	-	-	?	?
		EX	EX+CHO					↑	-	↓↔	-	-	-	?	?
		PRO	same as above	~15				-	-	↓↔	-	-	-	?	?
		PLA	CHO					-	-	↓↔	-	-	-	?	?
Onambélé-Pearson 2010 PMID: 20431985 PMID: 20431985	29	High vs Low intensity RET	CHO+AA		BIA	Low: 40% 1RM, High: 80% 1RM		↔	↔	↔	↔	↔	↔	-	-
Eliot 2008 Bemben 2010 PMID: 20126965 PMID: 18309444	(42) UT M, High LM	PRO	35g whey + 25g CHO (gatorade)	35	DEXA, Multifrequency BIA	WBR, 3x8, 80% 1RM	3d/wk, 14wk	-	-	↑	-	↓	↑	0.95	0.09
		PRO+CrM	35g whey + 5g CrM + 25g CHO (gatorade)							↑	-	↓	↑	1.03	0.22
		CrM	5g CrM + 25g gatorade							↑	-	↓↓	↑	0.92	-0.1
		PLA	CHO 25g gatorade							↔	-	↓	↑	0.94	0.01
Iglay et al, 2007, 2009 PMID:17413099 PMID:19214338	(30) UT	PRO	Egg + meat + dairy (diet), 17% Pro	~20	DXA, 24h creatine	WBR 80% 1RM	3d/wk, 12wk	-	↔	↑	↑	↓	↑	1.2	0.1
		PLA	Low-protien diet Y, 12% PRO	-				-	↔	↑	↑	↓	↑	0.9	-0.2
Verdijk et al, 09 PMID: 106243	(34) UT	PRO	Casein	20	DXA, CSA, CT	Legs only 60–80% 1RM, KE & press	3d/wk, 12wk	-	↑	-	↑	-	↑	1.1	0
		PLA	Water	-				-	↑	-	↑	-	↑	1.1	0
Maesta 2007 PMID: 17084566	(46) UT W overweight	Soy+RET	25g soy pro	25	BIA	WBR 8 Exs, 1x15 reps 40–50% 1RM to 3x8–12 60–80% 1RM	3d/wk, 16wk	-	-	↑ ↑ ↔	-	↓	-	↔?	-
		Soy		-				-	-	↑	-	-	-	↔?	-
		RET+PLA	25 g of maltodextrin	-				-	-	↑	-	↓	-	↔?	-
		PLA		-				-	-	↔	-	-	-	↔?	-
Candow 2006 PMID: 16767436	38(29) M (59-76y)	PRO B4	0.3 g PRO/kg bw Myoplex ~27gCHO	~25.8	BOD-POD, Muscle thickness	3X10, 70% 1RM for LP & BP & 10RM other Exs	3d/wk, 10wk	-	↑	↑	-	-	↑	1.30	-0.2
		PRO After						-	↑	↑	-	-	↑	1.36	0.2
		PLA B4 & after	0.63 g cho/kg body mass	54.2				-	↑	↑	-	-	↑	1.47	0.2
Haub 2002, 2005 PMID:12197993, 15931612 PMID:	(21) 65 ± 5 y	Beef	beef-containing (BC) diet	~53	Biopsy, BOD-POD, CT	2x8, 1xfail, ~80% 1RM	3x/wk, 12wk	-	↑	↔	-	↔	↑	1.1	0.15
		LOV (soy)	lactoovovegetarian (LOV) diet					-	↑	↔	-	↔	↑	1.1	0.1
Carter 2005 PMID: 16236227	(42) UT (48-72y)	PRO	35g whey	35	DEXA, Multifrequency BIA	PRT, 3x8, WBR 80% 1RM	3d/wk, 16wk	-	↑	↑	↑	-	↑	?	?
		PRO+Cr	35g whey + 5g Cr	35				-	↑	↑	↑	-			
		Cr	5g Cr	-				-	↑	↑	↑	-			
		PLA	CHO N	-				-	↑	↑	↑	-			
Castaneda 2001 PMID: 11730397	(26) UT, w/ moderate renal insufficiency (17M, 9W), >50y	Low-PRO + RET	low-PRO diet plus resistance training (n = 14)	~	Total body K, Biopsy, CT	Keiser, 5 machines, 3x8, ~80% 1RM	3x/wk, 12wk	-	↑	↑	-	-	↑	0.84	-0.22
		Low-PRO	low-PRO diet (0.6 g/kg of body weight per day)	~				-	↓	↓	-	-	↔	0.84	-0.22
Esmark 2001 PMID: 11507179	(13M) 74 ± 1, BMI 25	Immediate	oral PRO in liquid form (10 g PRO, 7 g CHO, 3 g fat)	10	MRI, DEXA	warm up on cycle, PRT bilateral LP, (lat) pulldown & KE	?x/wk, 12wk	-	↑	↑1	-	↔	↑	1.1	0
		2hr		10				-	↔	↓1	-	↔	↑	1.1	0
GODARD 2001 PMID: 12131252	(26) (>65y)	PRO	12 g EAA & 72 g fructose & dextrose, 400 mL H20	-	CT, R thigh	warmup cycle, 2x10,1xfail, KE 80% 1RM	3x/wk, 12wk	-	↑	-	-	-	↑	~17% of diet	NC
		PLA	none	-				-	↑	-	-	-	↑	~17% of diet	NC
Campbell 1995 PMID: 7611390	(12) UT	PRO	Milk (diet)	63	CT, HW	WBR, 2x8, 1xfail-12, 80% 1RM, nonsequential days/wk, Keiser,	3d/wk, 12wk	-	↔	↑↑ ?	-	-	-	1.6	?~0.9
		PLA	Low-PRO diet (iso-kcal)	-					↔	↑	-	-	-	0.8	?~0.10

Arrows denote direction of change. ↑, significantly increased; ↓, significantly decreased; ↔, no change; ↔↑, trend to increase; ↔↓, tend to decrease; ↔. Red color arrows represent a group diference. Blue arrows represent an effect of feeding . RM, repetition maximum; LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Ecc, eccentric contractions; Con, concentric contractions; O, old; Y, young; M, men; W, women; OW, overweight; h, hour; m, minutes; sec, seconds; WB, whole body; PRT, Progressive resistance training; TR, trained, RT, resistance trained, ; RE, resistance exercise; ST, strength trained; ET, endurance trained.LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Si, Single leg; Rec, recreationally active; TR, Trained; MP, Military Press; UT, Untrained; BCAA, Branch Chain Amino Acids; HMB, A-hydroxy-A-methylbutyrate; CrM, Creatine Monohydrate; EAA, Essential Amino Acids; CHO, Carbohydrate; PRO, Protein; PLA, placebo; WH, Whey; WHI, Whey Protein Isolate; WHC, Whey Protein Concentrate; Cas, Casein; FFM, fat-free milk; lac, lactose; Con, control; LOV,lactoovovegetarian; HP, high protein; Leu, leucine; AA, amino acids; MCV, maximal voluntary contraction; Glu, glutamine; LBM, Lean body mass; pi, post-ingestion; DXA, dual-xray absorbmerty; HW, hydrostatic weighing; BodPod, air displacement plethysmography; BIA, bioelectrical impedance; MRI, magnetic resonance imaging, CT, computed tomography; US, ultrasound; AnTH, anthropometry, AET, aerobic training; ISO, isokinetic;KEPT, knee extension peak torque;BW, body weight; wk, week; TTE, treadmill time to exhaustion; K, potassium; fCSA, myofiber cross-sectional area, soln, solution; NC, no change

EXERCISE TYPE/INTENSITY AND "TRAINING" STATUS

As proposed by Pasiakos et al. [353], the intensity, duration and volume of the PRT and the training may play a role in the added effectiveness of PRO supplements on augmenting these outcomes, at least in young adults. We were unable to determine any clear pattern to suggest that the intensity, duration and volume of RET may interact more favorably with protein supplementation in older adults. The wide range of exercise interventions used (from group exercise with therabands to complex progressive strength and power training programs) provided a clear benefit, indicating exercise, is the most potent and effective stimulus at promoting muscle heath in older adults. However, future research should examine the possibility that some exercise modalities or interventions may be more effective at combating the age-related anabolic resistance to enhance amino acid sensitivity in older adults.

Pasiakos et al. [353], Cermak et al. [324] but not Schoenfeld et al. [349] suggested that resistance trained individuals were more likely to demonstrate a benefit from added PRO during resistance training. The prevailing thought behind this observation is that neural improvements (motor unit synchronization and reduced antagonist activation) are the primary factor driving the strength gains during the beginning of an RET program [427]. Thus a potential explanation for the relative lack of an effect of PRO/AA supplements in older adults could reside in the possibility that most of the older adults studied would be considered "untrained" the sample size was too low or the training duration was not long enough to delineate an effect [78, 353]. Kosek et al. among others suggest that the primary determinant of improved strength in adults as compared to young adults, is attributable to non-hypertrophic adaptations, but rather neural factors and

this seems to be independent of motivation or a lack of familiarization [428]. In fact, although, several of these studies include "healthy" older adults several RCTs targeted more frail individuals (**Table 1.14**). Regardless, this concept has led to the frequent conclusion that if an intervention or protein supplement did not see an effect, if must be that the training duration was of insufficient duration. Certainly, this theory may be very applicable to the enhancement of strength gains or functional improvements with RET, but is likely to have limited bearing on hypertrophy. Indeed, very few studies have demonstrated improved strength with PRO/AA supplementation in young and especially older adults [388, 393, 400]. Holm et al. exhibited a weak trend for nutrients (PRO, CHO and micronutrients) to augment strength during RET in postmenopausal women [393], which is in agreement with another study [400]. Daly reported an 18% greater increase in leg extension strength in the red mea-consuming group [388]. We are unaware of any effect of PRO/AA on enhancing the adaptive process of motor unit recruitment. However, because some AA's are direct precursors of neurotransmitters, BCAA supplementation has been shown to improve CNS function [429] and may benefit older adults with fatigue when an AA imbalance is present. If the strength or functional test is sufficiently difficult, older adults could theoretically benefit from AA supplementation and/or a greater AA reservoir (hypertrophy) [430].

PROTEIN TYPE

Although the protein source (whey, milk, egg, meat or soy protein) used has varied between studies, whey, milk, egg, meat or soy protein (**Table 1.14**), only 2 studies have directly compared protein type in RET studies in older adults [395, 419]. These were not supplement studies per se; rather diet manipulation studies examining the effect

of a meat vs. a meat-free diet on RET induced changes in muscle growth and body composition. One study determined that a meat containing diet was more beneficial in altering body composition and muscle growth than a lactovegetarian diet [395]. However, a follow-up study from the same laboratory conducted the study with firmer diet control and found that when both diets exhibited a protein intake of > 1 g/kg/day there was no difference in between these diets on altering body composition and muscle growth. This demonstrates as discussed in earlier sections, that protein source may be a trivial issue if the sources ingested are of higher quality and a sufficient dose is achieved during consumption. We are unaware of any studies directly comparing 2 or more protein types/sources and having a non-protein control group in older adults.

FUNCTIONAL IMPROVEMENTS

We felt it necessary to examine the changes in functional testing (timed get-up and go, sit–to-stand, FSST and other measures) with PRO supplementation during RET because these outcomes are more directly related to the loss of independence than slight changes in muscle mass or strength. Interestingly, in a varied population of older adults, no effect of protein supplementation was shown to enhance any functional test over resistance training without supplementation, except on two occasions [399, 401]. Only exercise training, independent, of supplementation, was able to significantly and consistently improve physical function in older adults.

Regional vs. Whole Body Lean Mass

A factor in need of consideration is that the primary outcome of most of chronic exercise training and supplementation studies, lean mass as assessed via dual x-ray

absorptiometry. This measure is often is given little or no information regarding standardization of the scanning protocol. Our own pilot findings and those published from others [431-433] suggest several variables need to be addressed to obtain precise measurements. Unfortunately, most studies only report total body lean mass to make conclusions regarding muscle mass and few mention appendicular lean mass. Arm and leg mean mass will more specifically reflect RET induced changes in muscle mass, than trunk or total body lean mass, which includes viscera and vital organs, and may change size in response to increased amino acid supply [8].

This may also partly explain why very few studies report an enhancement in strength or function with PRO/AA supplementation during RET. In fact, even some of those studies that do demonstrate an effect of protein on "estimates of muscle mass" (i.e. DEXA lean mass) do not demonstrate an enhancement in strength. There are several concerns regarding these findings. 1) These increases in lean mass do not constitute limb muscle increases, rather trunk or viscera; 2) this could be a result of an increase in the free amino acid pool and not protein; 3) The strength testing applied is not specific to the area where mass accrual has occurred. Regardless of these postulations, the end result is a lower force to mass ratio compared to the placebo group, which should be a concern to several athletic populations where the highest force to mass ratio is essential for optimal performance. Thus, this line of evidence suggests that dietetic counseling for said populations may advise avoidance of or awareness of the proper ratio of protein supplements to total caloric intake. If anything, this situation of extra "non-contractile" muscle, should be further explored to determine the location and composition of this accrual if there is any functional or physiological benefit from this excess tissue/AA

supply. This could mean examination of potential for a greater post-absorptive glucose disposal or presence of a greater amino acid reservoir acting as a buffer against acute periods of sickness, injury or disuse common with aging [430]. However, at a certain point such as hypertrophy plateau or advanced aging, nutritional interventions may not have an effect on improving outcomes, especially since a study in frail elderly men has demonstrated that addition of a potent anabolic stimulator, testosterone, is not effective [434].

Satellite Cells

As suggested in the section on acute responses to RE and PRO/AA supplementation, an effect of feeding whey protein may enhance satellite cell activity (**Table 1.15**). Chronic support of this concept was demonstrated by Olsen et al. in back in 2006 by a greater satellite cell content after 16 weeks of RET with PRO+CHO compared to CHO supplementation [435]. More recently, Farup and colleagues [382] demonstrated a fiber type specific satellite cell enhancement in PRO+CHO compared to CHO supplementation. This is an interesting an exciting area of investigation that warrants further investigation of various protein types and in aging conditions.

Table 1.15. Summary of the effect of protein supplements on satellite cells in the in vastus lateralis following resistance exercise training conducted in young humans

Reference	Feeding	Group	Training Program/ Exercise Session	Intensity:Training Status	Time since last exercise	Fiber size	Sat Cell #	Sat Cell: MHCI	Sat Cell: MHCII	Myonuclear # MHCI	Myonuclear # MHCII
Olsen et al. (2006)	6g CrM + 14g/d CHO, 80g CHO (w/ EX)	PRO	3x wk for 16wk , PRT incline legpress, knee extensionand hamstring curl. 3–5 sets of 6–12 repetitions	(6–12 RM loading) to 8-10 1RM and then 6-8 1RM		↑ 16.8%	↑↑	-	-	-	-
	14 g carb (day), 20 g PRO + 80 g carb (w/ EX)	PRO+Cr				↑ 7.9%	↑↑	-	-	-	-
	14 g carb (day), 80 g carb (w/ EX)	Cr+CHO				↑ 13.8%	↑	-	-	-	-
	No sup, no training	CHO				↔	↔	-	-	-	-
Farup 2014	Whey 19.5g +CHO (19.5g glucose) (half pre/post)	Con N=11	12 wk, 33 sessions,KE, (6-12 sets × 6-15 repetitions)	10-15RM, active	3-6 d Post	T1:↑, T2: ↑↑	-	↑↑	↑↑	↑	↑
		Ecc N=11				T1:↑, T2: ↔	-	↑	↔		↔
	CHO (39g glucose) (half pre/post)	Con N=11				T1:↑, T2: ↔	-	↑↑	↑↑		↔
		Ecc N=11				T1:↑, T2: ↔	-	↑	↔		↑
Farup 2014	Whey 28g+28g CHO	Ecc N=12	15x10 max Ecc (dyna)		Rest, 24, 48, 168h	-	↑24,↑↑48	↔	↑24,↑↑48	-	-
	Pla 56g CHO	Ecc N=12				-	↑	↔	↔	-	-
Snidjers 2014	Normal PRO (88g/d)	Norm N=10	6x10 LP & KE 75% 1RM		Rest, 12,24,48,72h	↔	↑ 12-72	↑ 12-72	↑ 24-72	↔	↔
	Low PRO (11g/d)	Low N=10				↔	↑ 12-72	↑ 12-72	↑ 24-72	↔	↔

Arrows denote direction of change. ↑, significantly increased; ↓, significantly decreased; ↔, no change; ↔↑, trend to increase; ↔↓, tend to decrease; ↔. Red color arrows represent a group diference. Blue arrows represent an effect of feeding. Arrows reresent change from rest (where available) . RM, repetition maximum; LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Ecc, eccentric contractions; Con, concentric contractions; O, old; Y, young; M, men; W, women; h, hour; m, minutes; sec, seconds; Tr, trained, RT, resistance trained, SNP, sodium nitoprusside; RE, resistance exercise; ST, strength trained; ET, endurance trained.LP, leg press; KE, knee extensions; S, squats; LE, leg extensions; Si, Single leg; TR, Trained; UT, Untrained; BCAA, Branch Chain Amino Acids; EAA, Essential Amino Acids; CHO, Carbohydrate; PRO, Protein; LBM, Lean body mass; pi, post-ingestion.

RELEVANCE OF ACUTE RESPONSES ON CHRONIC OUTCOMES

The acute physiologic responses to exercise and nutrition have garnered ample attention (discussed above). This is partly due to the mechanistic insight into and physiologic knowledge gained, which is inherently fascinating. The main reason why we know more regarding the acute responses is probably due the relative ease of completing these studies compared to chronic studies. Recent [243, 436] findings and occasional critique over the years have questioned the relevance of these types of studies.

Indeed, the prevailing theory for adaptation is that the recurring summation of molecular and physiological changes results in the ensuing phenotype [26, 437]. The literature supports this concept, generally, but we know very little about this adaptive process in specific situations and populations. The first step in understanding these changes has been the acute study, which has most frequently explored the immediate hours or occasionally the following day(s) after one exercise bout. We review here the literature examining if is there is a direct the relationship between acute changes (signal transduction, MPS) and long-term outcomes of muscle mass and strength. This is a

crucial point, as the acute studies summarize their findings with various exercise modalities and/or nutritional interventions with inferences towards chronic outcomes.

Baar et al. showed that p70S6K1 phosphorylation was tightly correlated with muscle mass accrual in rodents [438], and this prompted other studies to follow-up with investigation of this relationship in humans (**Table 1.16**). Tezris et al. demonstrated that when untrained participants conducted RE 2 hours after breakfast, the p70S6K1 phosphorylation 30min post-exercise was correlated with the hypertrophy from 8week of RET [98]. Also, Mayhew and colleagues demonstrated that % change in p70S6K1 phosphorylation (Thr421/Ser424) at 24h post exercise was correlated to mean myofiber CSA after 16wk of RET [439]. Additional support for the concept that mTORC1 activity following an acute bout of RE is predictive of increases in muscle mass have been recently demonstrated by Mitchell et al. with the fold change in p70S6K1 phosphorylation (Ser389), at 5h post-exercise and muscle CSA [172]. In the same study, the authors demonstrated that a greater pre-training androgen receptor content was also a strong predictor of muscle hypertrophy [172], which is agreement with previous research [171]. In a separate study [243], the author demonstrated that 4E-BP1 phosphorylation 1h post-exercise was also predictive of muscle hypertrophy, although mTOR and p70S6K1 phosphorylation was not predictive. They also provided evidence from a separate study that p70S6K1 phosphorylation (Ser389) was not predictive [440]. The evidence taken together suggests that there is a possible association with acute RE induced mTORC1 activity and muscle hypertrophy, at least in the *vastus lateralis* (**Table 1.16**). However, this compilation of literature is likely an underestimation of the field, as many studies have employed study designs enabling such comparison, but have not

reported data for such a relationship, presumably due to the difficulty in publishing so called “negative” findings. Certainly, it is obvious that mTORC1 activity plays a role in the hypertrophic response [82, 92, 124]. Future, research should seek to examine mechanisms explaining the factors and variability modifying this relationship.

Table 1.16. Summary of studies suggesting or demonstrating an association with acute resistance exercise inducted MPS and muscle hypertrophy in the vastus lateralis

Acute FSR	Study	MPS Finding	Chronic effect	PE feeding	Hypertrophy Result	Acute MPS predict Chronic Phenotype?
Rasmussen 2000, Borsheim 2002	Effect and timing of oral EAA +CHO on MPS	↑	Bird 2010	Yes	EAA+CHO≧ EAA ≧PLA	Yes
Moore 2005	Effect of Ecc or Con contrations on PE MPS	↑ Ecc > ↑ Con	Moore 2012	Yes	Ecc = Con	no
Wilkinson 2007	Effect of milk, soy-milk on PE MPS	↑ Milk > ↑ Soy-milk	Hartman 2007	Yes	Milk > Soy-milk > Cho	Yes
Mayhew 2009	Effect of age on PE MPS & RET hypertrophy	↑ Young > ↔ old	same study	no	not predicitve	no
West 2009	Effect of high or low hormone on PE MPS	↑ High = ↑ Low	West 2010	yes	High = Low	Yes
Holm 2005	Effect of PEx protein-nutrieent on leg net balance in postmenopausal women	↑ net Bal Nutr > ↔ PLA	Holm 2008	Yes	↑↑Nutr > ↑ PLA	Yes
Holm 2010	Effect of exercise intensity & feeding on PE MPS	↑↑HI > ↑ Low	Holm 2008	Yes	↑↑HI > ↑ Low	Yes
Burd 2010	Effect of Ex intensity/vol on PE MPS	↑ High volume to fail > ↑Low volume to fail	Mitchell 2012	Yes	Low = High	no
Mitchel 2014	Effect of PE MPS on change in Muscle Size with RET	↑	same study	Yes	↑ MPS not predicitve of	no
Rahbek 2014	Effect of Whey/CHO sup & contraction mode on PE MPS & hypertrophy	PRO+CHO = CHO; ECC=CON	same study	Yes	PRO+CHO > CHO	no, but not directly examimed

MPS, Muscle protein synthesis; PE, post-exercise; Ex, exercise; PRO, protein; net bal, net-balance; EAA essential amino acids; Ecc, eccentric contractions; Con, concentric contractions; CHO, carbohydrate; Nutr, nutrition.

mTORC1 activation has been linked to muscle protein synthesis on many occasions (discussed in above sections). Thus in would seem intuitive that this direct estimate of the rate of muscle protein synthesis would be a stronger predictor of muscle mass accrual. However, a recent in-depth investigation [243] and a previous report [439] show that when using the same cohort of subjects to compare the acute FSR response to the change in muscle mass, this relationship, quantitatively, does not exist. This is a puzzling finding for some, as a recent viewpoint article [436] has highlighted that there have been several reports, in the same laboratory, but in different subject cohorts, where acute studies assessing MPS or net balance [34, 164, 246, 256] have reflected chronic outcomes [167, 344, 393, 441]. However, this is not always evident [33, 47, 384, 440, 442]. All these studies are shown in **Table 1.17** and once again, this amount of available evidence may be an underestimation due to lack of reporting. As similarly discussed in the previous section on the relationship between mTORC1 activity and MPS, it seems an obvious stretch in the powers of scientific observation to find an association between the 1 second snapshot of a phosphorylation status or the several hour post-exercise MPS assessment and muscle hypertrophy occurring over 2000 hours (the average 3 month clinical trial) of exercise training and daily activities. As suggested [436], there exist several reasons for this discordance. They include individual factors such as age, genetic, epigenetic, transcriptional adaptability, and nutritional status, antibody variability, level of physical activity and/or other environmental influences. Also, it is possible that variability in the outcomes, changes in protein breakdown or other factors may be involved. We have little or no information regarding which of these factors is most dominant or how they interact and future research should seek to elucidate what role

these factors play. It seems most evident that acute studies may be useful in presenting the general "hypertrophic" potential of a certain intervention. However, it is clear that there in an inherent variability in an individual's ability to respond to training, which we are only now beginning to understand.

Table 1.17. Summary of studies demonstrating an association with acute resistance exercise inducted intacelluar signaling or protein content and muscle hypertrophy in the vastus lateralis

Acute FSR	Study	MPS Finding	Chronic effect	PE feeding	Hypertrophy Result	Acute MPS predict Chronic Phenotype?
Rasmussen 2000, Borsheim 2002	Effect and timing of oral EAA +CHO on MPS	↑	Bird 2010	Yes	EAA+CHO≧ EAA ≧PLA	Yes
Moore 2005	Effect of Ecc or Con contrations on PE MPS	↑ Ecc > ↑ Con	Moore 2012	Yes	Ecc = Con	no
Wilkinson 2007	Effect of milk, soy-milk on PE MPS	↑ Milk > ↑ Soy-milk	Hartman 2007	Yes	Milk > Soy-milk > Cho	Yes
Mayhew 2009	Effect of age on PE MPS & RET hypertrophy	↑ Young > ↔ old	same study	no	not predicitve	no
West 2009	Effect of high or low hormone on PE MPS	↑ High = ↑ Low	West 2010	yes	High = Low	Yes
Holm 2005	Effect of PEx protein-nutrieent on leg net balance in postmenopausal women	↑ net Bal Nutr > ↔ PLA	Holm 2008	Yes	↑↑Nutr > ↑ PLA	Yes
Holm 2010	Effect of exercise intensity & feeding on PE MPS	↑↑HI > ↑ Low	Holm 2008	Yes	↑↑HI > ↑ Low	Yes
Burd 2010	Effect of Ex intensity/vol on PE MPS	↑ High volume to fail > ↑Low volume to fail	Mitchell 2012	Yes	Low = High	no
Mitchel 2014	Effect of PE MPS on change in Muscle Size with RET	↑	same study	Yes	↑ MPS not predicitve of	no
Rahbek 2014	Effect of Whey/CHO sup & contraction mode on PE MPS & hypertrophy	PRO+CHO = CHO; ECC=CON	same study	Yes	PRO+CHO > CHO	no, but not directly examimed

It is well known that physiologic adaptation to a given stress changes over time. What is rather clear is that the "law of diminishing returns" exerts strong precedent on the acute MPS response as an individual becomes more trained [356]. Interestingly, this effect has been suggested to occur rather quickly [436]. This data could theoretically suggest that the "upper limit" or "set point" of hypertrophy has been approached and that various mechanisms may start to attenuate the anabolic response. This becomes even more complicated with the reflection that this regulation may occur in other time periods over the course of exercise training.

Several investigations have sought to determine the effect of later time periods, repeated bouts, exercise habituation, and a few various durations of exercise training. Unfortunately, the majority of the acute MPS studies have focused on the immediate post-exercise time period. There exist several hours in the day and it is very likely that there are other time-frames, besides the immediate hours post-exercise and PRO/AA nutrition, where changes in MPS and MPB are regulated to control hypertrophy. As an example of this, although the acute post-exercise response may lessen in trained individuals, it appears that the resting post-absorptive MPS is increased in the trained state (**Table 1.7 & 1.8**). We know very little regarding the regulation of protein metabolism during those later time frames and diurnal response of protein turnover during exercise training and how that impacts overall phenotype change (hypertrophy or other outcomes). There likely exists a multifactorial role of PRO/AA stimulus on MPS and MPB or even processes of indispensable AA loss during exercise training. Training status alone could be a complicated variable suggesting differentiated responses based on sessions to years of training. Type of training (aerobic, resistance, concurrent) and when these sessions are applied during a periodized training program are also likely to illicit a variety of responses. Layering these variables together with factors intrinsic to the individual highlights the complexity of the situation. Since physiology adapts to both exercise and nutritional stimuli, it may also be of benefit to examine how altering or cycling PRO/AA form or dose can maintain the sensitivity of AA during RET. This reality frames a daunting test for investigators that may be impossible if the traditional approach of forward translation (basic science to human models) remains the dominant process. Obviously the cost to assess the layering of these variables and the time-course

and of these responses thoroughly would be enormous and ethically challenging given the current methods.

SUMMARY TO LITERATURE REVIEW

The compiled evidence from human research models indicates that the transcriptional, post-translational, physiologic and phenotypical response to exercise and nutrition is highly variable. This fact has provided a layer of ambiguity in our ability to make precise estimates of the effectiveness of PRO/AA and exercise interventions. We believe that this difficulty arises from a compulsory attempt to follow the overriding scientific paradigm of so called "forward" translation, which often does not translate well to clinical application. A scientific paradigm to understand and modify the human condition may be more effective by starting with the human condition and all its intrinsic variability and then work in "reverse" translation with various models to determine cause and effect. Anecdotal evidence provided by dieticians, physiologists, clincians, and exercise specialists could be used to better understand the variability in the human condition. With a common goal, improving human health, this information could be used to shape clinical and basic science research with "translational" effectiveness.

SPECIFIC AIMS

My goal was to determine the efficacy of protein blend ingestion following exercise on the enhancement of muscle growth and strength in humans. The results from this study will benefit the field of sports nutrition and also populations with highest risk for loss of muscle mass and function. The overall hypothesis was that nutritional supplementation with a blend of soy and dairy (whey and casein) proteins following resistance exercise will promote muscle hypertrophy to a greater extent than isolated whey protein supplementation, which is the current popular selection. This was tested acutely and chronically. The acute study hypothesis was that a blend of soy and dairy protein will improve the muscle protein anabolic response more than whey protein alone (matched for leucine content) when ingested following an acute bout of resistance exercise in young adults. The chronic study hypothesis was that nutritional supplementation with a blend of soy and dairy proteins during 12 weeks of resistance exercise training will increase muscle growth and strength to a greater extent as compared to isocaloric matched placebo (carbohydrate) or isolated whey protein supplementation. The primary rationale for this hypothesis is driven by the anabolic actions of similar leucine content and the different rates at which proteins are absorbed. Elevated presence of essential amino acids in the blood is necessary for muscle growth via their actions as substrates and signals for protein accretion [1]. Our main research question was: will protein blend supplementation following resistance exercise training increase muscle anabolism and strength more than the individual whey protein supplementation when matched for similar and sufficient leucine content? Thus, in young healthy adults, we tested the acute study specific hypotheses after one acute bout

of leg resistance exercise with supplementation and the chronic study specific hypotheses following 12 weeks of resistance exercise training with supplementation:

Acute Study Specific Aim: To determine in young men and women if a protein blend ingested following an acute bout of resistance exercise will improve:

Muscle protein synthesis: by prolonging the release and presence of amino acids, by increasing the duration of the synthetic response and anabolic signaling, by prolonging net balance across the leg and by increasing skeletal muscle amino acid transport and gene expression more than whey protein ingestion.

To determine whether the acute adaptations above are not a consequence of differences in leucine content, total protein given in the protein blend and whey protein were adjusted so that both supplements contain a similar amount of leucine. To address this aim we recruited 20 young men and women into a randomized, double-blind study to ingest ~20 grams of whey (N=10) or ~22 grams of the blend (50% casein, 25% whey and 25% soy protein) (N=10) following one bout of resistance exercise. Skeletal muscle protein anabolism was assessed with a stable isotopic infusion and muscle biopsies.

Chronic Study Specific Aim: To determine whether supplementation of a protein blend containing adequate leucine following resistance exercise training will:

Increase muscle mass, strength and muscle quality and stimulate muscle cell growth pathways more than supplementation of whey protein or isocaloric placebo.

To determine whether the chronic adaptations above are a consequence of the supplement nitrogen content (protein) and not due to increased energy consumption (calories) after exercise, we compared three iso-caloric supplement groups: 1) protein blend plus resistance exercise training, 2) whey protein plus resistance exercise training,

3) non-nitrogenous placebo (carbohydrate) plus resistance exercise training. To address this aim we recruited 60 young men into a double-blinded randomized control trial. These subjects were be divided into 3 isocaloric supplement groups (Blend, Whey or Placebo) and performed resistance exercise training. Treatments and exercise training were administered for 12 weeks. Muscle mass, strength and strength and a set velocity were measured at baseline, 6 weeks and after 12 weeks of resistance exercise training to determine whether the treatments induced gains in muscle size and/or function. Also, to gain insight into the mechanisms behind these adaptations, we assessed muscle satellite cells and myonuclear accretion, protein concentration and cell signaling pre and post-training.

CHAPTER 2

Protein Blend Ingestion following Resistance Exercise Promotes Human Muscle Protein Synthesis[1]

INTRODUCTION

An increase in amino acid availability following an acute bout of resistance exercise enhances skeletal muscle protein synthesis in humans [40, 41, 54, 252, 253, 255, 258]. In addition, intact protein ingestion in the form of soy, casein, whey, egg or beef increases amino acid supply to muscle, which further promotes muscle protein synthesis during post-exercise recovery [43, 65, 190, 218, 232, 233, 238, 241, 245, 246, 249, 291]. However, there is some disagreement about whether different protein sources produce superior effects on muscle protein synthesis. The primary points of contention include the overall protein quality (i.e., amino acid composition) of the protein source and its digestion rate (i.e., fast, intermediate, or slow).

High quality dairy (whey and casein) and plant (soy) protein sources contain all of the essential amino acids (EAA), and they each have distinct traits thought to offer an advantage for stimulating muscle protein synthesis [292, 443]. On average, ~20-25g of high quality protein contains ~8-10g EAA, which are critical for the regulation of muscle protein synthesis [300]. Whey contains a higher BCAA content, primarily leucine, compared to other high quality proteins [292], and its rapid digestion increases blood

[1]Protein Blend Ingestion Following Resistance Exercise Promotes Human Muscle Protein Synthesis. Reidy PT, Walker DK, Dickinson JM, Timmerman KL, Drummond MJ, Fry CS, Gundermann DM, Rasmussen BB. J Nutr. 2013 Apr;143(4):410-6.

[2] Soy-dairy protein blend and whey protein ingestion after resistance exercise increases amino acid transport and transporter expression in human skeletal muscle. Reidy PT, Walker DK, Dickinson JM,

amino acid concentrations shortly following ingestion [232, 234, 303, 304]. This effect is transient and returns to resting levels within two to three hours [303, 304] when consumed independently or following a bout of exercise [232, 234]. For these reasons whey protein has been considered to be superior compared to other isolated protein sources [234, 238, 245, 305, 306]. The hyperaminoacidemia occuring with whey ingestion stimulates additional amino acid oxidation, which could contribute to reduced nitrogen retention (i.e., whole body protein synthesis) [303, 304]. When a slowly digested protein such as casein is ingested, it produces a slower but more prolonged (~6 h) aminoacidemia that results in higher nitrogen retention and less oxidation [303, 304] and is effective in stimulating post-exercise muscle protein fractional synthetic rate (FSR) [65, 291]. When these milk proteins (whey and casein) are co-ingested, the slowly digested protein, casein, not the whey, contributes the amino acids for a prolonged protein synthetic effect across the leg [236]. Meanwhile, soy protein has an "intermediate" digestion rate [241, 312], contains key properties not associated with dairy proteins such as anti-oxidant/inflammatory activity [444, 445] and effectively stimulates post-exercise FSR [241, 245] and overall muscle accretion [342, 378].

We have recently demonstrated in a rodent model that a protein blend is effective in prolonging the FSR response when compared to single source proteins like whey [446]. Therefore, we hypothesized that a protein blend consisting of soy and dairy proteins would capitalize on the unique properties of each individual protein and would optimally deliver amino acids to promote muscle protein synthesis following resistance exercise. To address our hypothesis we conducted a randomized double-blind study in young adults to compare the effect of a protein blend (PB) (soy, casein and whey) vs. a single

protein isolate (whey protein: WP) ingested following a bout of high-intensity exercise on BCAA blood concentrations, mammalian target of rapamycin complex 1 (mTORC1) signaling and FSR during post-exercise recovery.

MATERIALS AND METHODS

Screening of participants.

We recruited nineteen healthy, young participants (17 male, 2 female; age range: 18-30y) for this double-blind, randomized clinical trial. Participant characteristics are shown in **Table 2.1**

Table 2.1 Participant characteristics[1]

	N	Age, *years*	BMI, kg/m^2	% Fat	FFM, *kg*	Lean Mass, *kg*
PB	10	23.1 ± 1.0	25.9 ± 0.8	24.3 ± 1.7	60.3 ± 3.5	57.3 ± 3.3
WP	9	25.1 ± 1.2	25.5 ± 1.0	24.1 ± 2.5	61.2 ± 3.0	57.9 ± 2.8

[1]Data are mean ± SEM. Protein blend (PB) and whey protein (WP). FFM, Fat-free mass.

The participants were recruited through locally posted flyers, newspaper advertisements, and by word of mouth. The participants were healthy and recreationally active, but were not engaged in any regular exercise training program (< 2 sessions high intensity aerobic or resistance exercise/week) at the time of enrollment. Screening of participants was performed on two separate days (>7 days apart) at the Institute for Translational Sciences-Clinical Research Center (ITS-CRC). The first screening day included 1 repetition maximum (1RM) testing, a clinical history, physical exam, and laboratory tests (complete blood count with differential, liver and kidney function tests, coagulation profile, fasting blood glucose, hepatitis B and C screening, HIV test, thyroid stimulating hormone, lipid profile, urinalysis, and drug screening). The second screening

day included a second 1 RM test and a dual-energy X-ray absorptiometry (DXA) scan (Hologic QDR 4500W, Bedford, MA) to measure lean and fat mass. 1RM testing was performed on a leg extension machine (Cybex-VR2, Medway, MA, USA) and was recorded as the highest weight lifted for a single repetition from the two testing days. All participants gave written informed consent before enrollment in the study. The study was approved by the Institutional Review Board of the University of Texas Medical Branch, and is in compliance with the Declaration of Helsinki as revised in 1983.

Study Design

Enrolled participants checked into the ITS-CRC at ~1700 h the day prior to the study. Participants refrained from exercise at least 72h before admission. The participants were given a standardized meal at 1900h prepared by the Bionutrition Division of the ITS-CRC with a macronutrient distribution of 20% protein, 60% carbohydrate, and 20% fat at 12 kcal·kg^{-1} body weight. Participants were provided water *ad libitum*. The participants were randomized to ingest protein blend (N=10 PB) or whey protein (N=9 WP) at 1h following a bout of high-intensity leg resistance exercise. Leucine content in the protein beverages was matched by adjusting the total amount given to control for the protein anabolic effect of leucine.

Experimental Protocol

All participants underwent the stable isotope infusion protocol **(Fig. 2.1)** at the same time of day (0600-1600h) on the day following admission. After an overnight fast (~10h), an 18G polyethylene catheter was inserted into the antecubital vein, from which background blood draws were taken followed by initiation of a primed, constant infusion

(~10h) of L-[ring-$^{13}C_6$] phenylalanine (Sigma-Aldrich, St. Louis, MO, USA). The priming dose for the labeled phenylalanine was 2 μmol·kg^{-1} and the infusion rate was 0.05 μmol·kg^{-1}·min^{-1}. A retrograde catheter was inserted (0700-0800h) into a hand vein on the contralateral arm and arterialized blood was extracted with the use of a heating pad prior to sampling. At two hours and four hours following initiation of the infusion muscle biopsies were taken from the lateral aspect of the *vastus lateralis* for the determination of resting mixed muscle FSR. All biopsies were taken with a 5mm Bergström biopsy needle under sterile procedure and local anesthesia (1% lidocaine). Following the second biopsy the participants were moved to a leg extension machine (Cybex-VR2, Medway, MA, USA) for high-intensity resistance exercise consisting of eight sets of ten repetitions at 55% (set 1), 60% (set 2) 65% (set 3) and ~70% (sets 4-8) of the participants previously determined 1RM with three min rest between sets. Three additional muscle biopsies were taken 1, 3 and 5 h after the completion of exercise. The nutritional supplements were ingested immediately following the 1h biopsy. The first and second, the third and fourth and the fifth muscle biopsies were sampled from three separate incisions on the same leg, respectively. To minimize multiple sampling, in a given area, skin incisions were separated by ~7 cm while biopsies taken from the same incision were angled ~5 cm from each other. This method has been previously utilized in our lab [29, 101, 447] and others [65, 108, 190]**.** Muscle tissue was immediately blotted, frozen in liquid nitrogen and stored at -80^{o}C until analysis. Blood samples were collected during the resting (0, 120, 180, 200, 240 min) and post-ingestion (-60, 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240 min) time periods (**Fig. 2.1)** for the determination of blood L-[ring-$^{13}C_6$] phenylalanine enrichment (see below), amino acid concentration.

The infusion study ended following the fifth muscle biopsy and participants were then given a standard meal.

Figure 2.1. Schematic of randomized double-blinded experimental protocol.

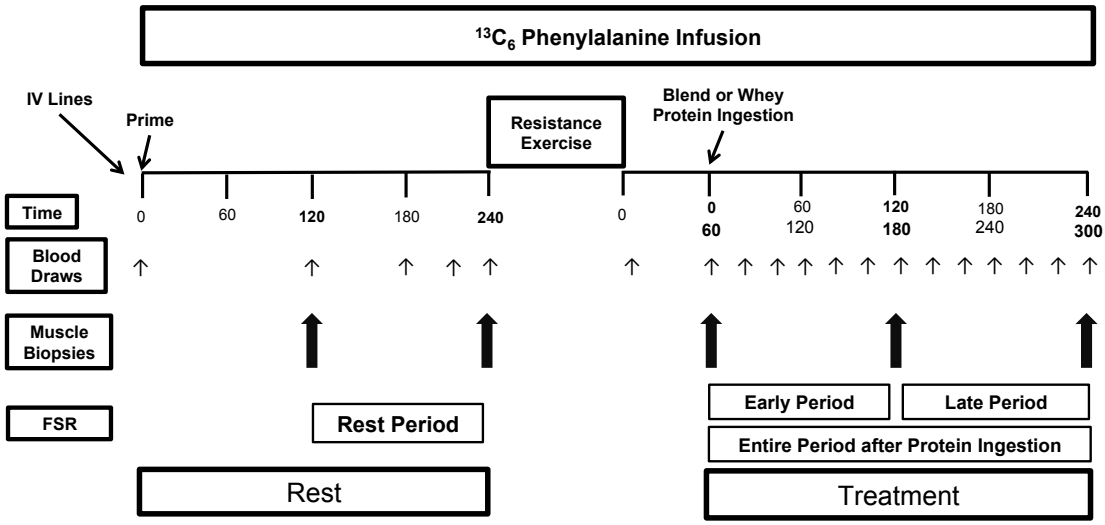


Fig 2.1. Participants ingested either the protein blend or whey protein one hour following completion of eight sets of knee extension resistance exercise. The small arrows represent blood draws whereas the large arrows represent biopsies.

Protein Beverage Intervention

The protein beverages (WP or PB) were consumed one hour following exercise. The beverages were dissolved in 300 mL of water and enriched (8%) with L-[ring-$^{13}C_6$] phenylalanine to maintain isotopic steady state in arterialized blood. The compositions of the beverages are shown in (**Table 2.2)**. To match leucine contents between the interventions, participants were given 0.30 or 0.35 g total protein·kg^{-1} lean mass for WP and PB respectively. The PB consisted of 19.3±1.1 g total protein (providing 1.8±0.1 g leucine, 8.7±0.5 g EAA) composed of 50% protein from sodium caseinate, 25% protein from whey protein isolate and 25% protein from soy protein isolate. The WP consisted of 17.7±0.9 g of protein (providing ~1.9±0.1 g leucine, 8.9±0.4 g EAA). The amount of

protein given in each group was based on the 8.6 g of EAA in intact protein demonstrated to maximize the FSR response following resistance exercise [233].

Table 2.2. Composition of the protein blend and whey protein beverages.[1]

	PB	WP
	g/100g product	
Protein	87.1	87.3
Fat, acid hydrolysis	2.09	0.80
Ash	3.62	2.82
Moisture	6.57	7.26
Alanine	3.38	4.66
Serine	4.60	4.38
Aspartic Acid	7.96	10.1
Cysteine	0.99	2.25
Glutamic Acid	18.7	17.1
Glycine	2.04	1.51
Proline	7.61	5.98
Tyrosine	4.02	2.71
Arginine	3.55	1.93
Isoleucine[2]	4.73	5.85
Leucine[2]	8.09	9.53
Lysine[2]	6.79	8.64
Methionine[2]	2.18	2.06
Phenylalanine[2]	4.13	2.83
Threonine[2]	4.38	6.51
Tryptophan[2]	1.06	1.38
Valine[2]	5.45	5.56
Histidine[2]	2.18	1.54
Total EAA	39.0	43.9

[1]Protein blend (PB) and whey protein (WP)
[2]Represent the EAA (Essential Amino Acids)

Free Blood Amino Acid Concentration and Plasma Glucose, Lactate and Serum Insulin

Concentrations of phenylalanine and the branch-chained amino acids (leucine, isoleucine, and valine) were measured in deproteinized whole blood using Gas chromatography-mass spectrometry (GCMS) as previously described using an internal standard solution [21, 448]. Serum concentrations of insulin were determined with an enzyme-linked immunosorbent assay (Millipore, St. Charles, MO) according to the manufacturer's instructions at rest, before and for several time points following beverage ingestion. Also, plasma glucose and lactate concentration was measured using an automated glucose and lactate analyzer (YSI, Yellow Springs, OH).

Muscle Protein Synthesis and Enrichments

Muscle proteins and muscle intracellular free amino acids were extracted from biopsy samples as previously described [29]. GCMS (GCMS, 6890 Plus CG, 5973N MSD, 7683 autosampler, Agilent Technologies, Palo Alto, CA) measurements were made to determine muscle bound and intracellular free concentrations with the internal standard method through the use of tracer enrichments for L-[ring-$^{13}C_6$] phenylalanine and appropriate internal standards (L-[^{15}N] phenylalanine). Measurements were determined as previously described [21]. Mixed-muscle protein-bound phenylalanine enrichment was analyzed by GCMS after protein hydrolysis and amino acid extraction [29, 300], using the external standard curve approach [20]. We calculated muscle protein synthesis as FSR by measuring the incorporation rate of the phenylalanine tracer into the proteins (Δ protein bound enrichment over time) and using the precursor-product model to calculate the synthesis rate:

$$FSR = (\Delta E_p/t)/[(E_{M(1)} + E_{M(2)})/2] \bullet 60 \bullet 100$$

where ΔE_p is the increment in protein-bound phenylalanine enrichment between two sequential biopsies, *t* is the time between the two sequential biopsies, and $E_{M(1)} + E_{M(2)}$ are the phenylalanine enrichments in the free intracellular pool in the two sequential biopsies. Data are expressed as percent per hour (%/ h).

SDS-PAGE and Western Blot Analysis

Immunoblotting was performed as previously described [29]. In brief, 20-50mg of frozen muscle tissue was processed and assayed for total protein content. After further processing, each sample (50μg of total protein) was loaded in duplicate onto a 7.5% or 15% polyacrylamide gel (Criterion; Bio-Rad) and subjected to electrophoresis at 150 V for 70 min. Following electrophoresis, proteins were transfer to a polyvinylidene difluoride membrane (Bio-Rad) which was then blocked in 5% non-fat dried milk. Membranes (blots) were then incubated with a single primary antibody overnight at 4°C. Rabbit polyclonal primary antibodies (Cell Signaling, Beverley, MA) used were the following: Akt (Ser308), mTOR (Ser2448), S6K1 (Thr389), 4E-BP1 (Thr37/46), ribosomal protein S6 (Ser240/244). Blots were incubated with secondary antibody (Amersham Bioscience) washed, and then a chemiluminescent solution (ECL plus; Amersham BioSciences, Piscataway, NJ, USA) was applied. Optical density measurements were then immediately obtained with a digital imager (Bio-Rad) and densitometric analysis (Quantity One software, version 4.5.2; Bio-Rad) was performed. Following detection of phosphorylated proteins, blots were stripped of primary and secondary antibodies and then re-probed for total protein, which was determined for each

blot. Data were normalized to an internal control and expressed phosphorylated:total protein.

Statistical Analysis.

All values are expressed as Mean ± SEM. Data were transformed using the Box-Cox set of transformations to stabilize the variance and make the data approximately normally distributed. To test differences between groups, the data were modeled using an ANCOVA model with resting values as a covariate. The testing of differences was thus accomplished through a t-test of the parameter indicating the difference between groups. Comparisons with resting values were based on inference of the intercept in the ANCOVA model after centering the response and resting variables. Each time point was modeled separately. Significance was set at $p < 0.05$. All calculations were done in R [449].

RESULTS

Subject Characteristics

Descriptive characteristics for all participants are shown in **Table 2.2**. The participants had similar one repetition maximum (1RM) values of 119±10 and 130±10 kg and their total average weight lifted was 63±2 and 62±2% of their 1RM for PB and WP respectively. There were no differences between groups.

Insulin, Glucose and Lactate

Serum insulin concentrations were significantly elevated ($p < 0.05$) above rest until 40 and 60 min following ingestion for PB for WP, respectively. (**Table 2.3**). There were no differences between groups. Plasma glucose concentrations were unchanged

following protein ingestion. Plasma lactate concentrations were significantly elevated ($p < 0.05$) above rest until 60 min following ingestion for PB and 80 min for WP. Further, lactate concentrations tended to be lower at 60 min ($p = 0.07$) and were lower 80 min ($p < 0.05$) post-ingestion for PB relative to WP.

Table 2.3. Serum insulin, plasma lactate and glucose concentrations after completion of resistance exercise[1]

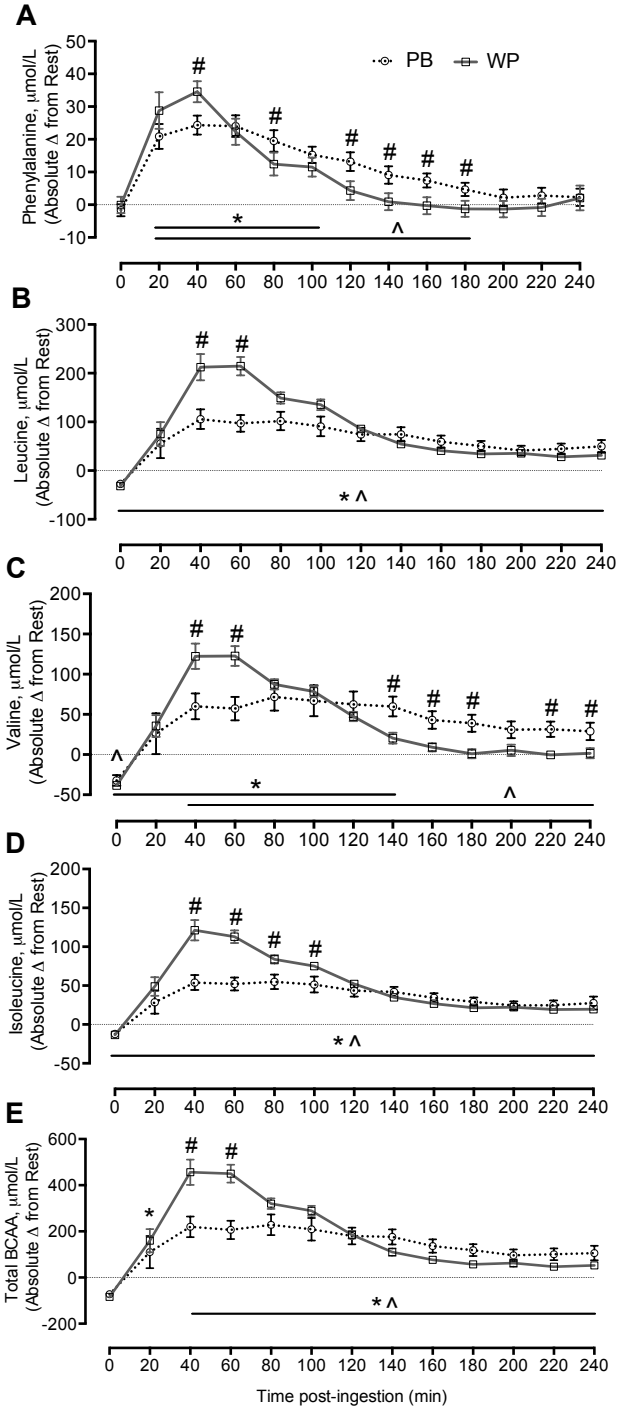
		Time post-ingestion (min)						
	Rest	**0**	**20**	**40**	**60**	**80**	**100**	**140**
Insulin				*pmol/L*				
PB	27 ± 4	35 ± 9	60 ± 14*	68 ± 10*	43 ± 7	29 ± 4	27 ± 3	22 ± 2
WP	24 ± 2	28 ± 5	60 ± 9*	70 ± 14*	49 ± 8*	31 ± 6	21 ± 3	16 ± 3
Lactate				*mmol/L*				
PB	0.80 ± 0.04	2.18 ± 0.31*	1.48 ± 0.15*	1.15 ± 0.11*	1.03 ± 0.08*##[2]	0.88 ± 0.06#	0.89 ± 0.09	0.82 ± 0.08
WP	0.85 ± 0.07	2.47 ± 0.32*	1.69 ± 0.22*	1.45 ± 0.16*	1.31 ± 0.11*	1.16 ± 0.13*	0.98 ± 0.11	0.94 ± 0.18
Glucose				*mmol/L*				
PB	4.90 ± 0.08	5.18 ± 0.29	4.99 ± 0.21	5.05 ± 0.13	4.98 ± 0.08	4.96 ± 0.09	4.90 ± 0.08	4.89 ± 0.07
WP	4.93 ± 0.07	5.12 ± 0.22	5.04 ± 0.14	5.05 ± 0.13	4.96 ± 0.09	4.96 ± 0.08	4.97 ± 0.07	4.95 ± 0.06

[1]Serum insulin, plasma lactate and glucose concentrations in young adults at rest during the post-exercise recovery period following ingestion of the protein blend (PB) or whey protein (WP) 1 h after completion of resistance exercise1Data are mean ± SEM, n=9 (WP) or 10 (PB). *Different from Rest, $P < 0.05$. Symbols indicate different from PB: #$P < 0.05$,
[2]Symbols indicate different from PB: ##$P = 0.07$.

Blood Amino Acid Concentrations

Phenylalanine concentrations were elevated ($p < 0.05$) from rest in the WP group until 100 min following ingestion whereas phenylalanine in the PB group remained elevated ($p < 0.05$) to 180 min (**Fig. 2.2**). Phenylalanine concentrations were significantly greater in the WP group at 40 min and in the PB group at 80, 120, 140, 160 and 180 min after ingestion ($p < 0.05$). Leucine and Isoleucine concentrations were elevated ($p < 0.05$) from rest in both groups for the duration of post-exercise recovery. The WP group displayed higher peak leucine concentrations at 40 and 60 min after ingestion and higher isoleucine concentrations at 40, 60, 80 and 100 min after ingestion as compared to the PB group ($p < 0.05$). Valine concentrations were elevated ($p < 0.05$) from rest in the WP group until 140 min following ingestion whereas in the PB group valine levels remained elevated ($p < 0.05$) for the duration of post-exercise recovery. Valine concentrations where higher in the WP group at 40 and 60 min and the PB group had higher concentrations at 140, 160, 180, 220 and 240 min after ingestion ($p < 0.05$). Total BCAA concentrations were elevated ($p < 0.05$) from rest in both groups for the duration of post-exercise recovery. Total BCAA concentrations were higher for the WP group ($p < 0.05$) at 40 and 60 min as compared to PB, whereas BCAA tended ($p = 0.06$) to be higher in the PB group at 180 min after ingestion.

Figure 2.2. Blood phenylalanine, leucine, valine, isoleucine, and total BCAA.



Changes from rest in blood phenylalanine (A), leucine (B), valine (C), isoleucine (D), and total BCAA (E) concentrations in young adults during the post-exercise recovery period following ingestion of the protein blend (PB) or whey protein (WP) 1 h after completion of resistance exercise. Data are mean ± SEM, n=9 (WP) or 10 (PB). #Different from PB at that time, $P < 0.05$; *Different from resting values for WP, $P < 0.05$; ^Different from resting values for PB, $P < 0.05$.

Blood and Muscle Intracellular Enrichments

Blood phenylalanine enrichments did not change over time ($p > 0.10$). However, the enrichments at 180 min post-ingestion were higher in WP than in PB ($p < 0.05$). Muscle intracellular phenylalanine enrichments were steady state during the treatment period, but during the resting period the enrichments increased ($p < 0.05$) over time in both groups. There were no group differences in the muscle intracellular phenylalanine enrichments ($p > 0.10$) during the treatment period, but at -120 min the enrichments tended to be lower ($p = 0.07$) in PB than in WP (Appendix **Figure A.2.1)**.

Muscle mTORC1 Signaling

There were no group effects for the phosphorylation status of mTORC1 (Ser 2448), Akt (Thr308), 4E-BP1 (Thr37/42) rpS6 (Ser240/244) and S6K1 (Thr389) at rest (data not shown) or post-exercise ($p < 0.05$) (**Table 2.4** and representative blots Appendix **Figure A.2.2)**. Compared to rest, there were increases in phosphorylation for mTORC1, rpS6 and 4E-BP1 at 2 and 4 h post-ingestion in both groups ($p < 0.05$). In the PB group the phosphorylation of S6K1 was significantly increased ($p < 0.05$) at 2 and 4 h post-ingestion whereas the S6K1 phosphorylation in the WP group only tended ($p = 0.07$) to increase at 2 h post-ingestion. Akt phosphorylation increased ($p < 0.05$) at 2 h post-ingestion in both groups

Table 2.4. Western-blot analyses of synthesis-associated signaling after completion of resistance exercise.[1]

Time post-ingestion	2h		4h	
	PB	WP	PB	WP
	Phosphorylated/Total, Fold of Rest			
Akt Ser^{308}	1.17 ± 0.14*	1.27 ± 0.16*	1.06 ± 0.15	0.87 ± 0.18
mTORC1 Ser^{2448}	3.51 ± 1.48*	3.01 ± 0.46*	2.78 ± 0.68*	2.83 ± 0.47*
p70S6K1 Ser^{389}	21.3 ± 7.25*	12.7 ± 3.12**	11.9 ± 3.76*	6.20 ± 1.30
rpS6 $Ser^{240/244}$	3.32 ± 1.33*	2.38 ± 0.85*	1.95 ± 0.43*	1.55 ± 0.55*
4E-BP1 $Thr^{37/42}$	1.27 ± 0.09*	1.34 ± 0.17*	1.27 ± 0.10*	1.17 ± 0.10*

[1]Western-blot analyses of synthesis-associated signaling proteins in young adults during the post-exercise recovery period following ingestion of the protein blend (PB) or whey protein (WP) 1 h after completion of resistance exercise.1Data are mean ± SEM, n=9 (WP) or 10 (PB). *Different from resting values for that group, $P < 0.05$; **Different from resting values for that group $P = 0.07$.

Fractional Synthetic Rate

Resting muscle protein synthesis (mixed-muscle FSR: **Figure 2.3**) was not different ($p > 0.10$) between the PB and WP groups (**Fig. 2.5**). The post-exercise FSR was elevated from resting values for the Early (0-2h) ($p = 0.001$), Late (2-4h) ($p = 0.030$) and Entire (0-4h) ($p < 0.001$) post-protein ingestion periods in the PB group. In the WP group, post-exercise FSR was elevated from resting values only in the Early ($p = 0.026$) and Entire ($p = 0.002$) periods, but not the Late ($p > 0.10$) period. There were no group effects at any time point ($p > 0.10$).

Figure 2.3. Fractional synthetic rate

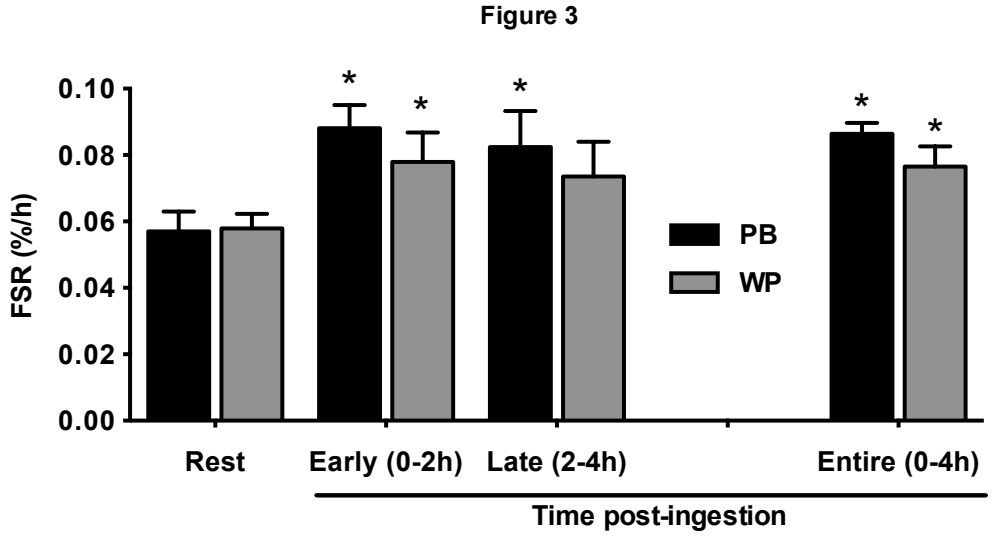


Fig 2.3. Fractional synthetic rate (% per hour) of the *vastus lateralis* in young adults during the post-exercise recovery period following ingestion of the protein blend (PB) or whey protein (WP) 1 h after completion of resistance exercise. Data are presented at Rest and Early (0-2h), Late (2-4h) and Entire (0-4h) post-ingestion periods. Data are mean ± SEM, n=9 (WP) or 10 (PB). *Different from resting values for that group, $P < 0.05$.

DISCUSSION

Proteins from milk (casein and whey), soy, beef and egg are effective in stimulating post-exercise muscle protein synthesis [43, 65, 218, 232, 233, 238, 241, 245, 246, 249, 291, 292]. Several studies have focused on whey protein and its effects in promoting lean mass gain [292, 305] due to its suggested superiority to other isolated protein sources [241]. Our data is novel in that it utilizes the proteins from soy, whey and casein with different digestion rates (amino acid release profiles) after an acute bout of resistance exercise. We show for the first time, that a soy-dairy protein blend (25% soy, 25% whey and 50% casein) is capable of stimulating muscle growth to a similar extent as whey protein through a marked elevation in muscle protein synthesis and skeletal muscle mTORC1 signaling. We compared this novel intervention against whey protein as the

single source of protein (single digestion rate) while maintaining similar absolute leucine content between the PB and WP. Previous research has only compared ingested proteins with a single digestion rate ("fast" vs. "slow") following resistance exercise [43, 65, 111, 232, 234, 239, 241, 245, 291]. Further, comparisons in these reports have not matched for the potent anabolic effect of leucine content. Additionally, the soy-dairy protein blend stimulated FSR into the Late post-exercise period, whereas WP only increased FSR from rest into the Early recovery period. In our hands a soy-dairy protein blend ingestion following exercise is capable of prolonging blood aminoacidemia, mTORC1 signaling and protein synthesis in human skeletal muscle.

Our data agrees with previous work suggesting that milk (a blend of casein and whey) offers advantages over a single source of protein such as soy to supplement resistance exercise [246, 344], yet no study until now has compared a blended protein source to isolated whey protein for muscle protein synthesis while matching leucine content. To date, the protein anabolic effect of whey protein ingestion following resistance exercise has only been tested against interventions examining other macronutrients [109, 218, 240, 254], supplemental amino acids [111, 251, 278] or other isolated protein sources [65, 235, 239, 241]. When compared to other high-quality protein sources following resistance exercise, whey has been suggested to be superior to isolated soy protein [241, 245] and micellar casein, [239, 241] which is the least soluble, most slowly digested, form of casein [450]. However, in these studies the leucine content, a key anabolic agent, was not matched between interventions, which may skew the results, especially in the aging population [286] where an adequate leucine content may be especially needed. However, a slightly more soluble form of casein, caseinate,

can initiate a comparable anabolic response to whey protein when ingested following resistance exercise [65, 235]. Our data further suggest the efficacy of co-ingesting rapidly and slowly digested proteins as a protein blend for promoting muscle protein synthesis following exercise.

High quality dairy (whey and casein) and plant (soy) protein sources contain all the EAA and have individual traits thought to offer a unique advantage for muscle growth [292, 443, 451]. One of the most supporting tenets favoring whey protein has been the higher BCAA content [305, 352], particularly leucine [292, 305]. Yet, the rapid hyperaminoacidemia of whey protein is short-lived [232, 234, 303, 304] as we demonstrated for phenylalanine and valine. Both protein supplements demonstrated a prolonged aminoacidemia as shown with leucine and isoleucine. The ingestion of WP demonstrated a peak in blood amino acid concentrations at 40 min post-ingestion that was greater than that observed in the PB group. Interestingly, this spike in substrate had no additional effect on the muscle FSR compared to the PB and did not further prolong the WP FSR response into the Late period. The PB exhibited a smaller initial peak than WP, but demonstrated proof of concept in that it remained elevated above resting values for up to three hours post-exercise for phenylalanine and four hours post-exercise for valine. It is possible that the prolonged substrate availability observed with the slower released proteins, casein [303, 304] and soy [241, 312], may explain the prolonged FSR response in the PB. The prolonged aminoacidemia may be attributed to the slower digestion of caseinate and soy protein isolate as compared to whey protein isolate. It is important to note that although we utilized a form of casein protein (i.e., caseinate) with a

more rapid digestion than micellar casein, we were still able to prolong the amino acid response as proof of concept.

The mechanisms for the prolonged FSR following exercise and nutrition (protein or amino acids) are unclear. One suggestion is that this could occur through early hyperactivation of the "leucine threshold" during a short time frame. West and colleagues [234] gave 25g of whey protein to demonstrate that the rapid digestion rate of whey protein given through a single bolus was more beneficial for stimulating muscle protein synthesis than repeated small boluses. This theory is supported by the work from our laboratory demonstrating that excess leucine provides further stimulation in the anabolic machinery [230]. Further, extra leucine given with a large bolus of amino acids was capable of stimulating FSR out into the late (3-6hr) period following resistance exercise [41]. Given that enough substrate is present [111], the increased Late (3-5hr) FSR response with Whey protein can occur without concomitant hyperaminoacidemia [234] in the later time periods, which suggests a strong early signal as a mechanism. Similar to the trend shown elsewhere [65] we did not see this pattern following ingestion of whey contrary to other studies [233, 234]. However, we were able to demonstrate a prolonged effect with the PB similar to that seen with caseinate ingestion [65]. The discrepancy in the literature regarding the prolonged effect of whey may be a factor of the total protein or the leucine content. Previous studies gave 25g of whey protein (3g leucine, 11.5g EAA) [111, 234], whereas we and others [65] gave approximately 17.7g of whey protein containing (~1.9g leucine and ~8.8g EAA) a dose previously demonstrated to produce a maximal response following exercise [233].

The prolonged FSR response with casein or our PB may occur through a continuous

and prolonged signal stimulating the mTORC1 pathway and translation initiation. Certainly, we saw similar patterns overall in mTORC1 cell signaling, yet only PB was able to prolong S6K1 phosphorylation, possibly because WP would have had a maximal signal about one hour following ingestion [234].

Regarding chronic exposure to supplementation of isolated protein sources following resistance exercise training, whey protein has tended to demonstrate advantages for muscle accretion in young healthy males [292, 305, 342, 343, 452]. The few studies with other protein sources have demonstrated that soy [342, 378, 453] or casein protein [377] is effective in stimulating muscle accretion in a variety of populations. There is a need for future research to test the efficacy of protein blends against whey protein supplementation for promoting muscle growth during exercise training.

CONCLUSION

In summary, our data and others [246] further support the use of a blended protein supplement following resistance exercise as compared to an isolated protein. A blended protein supplement containing sufficient essential amino acid content, several digestion rates and a prolonged aminoacidemia clearly promotes muscle protein synthesis during post-exercise recovery. Future applications of utilizing protein blends to promote or maintain muscle mass may include studies in aging and other muscle wasting clinical populations such as cancer patients where the use of blended protein has demonstrated a positive effect [454].

CHAPTER 3

Soy-dairy Protein Blend and Whey Protein Ingestion After Resistance Exercise Increases Amino Acid Transport and Transporter Expression in Human Skeletal Muscle[2]

INTRODUCTION

We have recently demonstrated a prolonged post-exercise aminoacidemia, mixed muscle protein synthesis rate (MPS), and mTORC1 signaling response with post-exercise ingestion of a soy-dairy protein blend [225]. Despite a significant increase in MPS with the protein blend at 3-5h post-exercise, there was no detectible difference ($p=0.12$) in mixed MPS between groups (whey versus blend). The purpose of the current study is to determine if different rates of digestion and subsequent prolonged changes in amino acid availability over time would create detectable differences in amino acid transport kinetics, mRNA expression, and myofibrillar protein synthesis during this later recovery period.

The combination of resistance exercise and increased amino acid availability is an effective and highly practical strategy for the promotion of skeletal muscle mass and strength [78, 253, 455, 456]. Resistance exercise and essential amino acids (EAA) or protein exert separate and combined effects on skeletal muscle protein synthesis (MPS) and mammalian/mechanistic target of rapamycin complex 1 (mTORC1) signaling [49,

[2] Soy-dairy protein blend and whey protein ingestion after resistance exercise increases amino acid transport and transporter expression in human skeletal muscle. Reidy PT, Walker DK, Dickinson JM, Gundermann DM, Drummond MJ, Timmerman KL, Cope MB, Mukherjea R, Jennings K, Volpi E, Rasmussen BB. J Appl Physiol (1985). 2014 Jun 1;116(11):1353-64.

54, 59, 69, 108, 252, 253, 356]. Interestingly, using stable isotopic methods, innovative studies demonstrated that resistance exercise in the fasted state and in combination with increased amino acid availability enhance the transport rate of amino acids from the circulation into the muscle cell [69, 253, 274].

Amino acid transporters facilitate amino acid flux across the muscle cell membrane to activate mTORC1 [457], which is thought to be essential in regulating muscle protein synthesis [458]. Changes in amino acid availability stimulate the system A amino acid transporter SNAT2/SLC38A2, the cationic amino acid transporter 1 CAT1/SLC7A1[459] and the system L amino acid transporter LAT1/solute-linked carrier (SLC)7A5 (which forms a heterodimer with CD98/SLC3A2) [229, 460, 461]. LAT1/SLC7A5 and SNAT2/SLC38A2 function cooperatively to transport large neutral amino acids into the cell [461, 462] whereas proton-assisted transporters (PAT) such as PAT1/SLC36A, are thought to play a role in stimulating protein synthesis after amino acids such as leucine reach sufficient quantities in the cell to activate mTORC1 [463, 464].

More recently, our laboratory has demonstrated that human skeletal muscle amino acid transporter expression, transport rates, mTORC1 activation and MPS is stimulated by the separate [140, 229] and combined [261] effects of exercise and EAA supplementation. Protein ingestion is also an effective means to increase amino acid supply and to augment the muscle protein anabolic response to exercise [108, 232, 233, 241, 465, 466]. However, proteins differ on the basis of digestion rate and composition of EAA, which together impact the metabolic fate (i.e., oxidation or incorporation into proteins) of the ingested protein source [292, 443, 467]. Although many protein sources are considered to be of high quality, their varying amino acid composition may influence

their amino acid transport in the gut [468] and also at the muscle membrane [214]. Thus, protein ingestion represents a unique means to study amino acid transporter function in humans. This is an exciting area of investigation, yet only one study has examined human skeletal muscle amino acid transporter expression following resistance exercise and dietary protein ingestion [466]. Although several studies have examined muscle protein net balance with consumption of dietary protein following resistance exercise [232, 246, 247, 254, 277, 278, 281], no study has examined how the ingestion of dietary protein after resistance exercise stimulates skeletal muscle amino acid transport rates during post-exercise recovery.

Amino acid transporters play a key role in muscle protein metabolism and activation of mTORC1 signaling by altering the delivery of substrate (amino acids) and/or by acting as a transporter/receptor (transceptor) of anabolic signaling [469, 470]. Because of the sensitivity of skeletal muscle amino acid transporters to amino acid availability [229] we sought to examine if the prolonged hyperaminoacidemia associated with the ingestion of a blend of plant (25% soy) and dairy (50% casein; 25% whey) proteins (with varying digestion rates) would prolong the skeletal muscle net protein balance across the leg (an indicator of overall muscle protein anabolism) as compared to rapidly digested whey and whether this would influence amino acid transporter expression and amino acid transport into muscle. We hypothesized that the prolonged hyperaminoacidemia from ingesting a blend of proteins would reduce markers of protein breakdown and enhance overall muscle protein anabolism, myofibrillar protein synthesis, amino acid transport into muscle, and amino acid transporter expression as compared to the ingestion of a rapidly digested protein.

Materials and Methods

Screening of Participants.

Sixteen healthy, young subjects (age range: 19-30y) participated in this double-blind, randomized clinical trial. Subject characteristics can be found in **Table 3.1.** The subjects were a subset of volunteers that participated in a previous study [225]; however, none of the data presented herein has been previously published. The participants were recruited through locally posted flyers, newspaper advertisements, and by word of mouth. The participants were healthy and recreationally active, but were not engaged in any regular exercise-training program (< 2 sessions high intensity aerobic or resistance exercise/week) at the time of enrollment. Screening of participants was performed on two separate days (>7 days apart) at the Institute for Translational Sciences-Clinical Research Center (ITS-CRC). The first screening day included 1 repetition maximum (1RM) strength testing, a clinical history, physical exam, and laboratory tests (complete blood count with differential, liver and kidney function tests, coagulation profile, fasting blood glucose, hepatitis B and C screening, HIV test, TSH, lipid profile, urinalysis, and drug screening). The second screening day included a second 1RM test and a dual-energy X-ray absorptiometry (DXA) scan (Hologic QDR 4500W, Bedford, MA) to measure lean and fat mass. A leg extension machine (Cybex-VR2, Medway, MA, USA) was used to establish a 1RM and the value was recorded as the highest weight lifted for a single repetition from the two testing days. All participants provided written informed consent before enrollment in the study. The study was approved by the Institutional Review Board of the University of Texas Medical Branch, and is in compliance with the Declaration of Helsinki as revised in 1983.

Table 3.1. Subject and Exercise Characteristics

Subject							
	N	**Age, y**	**BMI, kg·m^{-2}**	**% Fat**	**Lean Mass, kg**	**Leg Volume, L**	**Leg mass, kg**
Blend	8	22.3 ± 1.0	26.6 ± 0.8	23.9 ± 1.4	59.5 ± 2.5	10.9 ± 0.5	11.3 ± 0.5
Whey	8	23.6 ± 1.0	25.0 ± 1.3	25.1 ± 2.7	56.6 ± 3.0	10.4 ± 0.5	10.8 ± 0.6
Exercise							
		1RM, kg	**Total weight lifted, kg**		**% -1RM Mean**	**Time, min**	
Blend		124 ± 7	6265 ± 353		65 ± 1	26 ± 1	
Whey		126 ± 11	6302 ± 527		63 ± 1	27 ± 2	

Subject and exercise characteristics of participants randomized to receive Whey (N=8) or Blend (N=8) at 1h post-exercise. Mean ± SE.

Study Design

Subjects were admitted to the UTMB ITS-CRC at ~1700h the day prior to the study. Subjects were instructed to refrain from exercise at least 72h before admission. The subjects were given a standardized meal at 1900h prepared by the Bionutrition Division of the ITS-CRC with a macro-nutrient distribution of 20% protein, 60% carbohydrate, and 20% fat at 12 kcal/kg body weight. Subjects were provided water *ad libitum*. The subjects were randomized to ingest a soy-dairy protein blend (N=8) or whey protein (N=8) at 1h following a bout of high-intensity leg resistance exercise.

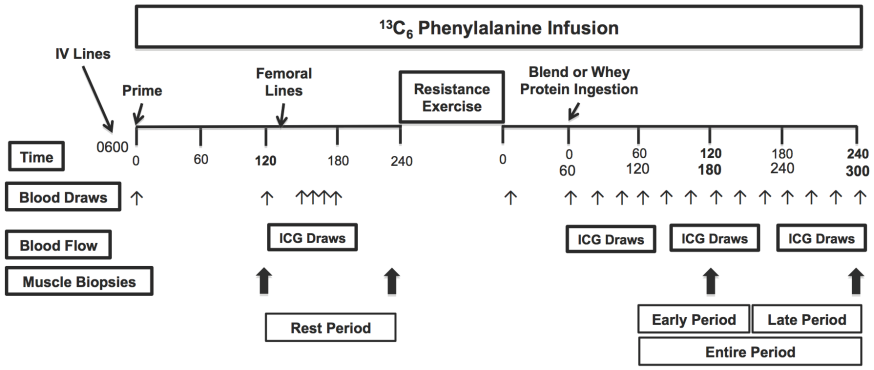
Experimental Protocol

All subjects underwent the stable isotope infusion protocol (**Fig 3.1**) at the same time of day (0600-1600h) on the day following admission. After an overnight fast (~10h), an 18 G polyethylene catheter was inserted into the antecubital vein, from which background blood draws for the measurement of phenylalanine concentration/enrichment and indocyanine green (ICG; Cardio-Green, Becton Dickinson and Co., Cockeysville, MD) concentration. This was followed by initiation of a primed, constant infusion (~10h) of L-[ring-$^{13}C_6$] phenylalanine (Sigma-Aldrich, St. Louis, MO, USA). The

priming dose for the labeled phenylalanine was 2 μmol/kg and the infusion rate was 0.05 μmol/kg/min. A retrograde catheter was inserted (0700-0800h) into a hand vein on the contralateral arm and arterialized blood was extracted with the use of a heating pad prior to sampling. A catheter was inserted (0900-1000h) into the femoral artery and vein (retrograde) of one leg for blood sampling. The femoral arterial catheter was also used for the infusion of ICG. At ~1030h a continuous infusion of ICG dye (0.5 mg/min) was started in the femoral artery and was maintained for 7 min to measure leg blood flow in each sampling period. Plasma ICG concentration was measured in blood samples during the resting period and several times following protein ingestion (see below) from the femoral and wrist veins. At approximately 2 and 4h following initiation of the infusion muscle biopsies were taken from the lateral aspect of the *vastus lateralis* for the determination of resting (Rest) intracellular phenylalanine enrichment and concentration. All biopsies were collected with a 5mm Bergström biopsy needle under sterile procedure and local anesthesia (1% lidocaine). Following femoral catheter placement and a series of blood draws the participants were moved to a leg extension machine (Cybex-VR2, Medway, MA, USA) for high-intensity resistance exercise consisting of eight sets of ten repetitions at 55% (set 1), 60% (set 2) 65% (set 3) and ~70% (sets 4-8) of the participants previously determined 1RM with three min rest between sets. Exercise characteristics can be found in **Table 3.1.** The nutritional supplements were ingested 1h following exercise. Two additional muscle biopsies were collected 2 and 4h after protein ingestion (corresponding to 3 and 5h after exercise) to represent Early and Late post-exercise periods (**Fig. 3.1**). The measurements taken during the 1-2, 2-3, 3-4, 1-2.5, 2.5-4 and 1-4h post-ingestion were averaged to represent the 2h, 3h, 4h, Early, Late and Entire

periods, respectively (**Fig 3.1**). The first, second, third and fourth muscle biopsies were sampled from two separate incisions on the same leg, respectively. To minimize multiple sampling in a given area, skin incisions were separated by ~7 cm while biopsies collected from the same incision were angled ~5 cm from each other. This method has been previously utilized in our lab [29, 101, 447] and others [65, 108, 190]**.** Muscle tissue was immediately blotted, frozen in liquid nitrogen and stored at -80^{o}C until analysis. Blood samples were collected before the infusion, during the resting and post-exercise/post-ingestion time periods (**Fig. 3.1**) for the determination of blood enrichment (see below) and amino acid concentration. The infusion study ended following the fourth muscle biopsy and participants were then given a standard meal.

Figure 3.1. Study Design



Protein Supplements

The protein beverages (Whey or Blend) were ingested at 1h post-exercise. The beverages were dissolved in 300 ml of water and enriched (8%) with L-[ring-$^{13}C_6$] phenylalanine to in an attempt to maintain isotopic steady state in arterialized blood. The composition of the beverages is similar to that we previously reported [225]. To match leucine and EAA content between the interventions, participants were given 0.305 or

0.337g total protein·kg^{-1} lean mass for Whey and Blend respectively. The amount of protein given in each group was based on the 8.6g of EAA in dietary (non-hydrolyzed) protein demonstrated to maximize the MPS response following resistance exercise [233]. The Blend consisted of 20.1±0.9g total protein (providing 1.9±0.1g leucine, 1.0±0.1g phenylalanine, 1.3±0.02g valine and 9.0±0.4g EAA) composed of 50% protein from sodium caseinate, 25% protein from whey protein isolate and 25% protein from soy protein isolate. Whey consisted of 17.3±0.9g of protein (providing 1.9±0.1g leucine, 0.6±0.1g phenylalanine, 1.1±0.01g valine and 8.7±0.5g EAA) composed of 100% whey protein isolate.

Phenylalanine Amino Acid Concentration, ICG, Lactate, Glucose and Insulin

Concentrations of phenylalanine (femoral artery and vein) were measured in the blood using gas chromatography-mass spectrometry (GCMS) as previously described using an internal standard [21, 448]. Plasma glucose and lactate concentration was measured using an automated glucose and lactate analyzer (YSI, Yellow Springs, OH) at rest, immediately post-exercise and 0, (at ingestion), 20, 40, 60, 80, 100, 120, 180 and 220 minutes post-ingestion. Serum concentrations of insulin were determined with an enzyme-linked immunosorbent assay (Millipore, St. Charles, MO) according to the manufacturer's instructions at rest, immediately post-exercise and 0, 20, 40, 60, 80, 100 and 120 minutes post-ingestion. The serum ICG concentration to determine leg blood flow was measured spectrophotometrically (Beckman Coulter) at λ = 805 nm [471]. The phenylalanine concentrations and blood flow measurements taken during the 1-2, 2-3, 3-4, 1-2.5, 2.5-4 and 1-4h post-ingestion were averaged to represent the 2h, 3h, 4h, Early, Late and Entire periods, respectively (**Fig 3.1**).

Amino Acid Parameters and Transport Rates

We calculated skeletal muscle amino acid transport rates from the enrichments and concentrations of phenylalanine in the femoral artery and vein and from the enrichment of muscle tissue-free phenylalanine, using amino acid kinetics modeling as previously described [458, 472]. Phenylalanine is used in this model because it is not oxidized by muscle, which allows for the calculation and measurement of amino acid net balance across the leg and MPS. The following amino acid parameters were measured:

delivery to the leg, $F_{in} = C_A \cdot BF$ (Eq 1)

release from the leg, $F_{out} = C_V \cdot BF$ (Eq 2)

net balance across the leg, $NB = (C_A - C_V) \cdot BF$ (Eq 3)

transport into muscle, $F_{M,A} = \{ [(E_M - E_V) / (E_A - E_M) \cdot C_V] + C_A \} \cdot BF$ (Eq 4)

transport from muscle, $F_{V,M} = \{ [(E_M - E_V) / (E_A - E_M) \cdot C_V] + C_V \} \cdot BF$ (Eq 5)

where, C_A and C_V are plasma phenylalanine concentrations in the femoral artery and vein, respectively; E_A, E_V, and E_M are phenylalanine enrichments (tracer/tracee ratio) in femoral arterial and venous plasma and in muscle, respectively; BF is leg blood flow. Data are presented per 100g leg lean mass. Similar values were obtained with correction by leg lean mass (from DXA) and leg volume (as demonstrated in [21]). Leg plasma flow was calculated from the steady state dye concentration values in the femoral and wrist vein as previously described [471, 473]. Leg blood flow was calculated by correcting the plasma flow by the hematocrit.

Muscle samples were processed as previously described [21], and muscle free tissue phenylalanine enrichments and concentrations were determined by GCMS. The

intracellular concentration of phenylalanine was then calculated from the tissue value, accounting for the ratio of intracellular to extracellular water [458].

Myofibrillar and Nuclear Protein Fraction Isolation

About 30–50 mg of frozen muscle tissue was placed in buffer [29] and homogenized (1:9, w/v) and centrifuged at 3,400 × *g* for 10 min at 4°C, followed by removal of the supernatant, which was used for western blotting for LAT1, SNAT2 and eEF2. The resulting pellet was then suspended in isolation buffer (1 M sucrose, 1 M Tris/HCl, 1 M KCl, 0.5 M EDTA, pH 7.4) containing protease and phosphatase inhibitors and centrifuged for 10 min at 4°C and 700 × *g*. After 3 series of PBS buffer suspensions and centrifugations at 15,000 × *g* for 5 min at 4°C, the pellet was re-suspended and agitated on ice for 2x20 min and in a 4°C sonication bath in high salt buffer (1:4, w/v). The slurry was centrifuged at 15,000 × *g* for 10 min at 4°C and the supernatant was taken as the nuclear extract which was assayed for protein concentration with the BCA protein assay (Pierce, Rockford, IL) and used for western blotting for activating transcription factor 4 (ATF4). The nuclear isolation was verified by examination of cytoplasmic and nuclear protein fractions run on the same gel and probed for antibodies specific to Histone H3 (for nuclear) and Hexokinase (for cytoplasmic). The resulting pellet was fully suspended in double distilled water and centrifuged at 15,000 × *g* for 5 min at 4°C. To precipitate the myofibrillar proteins, 1 ml of 0.3M NaOH was added to re-suspend the pellet and this heated at 50°C for 30 min with frequent vortexing. After centrifugation at 10,000 × *g* for 5 min at 4°C, the supernatant was collected and an additional 1 ml of 0.3M NaOH was added to re-suspend the pellet and this heated at 37°C for 10 min with frequent vortexing. After centrifugation at 10,000 × *g* for 5 min at 4°C, the supernatant

was collected and the collagen pellet was discarded. Precipitate was created by addition of 1 ml PCA to the collected supernatant and pelleted at 805 × *g* for 10 min at 4°C. This pellet was washed 2x with 70% ethanol and then hydrolysed overnight in 1.5 ml 6M HCL.

Western Blot Analysis

Western blot analysis was conducted as described previously [140]. Immunoblot data were normalized to an internal loading control, which was loaded on all gels for comparison across blots, and data are adjusted to represent fold change from basal. Antibodies utilized were LAT1/SLC7A5 (ab85226, Abcam, Cambridge, MA), SNAT2/SLC38A2 (Santa Cruz Biotechnologies, Santa Cruz, CA), ATF4 (Santa Cruz Biotechnologies, Santa Cruz, CA), Histone 3H (Cell Signaling), phospho-eEF2 (Thr-56) (Cell Signaling), total-eEF2 (Cell Signaling) and monoclonal alpha-tubulin (Sigma-Aldrich, St Louis, MO). LAT1/SLC7A5 and SNAT2/SLC38A2 were normalized to alpha-tubulin and ATF4 was normalized to Histone H3 to account for differences in loading.

Myofibrillar Protein Synthesis

Bound proteins from the myofibrillar fraction and muscle intracellular free amino acids were extracted from biopsy samples as described above. GCMS (GCMS, 6890 Plus CG, 5973N MSD, 7683 autosampler, Agilent Technologies, Palo Alto, CA) measurements were made to determine bound tracer enrichments for L-[ring-$^{13}C_6$] phenylalanine as previously described [21]. Using the external standard curve approach [20], muscle myofibrillar protein-bound phenylalanine enrichment was analyzed by

GCMS after protein hydrolysis and amino acid extraction [29, 300], We calculated myofibrillar protein synthesis as fractional synthesis rate (FSR) by measuring the incorporation rate of the phenylalanine tracer into the proteins (Δ protein bound enrichment over time) and using the precursor-product model to calculate the synthesis rate:

$$FSR = (\Delta E_p / t) / [(E_{M(1)} + E_{M(2)}) / 2] \bullet 60 \bullet 100$$

where ΔE_p is the increment in protein-bound phenylalanine enrichment between two sequential biopsies, t is the time between the two sequential biopsies, and $E_{M(1)} + E_{M(2)}$ are the phenylalanine enrichments in the free intracellular pool in the two sequential biopsies. Due to lack of tissue we were only able to calculate resting FSR with (N=4) in each group. Data are expressed as percent per h (%/ h).

RNA Extraction and Semiquantitative real-time PCR

RNA isolation, cDNA synthesis, and real-time qPCR were performed as we have previously described [229]. Total RNA was isolated by homogenizing 10-20 mg tissue with a hand-held homogenizing dispenser (T10 Basic Ultra Turrax, IKA, Wilmington, NC) in 1 ml of Tri reagent. The RNA was separated into an aqueous phase using 0.2 ml of chloroform and subsequently precipitated from the aqueous phase using 0.5 ml of isopropanol. RNA was washed with 1 ml of 75% ethanol, air-dried, and suspended in a known amount of nuclease-free water. RNA concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE) and RNA was DNase-treated using a commercially available kit (DNA-free, Ambion, Austin, TX). A total of 1 μg of RNA was reverse transcribed into cDNA according to the directions provided by the manufacturer (iScript, BioRad, Hercules, CA). Real-time

qPCR was carried out with an iQ5 Multicolor Real Time PCR cycler (BioRad). cDNA was analyzed with SYBR green fluorescence (iQ SYBR green supermix; BioRad). Primer sequences for genes of interest (LAT1/SLC7A5, CD98/SLC3A2 SNAT2/SLC38A2, PAT1/SLC36A1, CAT1/SLC7A1) have been previously published [229]. β_2-Microglobulin was utilized as a normalization/housekeeping gene. Relative fold changes were determined from the Ct values using the $2^{-\Delta\Delta Ct}$ method [474].

Statistical Analysis

All outcomes were assessed using standard ANOVA and ANCOVA models. With baseline as a covariate, an ANCOVA model for each outcome was used to determine possible differences between groups at each time point. To test marginal outcomes and differences across time points, a repeated measures ANOVA model was used in which a random-intercept model was used to account for subject-to-subject variability. Pairwise comparisons were calculated and tested using standard post-hoc contrast methods. All pairwise comparisons were done using contrasts in the ANOVA model, with Tukey testing for post-hoc adjustment. Assumptions of normality and homogeneity of variance were tested, and transformations were used as necessary to make all tests reliable. All calculations were done in SAS, version 9.3.

RESULTS

Subject and Exercise Characteristics

The subjects were effectively randomized as their baseline and exercise characteristics (**Table 3.1**) were not different ($p > 0.05$).

Plasma glucose in the femoral artery and vein (data not shown) increased from Rest only immediately post-exercise and were not different ($p > 0.05$) between groups. Plasma lactate in the femoral artery and vein (data not shown) increased from Rest for the first hour post-exercise and was not different ($p > 0.05$) between groups. Serum insulin (**Table 3.2**) was not different ($p > 0.05$) between groups and showed a time effect for an increase ($p < 0.05$) at 20, 40, 60 min post-ingestion compared to Rest. Compared to Rest, insulin was increased ($p < 0.05$) at 20 and 40 min post-ingestion in Blend, 20, 40, 60 min post-ingestion in Whey and decreased 140 min post-ingestion in Whey.

Table 3.2. Serum insulin

	Time Post-Ingestion							
	Rest	**0**	**20**	**40**	**60**	**80**	**100**	**140**
Blend	29.1 ± 4.6	38.9 ± 11.5	66.1 ± 17.4*	77.5 ± 10.1*	48.9 ± 6.8*	31.4 ± 4.4	27.2 ± 3.7	19.6 ± 2.8
Whey	24.6 ± 2.3	30.0 ± 4.9	62.4 ± 11.0*	70.8 ± 16.8*	51.9 ± 9.2*	36.6 ± 6.8	23.9 ± 3.1	17.3 ± 2.7*

Serum insulin (pmol·L-1) at rest, immediately post-exercise and post-ingestion (min) for subjects given Whey (N=8) and a Blend (N=8) at 1h post-exercise. Mean ± SE. *$p < 0.05$ vs Rest.

Arterial, Venous and Muscle Intracellular Phenylalanine Concentration

Phenylalanine arterial concentration increased in Blend at Early, 2h, 3h and Entire and in Whey at Early and 2h compared to Rest ($p < 0.05$). Phenylalanine venous concentration increased in Blend at Early, 2h, 3h and Entire and in Whey at Early and 2h compared to Rest ($p < 0.05$). Intracellular phenylalanine concentrations were similar between groups and there was an overall effect for a decreased concentration at Late compared to Rest ($p < 0.05$). There were no differences between groups for arterial, venous and muscle intracellular phenylalanine concentrations (**Table 3.3**).

Table 3.3. Phenylalanine concentrations, leg blood flow and phenylalanine net balance

		Time Post-Ingestion					
		1-2.5h	2.5-4h	1-2h	2-3h	3-4h	1-4h
	Rest	Early	Late				Entire
Arterial							
Blend	57.2 ± 1.3	70.8 ± 2.0*[a]	60.0 ± 2.7	74.4 ± 2.4*[bc]	63.8 ± 2.6*[d]	58.5 ± 3.0	65.6 ± 1.9*
Whey	59.0 ± 2.6	64.0 ± 3.0*[a]	56.6 ± 2.9	68.1 ± 3.0*[bc]	57.5 ± 2.8	56.0 ± 3.1	60.6 ± 2.9
Venous							
Blend	62.6 ± 1.7	71.2 ± 1.8*[a]	63.0 ± 2.0	72.7 ± 2.2*[bc]	67.2 ± 1.8*[d]	61.3 ± 2.4	67.1 ± 1.3*
Whey	63.0 ± 2.5	66.3 ± 3.1*[a]	60.0 ± 2.7	70.0 ± 3.3*[bc]	60.8 ± 2.9	59.0 ± 2.7	63.6 ± 2.9
Blood Flow							
Blend	2.41 ± 0.22	3.47 ± 0.98	3.23 ± 0.49	3.57 ± 1.23	3.26 ± 0.59	3.27 ± 0.46	3.37 ± 0.73
Whey	2.66 ± 0.41	3.23 ± 0.52	3.79 ± 0.55	3.11 ± 0.45	3.65 ± 0.64	3.75 ± 0.57	3.50 ± 0.53
Net Balance							
Blend	-12.3 ± 1.5	2.3 ± 4.1*[a]	-8.0 ± 2.4	9.3 ± 5.7*[bc]	-8.6 ± 3.7	-8.1 ± 2.4	-2.3 ± 2.7*
Whey	-10.5 ± 1.8	-6.5 ± 2.8	-12.0 ± 4.3	-3.5 ± 4.4	-12.7 ± 4.5	-11.7 ± 4.5	-9.1 ± 2.8
Intracellular Muscle		**2h**	**4h**				
Blend	68.7 ± 2.8	77.0 ± 4.9	61.5 ± 3.0†				69.3 ± 3.2
Whey	70.5 ± 5.7	74.6 ± 9.7	60.4 ± 4.7†				67.5 ± 6.3

Femoral artery and vein blood and intracellular muscle phenylalanine concentration (nmol·ml^{-1}) leg blood flow (ml·min^{-1}·100 g leg muscle^{-1}) and phenylalanine net balance (nmol·min^{-1}·100 g lean leg mass^{-1}) across the leg at rest and post-ingestion for subjects given Whey (N=8) and a Blend (N=8) at 1h post-exercise. Values are Mean ± SE. Comparisons are: Rest vs. Early, Late, Entire, 2h, 3h & 4h; Early vs Late; 2h vs 3h vs 4h. *$p < 0.05$ vs Rest; †$p < 0.05$ main effect of time; [a]$p < 0.05$ Early vs Late; [b]$p < 0.05$ 2h vs 3h; [c]$p < 0.05$ 2h vs 4h; [d]$p < 0.05$ 3h vs 4h.

Phenylalanine Enrichment

Arterial tracer-tracee ratio (TTR) was elevated across both groups at Late, 3h, 4h and Entire compared to at Rest (effect of time: $p < 0.05$; **Table 3.4**). This effect was driven largely through an increased arterial TTR in Whey at all post-ingestion time points compared to Rest ($p < 0.05$). Venous TTR was elevated across both groups at all post-exercise time points compared to Rest (effect of time: $p < 0.05$). There was a group difference in venous TTR at 3h ($p < 0.05$). Muscle TTR was increased across both groups at all post-exercise time points compared to Rest (effect of time: $p < 0.05$) (**Table 3.4**). Overall these data only show minor perturbations in the steady state conditions at rest and post-exercise conditions, which permitted us to calculate amino acid transport into and out of leg muscle.

Table 3.4. Phenylalanine enrichments

		Time Post-Ingestion					
		1-2.5h	2.5-4h	1-2h	2-3h	3-4h	1-4h
	Rest	Early	Late				Entire
Femoral Artery							
Blend	7.92 ± 0.10	7.80 ± 0.19	8.02 ± 0.21	7.77 ± 0.16	7.87 ± 0.32	7.99 ± 0.23	7.88 ± 0.16
Whey	7.74 ± 0.15	8.30 ± 0.28*	8.42 ± 0.24*	8.18 ± 0.26*	8.45 ± 0.24*	8.42 ± 0.25*	8.35 ± 0.26*
Femoral Vein							
Blend†	5.78 ± 0.17	6.65 ± 0.18a	6.62 ± 0.11	6.75 ± 0.18	6.50 ± 0.18	6.68 ± 0.12	6.64 ± 0.1
Whey†	5.97 ± 0.20	7.00 ± 0.15a	7.09 ± 0.21	6.92 ± 0.16	7.14 ± 0.21#	7.06 ± 0.20	7.04 ± 0.18
Intracellular Muscle							
Blend†	4.50 ± 0.22	5.95 ± 0.14*	5.98 ± 0.07*	5.93 ± 0.11*	5.95 ± 0.06*	6.02 ± 0.11*	6.00 ± 0.06*
Whey†	4.69 ± 0.21	6.18 ± 0.18*	5.95 ± 0.28*	6.13 ± 0.20*	6.01 ± 0.25*	5.88 ± 0.32*	6.10 ± 0.29*

Phenylalanine enrichments as tracer to tracee ratio (%) at rest and post-ingestion for subjects given Whey (N=8) and a Blend (N=8) at 1h post-exercise. Values are Mean ± SE. Comparisons are: Rest vs. Early, Late, Entire, 2h, 3h & 4h. *$p < 0.05$ vs Rest; #$p < 0.05$ group effect at time point; †$p < 0.05$ main effect of time.

Amino Acid Transport Rates, Net Balance and Transporter mRNA Expression

Blood flow was not different between groups or across time ($p > 0.05$; **Table 3.3**). Phenylalanine Net Balance across the entire leg became positive at Early and 2h and was less negative at 3h in the Blend compared to Rest ($p < 0.05$). With the period analysis (**Table 3.3**), there was no change in Net Balance with Whey. In the point analysis (**Fig. 3.2**), the Net Balance became less negative at 0 min in Whey and was positive at only 20 and 40 min post-ingestion as compared to Rest ($p < 0.05$).

Figure 3.2. Phenylalanine net balance

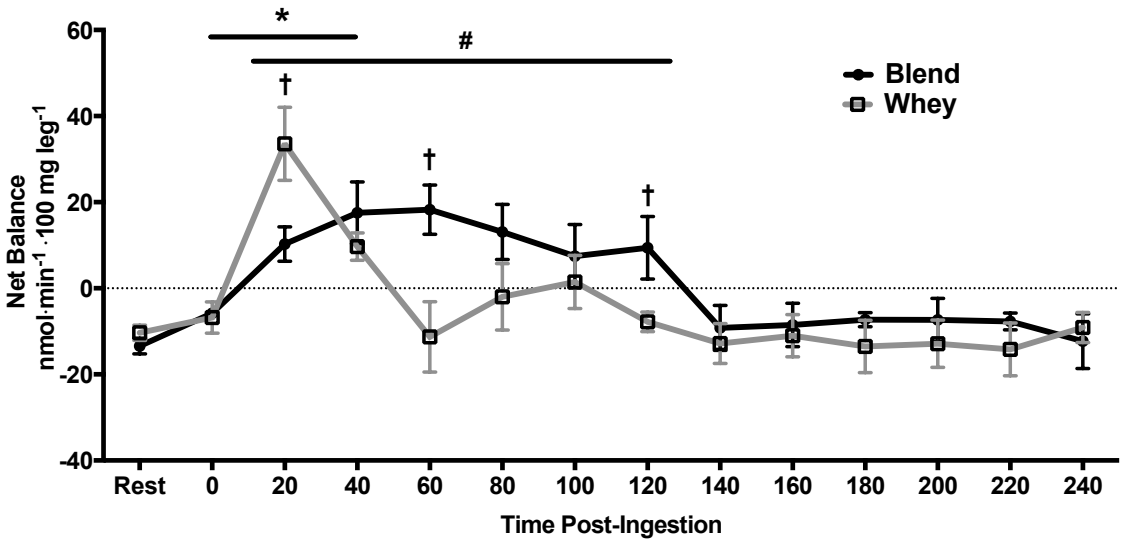


Fig 3.2. Phenylalanine net balance at Rest and during 4h post-ingestion for subjects given Whey (N=8) and a Blend (N=8) at 1h post-exercise. Mean ± SE: *Whey vs. rest, $p < 0.05$; #Blend vs. rest, $p < 0.05$; Blend vs. Whey, †$p < 0.05$.

With the Blend, the Net Balance became positive at 20, 40, 60, 80, 100 and 120 min post-ingestion compared to Rest ($p < 0.05$). This positive net balance caused an overall time effect at 20, 40, 60, 80, 100 and 120 min post-ingestion compared to Rest ($p < 0.05$) in the Blend. There was a group difference and a more positive Net Balance in Blend than

Whey at 60 and 120 min post-ingestion and a group difference and a more positive Net Balance in Whey than Blend at 20 min post-ingestion ($p < 0.05$). Phenylalanine delivery to the leg was not different between the groups and there was an overall time effect at Early ($p = 0.058$), Late, 3h, 4h and Entire compared to Rest ($p < 0.05$) (data not shown). Phenylalanine release from the leg was not different between the groups and there was an overall time effect at Late, 3h ($p = 0.054$), 4h and Entire ($p = 0.053$) compared to Rest ($p < 0.05$) (data not shown).

For both groups combined, inward transport of phenylalanine into leg muscle increased at Early, Late, 2h and 3h compared to Rest (effect of time: $p < 0.05$). Phenylalanine inward transport increased in Blend during Early, 3h and Entire and in Whey during Early, 2h and Entire compared to Rest ($p < 0.05$). There were no group differences ($p > 0.05$). For Blend, outward transport of phenylalanine increased at Early ($p = 0.050$), Late, 2h and 3h ($p = 0.056$) compared to Rest (effect of time: $p < 0.05$). Phenylalanine outward transport increased in Blend during the Early, 3h and Entire and in Whey during Early, 2h and Entire compared to Rest ($p < 0.05$). There were no group differences (**Table 3.5, Fig 3.3 A-B**).

Table 3.5. Phenylalanine transport by hour

		Time Post-Ingestion		
	Rest	**1-2h**	**2-3h**	**3-4h**
Inward Transport				
Blend	83.0 ± 12.1	115.2 ± 17.5	178.4 ± 53.5*	124.1 ± 23.2
Whey	82.1 ± 12.9	124.8 ± 14.2*	104.6 ± 18.4	117.8 ± 30.1
Outward Transport				
Blend	92.3 ± 13.1	105.9 ± 15.5	187.0 ± 52.7*	132.2 ± 23.8
Whey	92.7 ± 14.0	128.3 ± 14.3*	117.3 ± 20.2	129.4 ± 29.3

Phenylalanine transport rates (nmol·min-1·100 g lean leg mass-1) across the muscle membrane at rest and post-ingestion for subjects given Whey (N=8) and a Blend (N=8) at 1h post-exercise. Values are and Mean ± SE. Comparisons are: Rest vs. Early, Late, Entire, 2h, 3h & 4h. *$p < 0.05$ vs Rest.

Figure 3.3. Phenylalanine transport by early, late and entire period

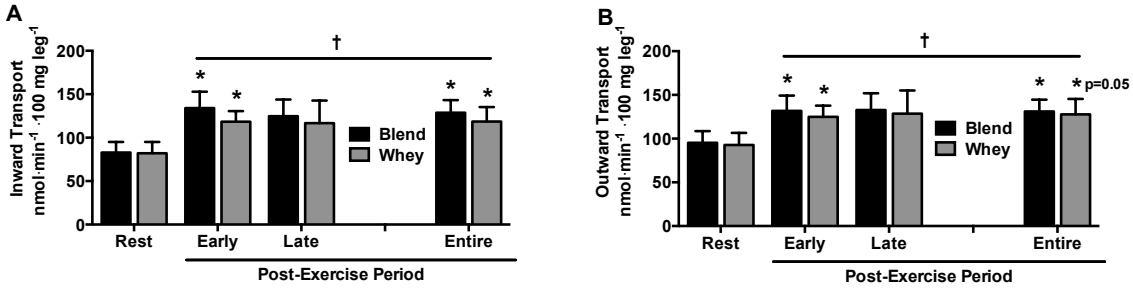


Fig 3.3.Phenylalanine inward (A) and outward (B) transport averages during Rest, Early, Late and Entire periods for subjects given Whey (N=8) and a Blend (N=8) at 1h post-exercise. Mean ± SE: †effect of time, $p < 0.05$; *different from rest, $p < 0.05$.

CD98/SLC3A2, PAT1/SLC36A1 and CAT1/SLC7A1 mRNA expression were elevated at 2 and 4h post-ingestion as compared to Rest for both groups ($p < 0.05$). LAT1/SLC7A5 mRNA expression was elevated ($p < 0.05$) at 2 and 4h post-ingestion as compared to Rest for Blend and only at 2h for Whey. However, there was a trend ($p = 0.06$) for LAT1/SLC7A5 mRNA expression to be elevated 4h post-ingestion as compared to Rest in Whey. SNAT2/SLC38A2 mRNA expression was elevated at 2h post-ingestion as compared to Rest for both groups ($p < 0.05$). CAT1/SLC7A1 mRNA expression was greater at 4h than at 2h post-ingestion for both groups ($p < 0.05$). With Whey only PAT1/SLC36A1 mRNA expression was greater at 4h than at 2h post-ingestion ($p < 0.05$; **Fig 3.4 A-E**).

Figure 3.4. mRNA expression of select amino acid transporters

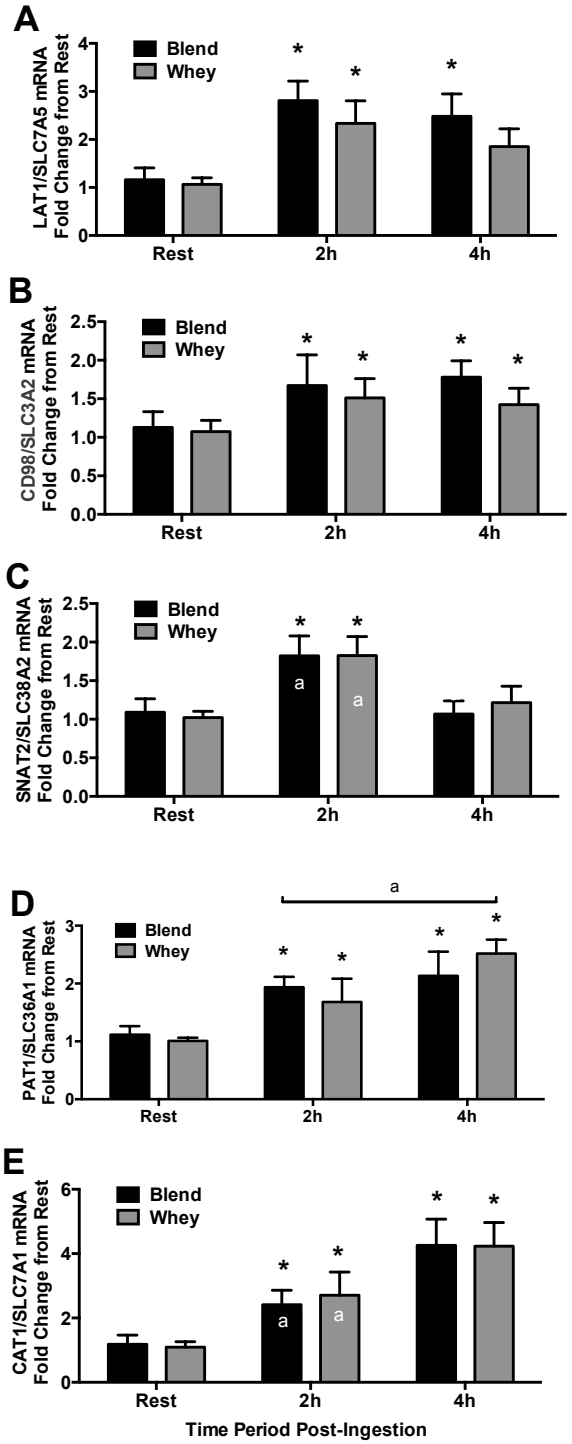


Fig 3.4. mRNA expression of LAT1/SLC7A5(A), CD98/SLC3A2(B), SNAT2/SLC38A2(C), PAT1/SLC36A1(D) and CAT1/SLC7A1(E) during Rest, Early, and Late periods for subjects given Whey (N=8) and a Blend (N=8) at 1h post-exercise. Mean ± SE: *different from rest, $p < 0.05$; a2h vs 4h, $p < 0.05$.

LAT1 and SNAT2 protein expression was not different ($p > 0.05$) from Rest at any time point or between groups (**Table 3.6)**. Nuclear ATF4 protein expression (a known regulator of amino acid transporter expression) was not different ($p > 0.05$) between groups and was only elevated ($p < 0.05$) from Rest in the Blend at 2h post-ingestion (**Table 3.6)**.

Table 3.6. Protein expression of LAT1, SNAT2, ATF4 and eEF2

	Time Post-Ingestion	
	2h	**4h**
LAT1		
Blend	1.12 ± 0.11	0.81 ± 0.18
Whey	1.23 ± 0.36	1.19 ± 0.39
SNAT2		
Blend	1.22 ± 0.28	1.10 ± 0.31
Whey	1.20 ± 0.36	1.45 ± 0.60
Nuclear ATF4		
Blend	2.07 ± 0.77*	0.77 ± 0.11
Whey	1.28 ± 0.30	1.39 ± 0.48
p/t eEF2		
Blend	0.66 ± 0.18*	0.56 ± 0.06*
Whey	0.59 ± 0.08*	0.74 ± 0.12

Fold change from rest of protein expression of LAT1, SNAT2, ATF4 and eEF2 in the hours post-ingestion for subjects given Whey (N=8) and a Blend (N=8) at 1h post-exercise. Mean ± SE. *$p < 0.05$ vs Rest. ATF4 is presented as N=7 in each group.

Myofibrillar Protein Synthesis and Markers of Protein Turnover

We have previously shown that both Blend and Whey increase mixed muscle protein synthesis and mTORC1 signaling to a similar extent following resistance exercise [225]. However, to confirm that no group differences occurred during post-exercise recovery we compared post-exercise myofibrillar protein synthesis rates between Blend and Whey, and whether other markers of protein synthesis (eEF2 phosphorylation) and

breakdown (MAFbx and MuRF-1 mRNA) differed between groups. Resting myofibrillar protein synthesis was not different ($p = 0.662$) between Whey (0.035 ± 0.011 %/h) and Blend (0.0413 ± 0.008 %/h) so we pooled the resting data. Post-exercise myofibrillar protein synthesis increased above resting values in both groups ($p<0.05$) and was not different ($p = 0.333$) between Whey (0.093 ± 0.007 %/h) and Blend (0.081 ± 0.009 %/h) (**Fig 3.5**).

Figure 3.5. Skeletal muscle myofibrillar fractional synthetic rate

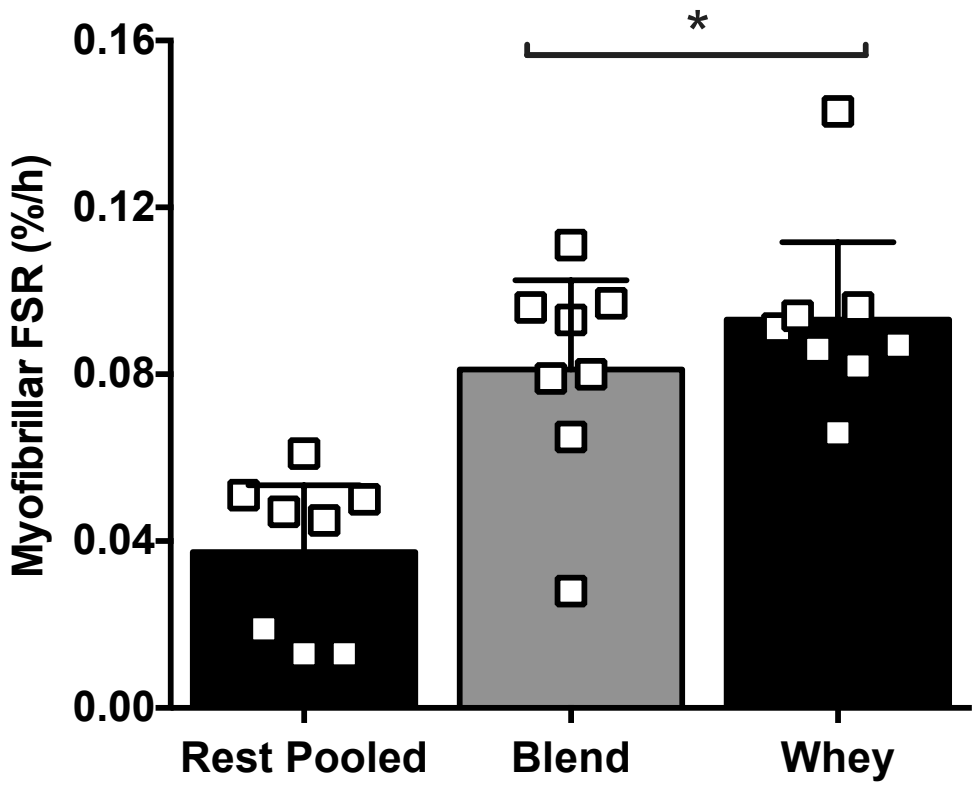


Fig 3.5. Skeletal muscle myofibrillar fractional synthetic rate in the *vastus lateralis* at rest (pooled from Whey (N=4) and a Blend (N=4) and during the post-exercise recovery period for subjects given Whey (N=8) and a Blend (N=8) at 1h post-exercise. Mean ± SE.

Phosphorylated eEF2 was not different ($p > 0.05$) between groups, but was reduced ($p < 0.05$) at 2 and 4h post-ingestion with the Blend, but only at 2h with Whey **(Table 3.7)**. mRNA expression of MuRF-1 was increased at 2h in Blend and 4h post-ingestion in both Whey and Blend compared to Rest ($p < 0.05$). There were no group differences for either MAFbx or MuRF-1 mRNA expression (**Fig 3.6 A-B**). mRNA expression of MAFbx was unaltered compared to Rest in both groups ($p < 0.05$). Representative immunoblots for protein expression data are shown in Appendix **Fig A.3.1**.

Figure 3.6. mRNA expression of MURF-1 and MAFbx

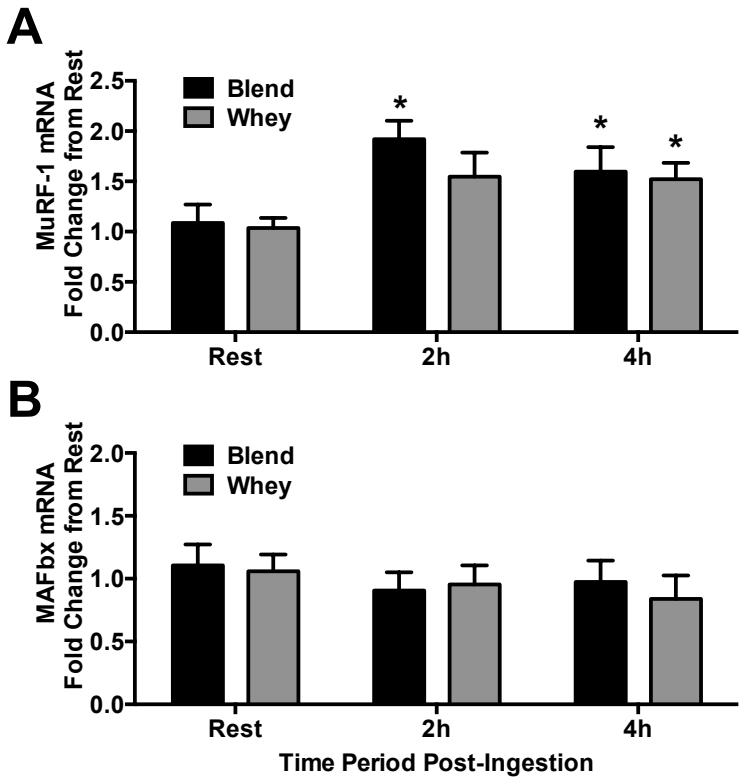


Fig 3.6. mRNA expression of MURF-1 (A) and MAFbx (B) during Rest, Early, and Late periods for subjects given Whey (N=8) and a Blend (N=8) at 1h post-exercise. Mean ± SE: *different from rest, $p < 0.05$.

DISCUSSION

In the current study, we utilized arterial and venous femoral catheterization of the leg and *vastus lateralis* muscle biopsies during infusion of a stable isotopically labeled amino acid tracer to comprehensively assess several measures of skeletal muscle amino acid transport and muscle protein anabolism in the post-exercise recovery period following the ingestion of a soy-dairy protein blend (Blend) or whey (Whey). Importantly, we examined how post-exercise protein ingestion impacts the immediate post-exercise recovery transport kinetics and also the adaptive response to expand the amino acid transporter machinery (mRNA expression). We report two novel findings: 1) increased post-exercise phenylalanine net balance (i.e., an indicator of overall muscle protein anabolism) across the leg was prolonged with Blend ingestion during the acute post-exercise recovery phase (0-2h post-ingestion) as compared to Whey; and 2) dietary protein ingestion of Blend and Whey increased post-exercise amino acid (phenylalanine) transport into muscle and mRNA expression of amino acid transporters associated with the regulation of mTORC1 signaling and muscle protein synthesis.

Similar to studies with resistance exercise and/or amino acids [69, 252, 253, 255, 258, 260], we observed increased amino acid flux across the muscle cell membrane with the post-exercise ingestion of dietary protein. As predicted, the prolonged aminoacidemia in the Blend delayed the amino acid flux to its highest point, 2-3hr post-ingestion, whereas in Whey it was highest at 1-2 hour post-ingestion. However, both groups experienced a similar increase when the values where averaged over the 1-2.5h and 1-4h periods, which is probably why we did not detect differences between groups in MPS. This transport data and the slight differences in mTORC1 signaling [225] suggest

that although the end result (MPS) could be the same, the mechanism to stimulate MPS may be different. The magnitude of amino acid transport rate was less with dietary protein compared to previous studies using crystalline amino acids [252, 253, 255, 258, 260]. Interestingly, Biolo et al. were not able to detect an increase in phenylalanine transport at a similar time following resistance exercise in the fasted state [69], however, they did demonstrate increased transport of lysine, leucine and alanine. In a follow-up study [253], these investigators provided an infusion of amino acids in a similar post-exercise recovery period and significantly increased amino acid concentrations, particularly phenylalanine, to twice the amount demonstrated in this study with ingestion of dietary protein. This suggests that phenylalanine transport is an effective means to assess the enhanced post-exercise protein anabolic response of exogenous amino acids. Further support for this concept, by the same researchers, demonstrated that insulin infusion alone following resistance exercise was insufficient to stimulate phenylalanine transport [274]. Although, insulin is thought to independently stimulate amino acid transport [475, 476], these reports suggest that post-exercise insulin action on muscle protein synthesis and amino acid transport requires excess amino availability in human skeletal muscle [448, 477]. Even with these differences in magnitude of the response, we arrive at a similar conclusion - that the increased amino acid availability (from dietary protein) following exercise is likely driving the increased phenylalanine transport in this model.

Changes in amino acid availability stimulate SNAT2/SLC38A2, CAT1/SLC7A1 LAT1/SLC7A5 and CD98/SLC3A2 [229, 459-461]. We found an increase in mRNA expression, from rest, of select amino acid transporters, (LAT1/SLC7A5, CD98/SLC3A2,

SNAT2/SLC38A2, PAT1/SLC36A1and CAT1/SLC7A1), concomitant with increased mTORC1 signaling and MPS at 3 and 5h of recovery from resistance exercise coupled with whey or protein blend ingestion 1h post-exercise. As compared to the previously examined fasted state post-exercise response in young adults [140], we see increases in SNAT2/SLC38A2, PAT1/SLC36A1, CD98/SLC3A2 and CAT1/SLC7A1 at 3h post-exercise (2h post-ingestion) with Whey and Blend. This further supports the sensitivity of these amino acid transporters to amino acid availability and their possible role in promoting MPS. By 5h post-exercise (4h post-ingestion) we demonstrated similar values to the fasted study [140] indicating that the prolonged amino acid availability and mTORC1 signaling in the blend or the strong initial anabolic signal from whey did not cause further stimulation via this mechanism. We have previously reported that a combination of essential amino acids and exercise [216] stimulate increased expression of similar amino acid transporters (LAT1/SLC7A5, SNAT2/SLC38A2), but not increased CD98/SLC3A2 and PAT1/SLC36A1 mRNA expression as we show in this study. This may be a factor of a difference in the level (20g EAA vs. ~9g) and type (EAA only vs. EAA and NEAA) of amino acid availability. CAT1/SLC7A1 expression tends to be greater at 3h post-exercise with protein ingestion compared to fasting recovery [140] or 20g EAA [216], which could be due to the NEAA in the ingested protein.

A recent study showed that 25g of whey protein ingestion following resistance exercise increased skeletal muscle amino acid transporter expression above rest at similar time points [466] compared to our study, however, the fold changes reported in that study were approximately double what we found in our study. Given the sensitivity of these transporter mechanisms to amino acid availability and muscle contraction [69, 216,

253, 478] this may be a reflection of the different dose of protein (25g Whey from [466] vs 20.1g Whey vs 17.3g Blend) or the overall content of leucine (3g from [466] vs 1.9g) ingested. Preliminary data from our laboratory suggest that when subjects ingest 10 grams of EAA with low (1.8g) vs high (3.5g) amounts of leucine the high leucine group exhibited greater stimulation of skeletal muscle amino acid transporter expression (unpublished observations). Interestingly, LAT1/SLC7A5 expression appears to be ~1-2 fold higher 3h post-exercise (2h post-ingestion) when 20g leucine-enriched EAA are ingested following resistance exercise [216] compared to fasted conditions [140] or here with protein ingestion suggesting the higher leucine content may be driving this response. Thus it may be that leucine content of a protein source is a key regulator of amino acid transporter expression. In addition, it is likely that increases in amino acid transporter protein expression occurred beyond the 5h post-exercise time point as observed in our previous resistance exercise study [229]. Amino acid transporter mRNA expression and amino acid transport kinetics are loosely linked outcomes during the short time frame of our acute study. We propose the changes in mRNA expression and eventual increases in protein expression are likely to have an impact when the muscle is exposed to a subsequent increase in amino acid availability (i.e, the next meal). More research in this area is needed as very little is known regarding the kinetics and functional relevance of amino acid transporters in human muscle biology.

The molecular mechanisms driving the increase in amino acid transporter expression are poorly understood. It has been suggested, from data collected in cell culture studies, that the nuclear transcription factor, AFT4, regulates gene expression of select amino acid transporters [475, 476] in conditions of amino acid deprivation [475],

overabundance [479] and presence of insulin [475, 479]. However, this relationship is not as pronounced in human skeletal muscle under physiological conditions of crystalline amino acid ingestion [229] or following resistance exercise in the fasted state [140]. Here we demonstrate nuclear ATF4 to slightly increase 2h post-ingestion of the Blend, which *may play a role* in promoting the increase in amino acid transporter gene expression. We did not see this same response in Whey, which may be a factor of the biopsy sampling time. Further evidence is needed to determine the role of ATF4 or other transcription factors (e.g. GCN2) in regulating amino acid transporter expression in human skeletal muscle in response to muscle contraction or amino acid availability.

As with previous studies [232, 247, 277, 278, 281], we also demonstrated that whey protein exhibits a rapid increase in amino acid net balance that is short-lived, returning to resting values around the first hour following post-exercise ingestion. As a novel feature, in this study, we demonstrated that the Blend had a less rapid rise in net balance across the leg, but was able to prolong a positive net balance to 2h post-ingestion. Additionally, the net balance in the Blend was greater than Whey at 60 and 120 min post-ingestion. This difference between groups could reflect a transient increase in the intracellular AA pool, potentially be due to a greater reduction in breakdown, in the blend, during 1-2h post-ingestion, which we unfortunately could not accurately assess due to the confounding influences of recent exercise and amino acid flux perturbations. This prolonged net balance is likely due to the intermediate digestion of soy and the prolonged digestion of casein. This prolonged hyperaminoacidemia is not just specific to phenylalanine (which had a slightly higher content in the blend), but valine as we have previously reported [225]. This suggests that a similar effect on amino acid net balance

could be occurring with other amino acids besides phenylalanine. As external support of our net balance data, milk [246, 247] or the slowly digested casein [281] can also prolong net balance up to 2h post-ingestion. A previous study demonstrated that a blend of fast (whey) and slowly (casein) digested proteins provided as fat-free milk had a prolonged post-exercise net balance as compared to a single protein provided as soymilk [246]. This provides further evidence that combining proteins with varying digestion rates can sustain the post-exercise net protein balance.

We also examined two key markers of muscle protein breakdown – the E3 ligases MuRF-1 and MAFbx. We found similar expression patterns for both atrogenes with Blend or Whey ingestion 3 and 5h following resistance exercise. Although MuRF-1 was up regulated in both groups, the expression level was ~ 2 fold less than what we have reported following resistance exercise in the fasted state [187]. As mentioned earlier, in reference to early net balance differences, any potential difference in breakdown between the groups may have occurred sometime before 3h post-exercise (2h post-ingestion). Thus after this time, the mRNA data suggest that the additional amino acid supply and/or equivalent insulin stimulus in both Blend and Whey were effective in reducing markers associated with post-exercise muscle protein breakdown which may also be an important part in the overall muscle protein turnover response to exercise combined with post-exercise protein intake.

Similar to our previous report with mixed muscle protein synthesis [225] we found no difference in post-exercise myofibrillar protein synthesis between Whey and Blend. As we have previously suggested [225], a blend of proteins with different digestion rates and prolonged aminoacidemia may have a different cellular response, but a similar effect

(MPS) compared to a bolus of whey protein when matched for leucine. In the protein blend (soy, whey and casein) it seems likely that there is an initial anabolic signal generated with initial whey digestion, albeit weaker than only a whey bolus, that is prolonged with stimulation from slower released soy and casein. These post-exercise rates of myofibrillar protein synthesis are comparable to those reported elsewhere for dietary protein ingestion following resistance exercise (37). Given the divergent results regarding muscle protein anabolism between net phenylalanine balance across the leg and *vastus lateralis* protein synthesis, it is important to note key differences in these methods. 1) The temporal differences in myofibrillar protein synthesis were assessed during the later period of recovery, 2-4h post-ingestion at a time when net balance was similar between groups; 2) Net balance assesses uptake of phenylalanine in all the muscles of the leg irrespective of the potential protein(s) being synthesized, which experience different or in some instances no activation with exercise, whereas the precursor product assessment of myofibrillar protein synthesis is only specific to the activity of that protein fraction in the *vastus lateralis*. Some of the mechanisms for the post-exercise muscle protein anabolism with protein blend ingestion are likely increased translation initiation, as we have reported [225], but also increased translation elongation as suggested by the decreases in eEF2 phosphorylation demonstrated in this study. These data offer further support for the hypothesis that a blend of protein with different digestion rates and prolonged aminoacidemia may have a different cellular response, but a similar effect (MPS) to that of rapidly digested whey.

When researchers supply a dose of protein well above the leucine threshold the amount of leucine probably has little additional effect on rates of MPS, which have already been

maximized [233]. On the other hand, because we did not oversupply protein (~20g protein; 9g EAA), we believe that matching the leucine content is essential in our investigation as leucine content plays a very important role in regulating MPS. Two recent studies have elegantly demonstrated that the leucine content in a supplement is a primary stimulator of MPS, especially when the total protein or content of other amino acids is low [217, 302]. By matching the proteins according to leucine content we ended up with a difference in total protein and calories between the ingested proteins. The difference in total protein ingested (<3g) was very minimal and is mostly composed of NEAA, which does not stimulate muscle protein synthesis. We have demonstrated that adding 120 kcals does not further stimulate muscle protein anabolism when sufficient EAA are provided [301]. Thus, the 10-20 kcal difference in total energy (in this study) is unlikely to have influenced the response.

Limitations to the study are as follows; 1) We did not assess the kinetics of other amino acids following resistance exercise, which could be variable [69]. 2) Due to the challenges of maintaining an isotopic steady state with multiple perturbations in kinetic parameters following the combination of exercise and dietary protein ingestion, we could only assess a later (2-5 h) post-exercise period, not the immediate post-exercise period (0-2 h) without violating the assumptions of our stable isotopic model for calculating amino acid transport rates. As such, this only allowed us to accurately calculate the transport model parameters of inward and outward transport. However, the measurement of the rate of amino acid transport into leg muscle is the focus of our study and a novel means to investigate the effects of dietary protein.

CONCLUSION

In summary, we found that the increase in post-exercise phenylalanine net balance across the leg (an indicator of muscle protein anabolism) was prolonged with ingestion of a protein blend compared to whey protein. We also report that ingesting a protein blend or whey protein enhances the rate of amino acid transport into muscle, increases select amino acid transporter (LAT1/SLC7A5, CD98/SLC3A2, SNAT2/SLC38A2, PAT1/SLC36A1, CAT1/SLC7A1) mRNA expression, and increases post-exercise myofibrillar protein synthesis. These results provide further support for the efficacy of ingesting a protein blend to increase and prolong post-exercise muscle protein anabolism. Further research is necessary to determine the efficacy of protein blend supplementation on muscle growth and strength during chronic resistance exercise training.

CHAPTER 4

The Effect of Soy-Dairy Protein Blend Supplementation during Resistance Exercise Training.

INTRODUCTION

Increased muscle size and strength are two of the many benefits of resistance exercise-training (RET) [320-323]. Many acute molecular and metabolic investigations claim the additive anabolic effect of protein/ amino acid supplementation following an acute RE session [43, 47, 52, 54, 56, 64, 65, 232, 240, 253], yet there is less certainty whether chronic protein supplementation during RET enhances muscle growth as compared to RET without protein supplementation [324, 349, 353]. Although, meta-analysis has determined an additive effect of protein supplements to independently enhance muscle size and strength [324], this effect is not universal [349, 353]. This incongruity may stem from dissimilarities in study design, choice and measurement of outcomes, target populations, exercise-training protocols and the timing, source and amount of the protein and/or placebo supplement [324, 347-349, 353].

Investigation of the most effective protein source for this enhancement has prompted acute [65, 189, 225, 232, 235, 238, 239, 241, 245] and chronic [333, 339, 342, 375, 377, 378, 385] clinical trials. Several isotopic tracer studies have clearly demonstrated that the rapid digestion rate and high leucine content of whey protein are two primary factors driving the protein anabolic response following post-exercise ingestion [111, 113, 234, 238, 239, 241, 245]. Further, some studies [113, 238, 239, 245,

246], but not all [65, 151, 235, 241], have suggested that whey protein is the gold standard compared to other high quality protein sources, soy or casein. A soy-dairy protein blend containing 25% whey protein, 25% soy protein and 50% caseinate demonstrated that when matching leucine content to whey protein, at a 1-2 g expense of more total protein, these supplements induce similar increases in mTORC1 signaling, mixed-muscle and myofibrillar protein synthesis when ingested following resistance exercise [189, 225]. Interestingly, these similar effects were observed despite differences in aminoacidema and amino acid transport profile between treatments.

Our hypothesis is that a high quality protein supplement (soy-dairy protein blend), containing adequate leucine, will provide an enhancement of lean mass and strength over isocaloric placebo and will elicit comparable effects to whey protein supplementation during 12 weeks of RET.

MATERIALS AND METHODS

Screening of Participants.

We recruited healthy male participants for this double-blind, randomized clinical trial. Participant characteristics are shown in (Table 4.1).

Table 4.1. Baseline participant characteristics[1]

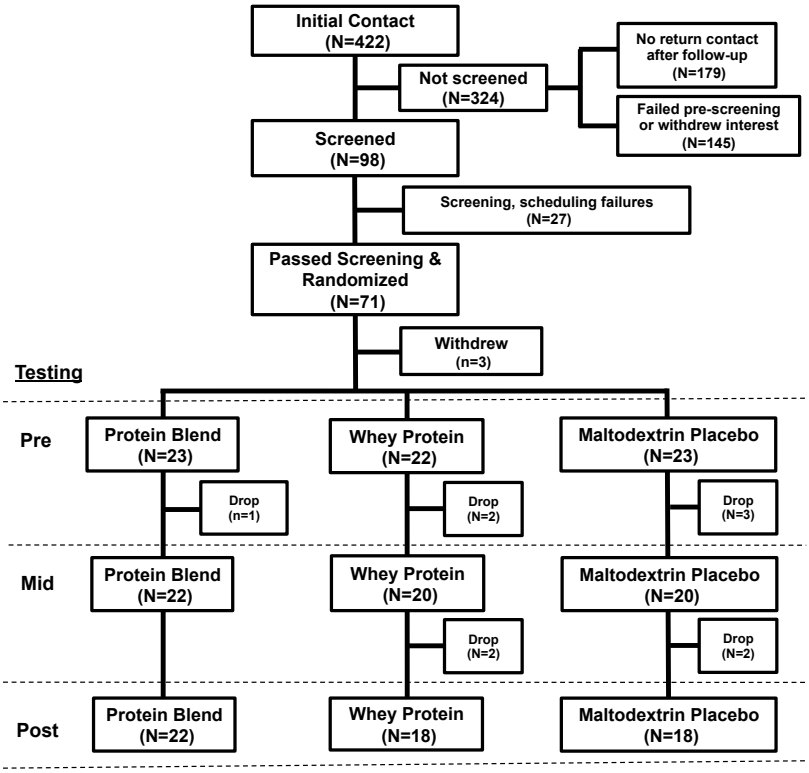
	PB (N=23)	WP (N=22)	MDP (N=23)
Characteristics			
Age, *years*	24.4 ± 0.9	24.8 ± 0.9	25.3 ± 0.9
Height, *cm*	179 ± 1.8	178 ± 1.7	176 ± 1.7
Weight, *kg*	78.0 ± 2.5	81.8 ± 2.5	76.6 ± 2.5
BMI, kg/m^2	24.4 ± 0.6	25.8 ± 0.7	24.6 ± 0.6
Strength 1RM, *kgs*			
Squat	109.4 ± 10.4	120.0 ± 10.6	123.7 ± 10.2
Knee extension	107.0 ± 6.0	115.0 ± 6.1	105.8 ± 5.8
Chest press	82.5 ± 6.9	90.4 ± 7.1	83.8 ± 6.8
Average	77.8 ± 4.0	83.1 ± 4.1	78.3 ± 3.9

[1]Data are mean ± SEM. Protein blend (PB), whey protein (WP) and Maltodextrin Placebo (MDP).

Participants were recruited through locally posted flyers, newspaper advertisements, and by word of mouth. After initial contact, prospective participants filled out a pre-screening questionnaire to determine eligibility and availability to participate. Individuals who could potentially participate were screened in the morning after an overnight fast at the Institute for Translational Sciences-Clinical Research Center (ITS-CRC) at the University of Texas Medical Branch. The screening day included 3-day food diary analysis, strength testing, a clinical history, physical exam, resting ECG, and laboratory tests (complete blood count with differential, liver and kidney function tests, coagulation profile, fasting blood glucose, hepatitis B and C screening, HIV test, thyroid stimulating hormone, lipid profile, urinalysis, and drug screening). Participants with clinical signs of malnutrition, on anabolic steroids or corticosteriods in the past 6 months, current tobacco users, admitted vegan or vegetarians, on a high-protein diet, high soy diet (> 2 servings of soy per day), high dairy diet (> 6 servings of dairy per day)

currently using protein supplements or having dairy allergies were excluded. The participants were healthy and recreationally active, but were not engaged in any regular exercise-training program (< 2 sessions high intensity aerobic or resistance exercise/week) at the time of enrollment. All participants gave written informed consent before enrollment in the study. The study was approved by the Institutional Review Board of the University of Texas Medical Branch, and is in compliance with the Declaration of Helsinki as revised in 1983. The consort diagram is Figure 4.1.

Figure 4.1. Consort diagram for exercise training study



Study Design

Following enrollment, participants completed a 10-14 day pre-training, run-in period that consisted of the pre-training study day at UTMB and then 3 non-consecutive days of exercise familiarization and baseline 1-repetition max (1-RM) strength testing at the University of Texas Medical Branch Alumni Fieldhouse. At the run-in, participants were given a study binder containing study information, food diary record instructions, supplement logs and visual analog scale (VAS) appetite questionnaires.

The pre-training study day included assessment of body composition, thigh muscle thickness, blood and serum collection, and isokinetic and isometric strength testing. 2-3 days later, the participants reported to the University of Texas Medical Branch Alumni Fieldhouse for familiarization/testing before beginning 12-weeks of training. At 6-weeks of training, participants were re-tested on all measures in the

morning following an exercise training day and after an overnight fast. Following 12-weeks of training, participants were re-tested 3 days following the final exercise session. For the post-testing, participants reported to the ITS-CRC at the same time in the morning as the pre-training study day to repeat the same tests and sample collection.

Clinical Testing

Participants reported to the ITS-CRC at the University of Texas Medical Branch in the morning following an overnight fast. They were instructed to refrain from any medication that affects muscle metabolism, caffeine, fish oil and alcohol for several days before testing. They were instructed to avoid strenuous or long duration exercise for 3 days before arrival and to drink a liter of water the night before. After arrival on the unit participants voided to ensure an empty bladder and bowel, and then lay supine for 30 min prior to assessment of body composition by DXA scan (dual-energy X-ray absorptiometry) (Hologic ADR 4500W, Bedford, MA). The same technician set the regions of interest for all the DXA scans. A catheter was placed in the antecubital vein for blood sampling.

To maintain a supine position, participants were transported to and from the CRC bed in a stretcher. After the DXA scan, Ultrasound (Phillips HDI 5000) of the vastus lateralis (VL) and vastus intermedius (VI) was conducted while the participant lay in bed as others have previously described [480] with some minor modifications [481]. Briefly, several B-Mode real time images of the VL and VI were taken in the mid-sagittal position at 50 and 75% of the femur length (from the anterior superior iliac spine to the superior border of the pattella). The ultrasound head position, pre and post-training, was placed relative to specific measured landmarks. The image that offered the sharpest contrast with the femur was chosen to ensure perpendicular placement of the scan head. Muscle thickness was assessed as the average distance from the superficial aponeurosis to

the femur at these two locations. The average of both sites, with 3 images at each site was used to assess muscle thickness. Preliminary testing, on the same individuals, revealed that the within-day and week-to-week coefficient for variation for measurements was 1.42±0.20 and 1.84±0.40%, respectively.

Peak torque of the knee extensors and knee flexors of the non-biopsied leg were subsequently determined by dynamometry (Biodex Medical, Shirley, NY). Participants were previously familiarized to the test at the screening. Briefly, participants were restrained in the dynamometer, with the anatomical access of the knee joint of their leg aligned with the mechanical axis of the dynamometer. Range of motion was measured from 90º to 10º (0º = full extension). After demonstration of proper technique and an explanation of the strength test protocol, participants performed practice contractions to warm-up and re-familiarize themselves with the dynamometer. Thereafter, isometric peak torque (extension and flexion) was determined at a 60◦ angle of knee flexion over 3 maximal voluntary contractions (5 seconds long) with 90 seconds rest between attempts. Then, isometric peak torque (extension and flexion) was determined from 4 attempts at an angular velocity of 120◦/sec. The coefficient of variation for these repeated measures of strength is <4%.

Following the strength test, participants were fed a meal before leaving the unit. All testing was repeated on the post-testing day in the same order as the pretesting day.

Supplementation

Participants were randomized to the MDP, WP or PB treatments. Immediately following each workout, under direct observation of the study personnel, the participants ingested either the placebo beverage or one of the protein supplements to which they were assigned. On the four resting (non-exercise) days each week the participants ingested the placebo or supplement one time between meals. Participants were instructed

to refrain from any other food or macronutrient-containing beverage for 2 hours before or after exercise or supplementation.

Whey and protein blend samples were provided by DuPont Nutrition & Health and were independently tested for amino acid profile (Table 4.2). The soy-dairy blend (PB) was composed of 25% soy protein isolate, 25% whey protein isolate, and 50% sodium caseinate. The whey (WP) treatment consisted of 100% whey protein isolate and maltodextrin placebo (MDP) was an isocaloric maltodextrin mixture. To assess the overall effect of protein supplementation, the PB and WP groups were combined as PRO. The dose for the two protein nutritional supplements was ~22 g protein/day. The dose has been chosen on the basis of the laboratory's preliminary data that this protein dose will contain an amount of leucine sufficient to acutely maximize protein synthesis for all protein supplements (i.e., ≧2 g of leucine) in young men. Therefore, the leucine content was 2.00 g for the PB and 2.31 g for the WP. Supplements were separated into individual ready-made packets for daily consumption and participants were given a 2-week supply. The personal trainer collected the empty supplement packets from each participant every 2 weeks. Supplements and placebo were given in powder form and dissolved in 300 ml water to ensure a rapid and predictable absorption.

Table 4.2. Composition of the nutritional treatments[1]

	PB	WP	MDP	PB	WP	MDP
	g/100g product			per serving		
Serving *g*				25.2	26.2	25.2
		%			*g*	
Protein	87.0	82.1	<0.10	21.9	21.5	0.0
Fat	2.8	2.5	0.3	0.7	0.6	0.1
Ash	3.7	2.5	<0.2	0.9	0.7	0.0
Moisture	5.9	7.0	5.4	1.5	1.8	1.4
Carbohydrate	0.6	5.8	94.2	0.2	1.5	23.7
Calories				95	98	96
		mg			*mg*	
Sodium	874.0	174.0	59.3	220.2	45.6	14.9
Potassium	545.7	631.7	10.4	137.5	165.5	2.6
Calcium	141.7	390.0	<0.01	35.7	102.2	0.0
Magnesium	32.2	75.8	4.8	8.1	19.9	1.2
Phosphorus	614.0	259.7	<0.01	154.7	68.0	0.0
		g			*g*	
Alanine	3.45	4.54	-	0.87	1.19	-
Arginine	3.74	1.95	-	0.94	0.51	-
Aspartic Acid	7.92	9.17	-	2.00	2.40	-
Cysteine	1.02	2.20	-	0.26	0.58	-
Glutamic Acid	18.27	15.90	-	4.60	4.17	-
Glycine	2.11	1.48	-	0.53	0.39	-
Histidine[2]	2.28	1.51	-	0.57	0.40	-
Isoleucine[2]	4.72	5.64	-	1.19	1.48	-
Leucine[2]	7.95	8.81	-	2.00	2.31	-
Lysine[2]	6.77	7.78	-	1.71	2.04	-
Methionine[2]	2.05	1.92	-	0.52	0.50	-
Phenylalanine[2]	4.14	2.65	-	1.04	0.70	-
Proline	7.23	5.55	-	1.82	1.45	-
Serine	4.63	4.22	-	1.17	1.11	-
Threonine[2]	4.37	6.24	-	1.10	1.63	-
Tryptophan[2]	1.14	1.32	-	0.29	0.34	-
Tyrosine	3.97	2.53	-	1.00	0.66	-
Valine[2]	5.40	5.20	-	1.36	1.36	-
Total EAA	38.83	41.07	-	9.78	10.76	-

[1]Protein blend (PB), whey protein (WP) and maltodextrin placebo (MDP)
[2]Represent the EAA (Essential Amino Acids

Nutritional Intake

Participants were instructed to maintain their habitual diet and to log a 3-day food diary on 3 occasions, pre-testing, mid-testing and post-testing. On each occasion participants were given detailed instruction and were told to record their normal diet in the week before the testing day on two weekdays and one weekend day with emphasis that one of the days was the day before testing. These records were entered into (Nutritional Data System for Research) NSDR 2012 to estimate energy intake and macronutrient composition.

Appetite

Participants were instructed to complete an appetite questionnaire to represent every day during the treatment period. They were instructed to reflect on how they felt during the time in between meals (in the immediate hours following supplementation) and how they felt right before the 1st meal after supplementation. The cumulative responses of each day were averaged to represent a 3-week time frame because responses during these blocks were similar and reflected each change in exercise intensity (strength was tested ever three weeks and intensity was adjusted accordingly). At each occasion, the questions addressed perceived hunger, thirst, and quantity of food desired, nausea and fullness. This questionnaire was in VAS format (scale 0-10) as previously described [482].

Resistance Exercise Training Protocol.

Following familiarization and 1-RM strength testing, participants began a 12-week whole-body progressive resistance exercise-training (RET) program (Supplemental Figure 1). All exercise-training sessions were performed at the Alumni Fieldhouse at the

University of Texas Medical Branch. Exercise sessions were performed on non-consecutive days, 3 times weekly, with 4 rest days per week under supervision of personal trainers. Participants were allowed to maintain their recreational physical activity, but instructed not to do any other strength training outside the study. RET was performed at and intensity of 60-80% of 1-repetition maximum (1-RM) and consisted of 3-4 sets of 8-10 repetitions performed to technical failure during the last set for each exercise. In week 1, 3 sessions were conducted at 3 sets of 10 repetitions at 60% 1-RM. In weeks 1-8, 2 sessions per week were performed an intensity of 70% 1-RM, where 3 sets of 10 repetitions were the last set was performed to momentary muscular failure. Each session consisted of whole body resistance exercise that lasted ~60-70 min. To reduce the risk of injury and overtraining one additional training session per week was conducted at 3 sets of 10 repetitions at 60% 1RM with the goal of not reaching momentary muscular failure. These sessions were scheduled to occur as the training sessions immediately before and after the 1-RM training days. In weeks 9-12, 2 sessions per week were performed an intensity of 80% 1-RM, where 4 sets of 8 repetitions were performed to momentary muscular failure. The 3rd session was performed at an intensity of 60% 1-RM as before. Each session consisted of whole body resistance exercise that lasted ~70-90 min. Resistance exercises included flat and incline chest press; leg press, curl and extension; seated pull-downs and rows; calf raises; and abdominal exercises. Participants rested for 1-2 minutes between exercises and individuals sets. 1-RM was directly tested on the chest press, leg press and the knee extension. 1-RM was estimated with 8-RM testing on the remaining exercises. Strength was re-tested at 3, 6 and 9 weeks so as participants strength increased, absolute training loads could be adjusted to maintain a relative training intensity between 60-80% 1-RM. 1-RM strength tested was performed again at the completion of the training program as the final exercise session. During and after each training session the personal trainer recorded the sets, reps and total weight lifted along with other relevant notes, which were entered into a secure database. To

allow for unforeseen life events, participants were given 13 weeks following the familiarization period to complete 36 exercise sessions. This allowed for 100% exercise compliance.

Figure 4.2. Resistance exercise training protocol design

	Run-IN		Exercise Training Protocol											
Week	**-2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Sets and Reps		**2x10**	**2 days: 3x10 (to failure) 1 day: 60% 1-RM (no failure)**								**2 days: 4x8 (to failure) 1 day: 60% 1-RM (no failure)**			
Intensity (% 1-RM)		**60%**	**70%**								**70%**	**80%**		
1-RM Testing		✓			✓			✓			✓			✓
Exercise Familiarization		✓												
Exercise Training		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Schematic of the resistance exercise training protocol.

Serum Testosterone

Testosterone was assayed from serum in duplicate on an Immulite 2000 Immunoassay System (Siemens, Erlangen, Germany) at the ITS-CRC core laboratory per the manufacturer's instructions.

Participant Assessment

The personal trainers were given an assessment to reflect their expert evaluation of their trainee's body type (somatotype), training status (familiarity with exercises), training history (based on pre-screening questionnaires), 6 & 12 week responder (evaluation of trainee's progress via strength, lean mass changes and visual appearance), and motivation (effort level applied). This subjective evaluation was conducted to complement tests for differences between treatments and to affirm that changes during

the study were due to the supplementation, not any other of the above variables known to influence the exercise adaptions.

Free Blood Amino Acid Concentrations

Concentrations of phenylalanine and the branch-chained amino acids (leucine, isoleucine, and valine) were measured in deproteinized whole blood using gas chromatography-mass spectrometry (GCMS) as previously described using an internal standard solution [21, 448].

Treatment, Nutritional Intake Log and Appetite Questionnaire Compliance

Treatment compliance was confirmed via supervision of post-exercise supplementation at the field-house, tallying the number of returned and empty packets and with documentation of the self-reported supplement log. Nutritional intake and appetite questionnaire compliance was determined for each treatment as the number of completed items as the percent of total possible items returned.

Statistical Analysis

Values are the raw values or model corrected estimates expressed as Mean ± SEM or Mean ± 95% CI. Data were transformed using the Box-Cox set of transformations to stabilize the variance and make the data approximately normally distributed. To test differences between treatments, the data were modeled using an ANCOVA model with baseline (pre) values as a covariate. Contrasts were used to test the difference between treatments, along with a Tukey adjustment for multiple comparisons. Each of the post-

baseline time-points was be analyzed separately, to allow for changes in variance at each time point. The data was then combined and analyzed using a mixed factors model, with time and group as fixed effects and differing within-group variances at each time point. If within each time point group variances differed significantly, transformations of the response were used to stabilize the variance. In the ANOVA Mixed Model subjects were set as a random effect, and treatment (PB, WP and MDP), and time (baseline, 6 weeks, 12 weeks or (baseline and 12 weeks as appropriate) were treated as fixed effects. To test the effect of protein supplementation we pooled the protein treatments WP and PB as PRO. An additional model was conducted with treatment effects of PRO and MP only. Significance was set at $p<0.05$ with trends at $0.05 > p < 0.1$. All calculations were done in R, with the exception of Pearson correlations, which were calculated with Graph Pad Prizm 6.0f for Mac (La Jolla California USA). All figures were generated with the same program.

RESULTS

Participant Characteristics

Descriptive characteristics at baseline for all participants are shown in Table 4.1. There were no differences between treatments at baseline for any variable ($p>0.10$).

Nutritional Intake

The average habitual energy and macronutrient intake (Table 4.3 and Appendix Table A.4.1) was stable over time in all conditions ($p>0.10$). Supplementation of protein increased protein intake in protein supplemented groups over MDP ($p<0.05$). Carbohydrate intake was not affected by time or treatment ($p>0.10$).

Table 4.3. Participant dietary intake (with supplementation) by treatment before (Pre), 6 weeks (Mid) and 12 weeks (Post) resistance exercise-training with nutritional supplementation1

TRT	Time Period			Main Effects
	Pre	Mid	Post	
Total Energy, *kcal (non-supplemented)*				
PB	2458 ± 158	2462 ± 158	2272 ± 161	t: 0.996 trt: 0.235, t x trt: 0.716
WP	2485 ± 179	2502 ± 189	2657 ± 194	
MDP	2223 ± 189	2186 ± 195	2204 ± 195	
Protein Intake, *g/d*				
PB%	101.3 ± 7.0	**129.4 ± 7.0***	**121.5 ± 7.1#**	t: 0.000, trt: 0.007, t x trt: 0.014
WP%	101.9 ± 7.1	**125.8 ± 8.1#**	**134.6 ± 8.3***	
MDP	95.1 ± 7.0	95.1 ± 8.3	93.2 ± 8.3	
Protein Intake, *g/kg/d*				
PB%	1.33 ± 0.10	**1.68 ± 0.10***	**1.54 ± 0.10**	t: 0.001, trt: 0.061, t x trt: 0.016
WP%	1.29 ± 0.10	**1.54 ± 0.11**	**1.64 ± 0.11**	
MDP	1.27 ± 0.10	1.22 ± 0.11	1.23 ± 0.11	
Carbohydrate Intake, *g/d*				
PB	274.2 ± 18.3	290.0 ± 18.3	272.4 ± 18.6	t: 0.481, trt: 0.560, t x trt: 0.915
WP	283.3 ± 20.7	291.3 ± 22.0	284.3 ± 23.0	
MDP	245.8 ± 21.0	272.2 ± 23.0	274.0 ± 23.0	
Carbohydrate Intake, *g/kg/d*				
PB	3.58 ± 0.24	3.71 ± 0.24	3.42 ± 0.24	t: 0.853, trt: 0.881, t x trt: 0.786
WP	3.54 ± 0.27	3.52 ± 0.28	3.46 ± 0.29	
MDP	3.27 ± 0.27	3.16 ± 0.29	3.31 ± 0.29	
Fat Intake, *g/d*				
PB	91.3 ± 7.9	96.9 ± 7.9	87.2 ± 8.1	t: 0.911, trt: 0.406, t x trt: 0.628
WP	100.4 ± 8.9	96.6 ± 9.4	106.1 ± 9.7	
MDP	92.4 ± 9.0	87.0 ± 9.7	83.4 ± 9.7	
Fat Intake, *g/kg/d*				
PB	1.19 ± 0.11	1.26 ± 0.11	1.08 ± 0.11	t: 0.649, trt: 0.811, t x trt: 0.485
WP	1.26 ± 0.12	1.18 ± 0.13	1.29 ± 0.13	
MDP	1.23 ± 0.12	1.13 ± 0.13	1.11 ± 0.13	

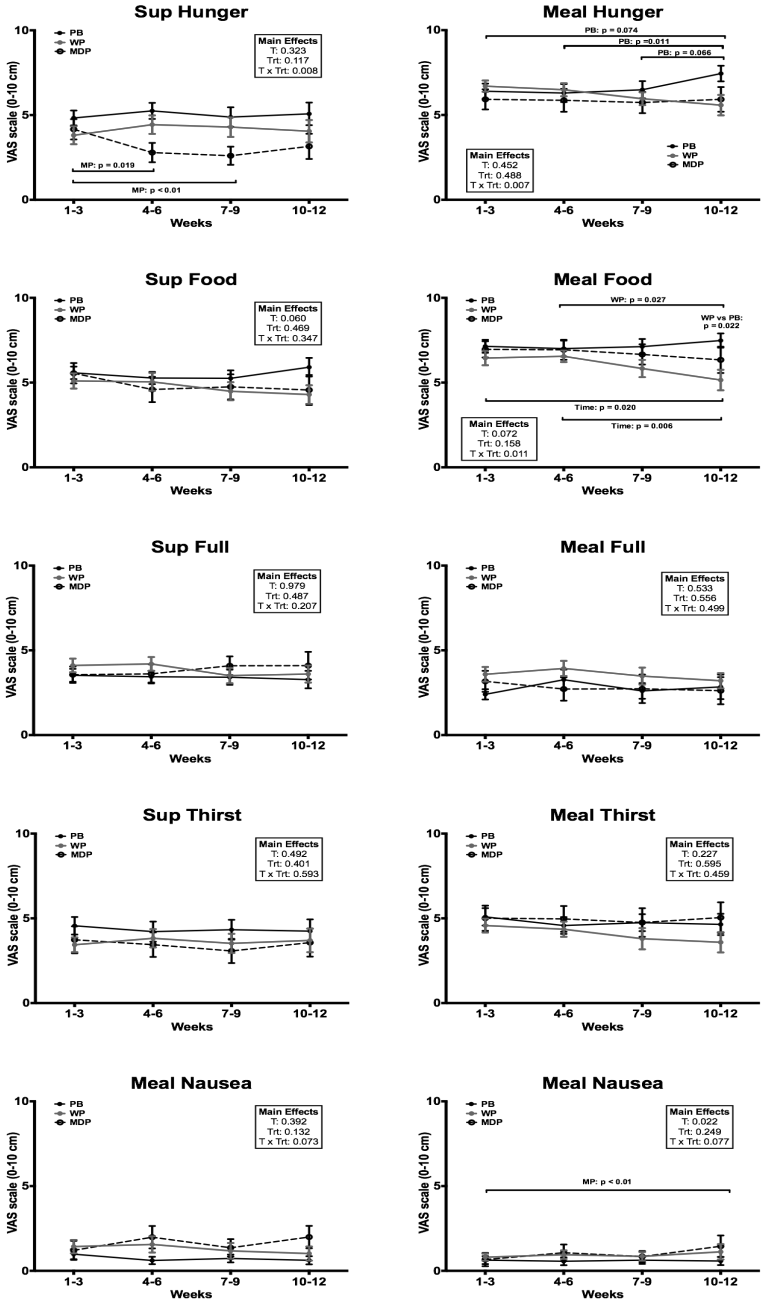
[1]Data are mean ± SEM, n=18 (WP), 22 (PB) & 18 (MDP). * P<0.05, # P<0.06 vs MDP at that timepoint. # P<0.06. % Effect of PRO. Bold P<0.05 vs Pre via ANCOVA.

Appetite

The average treatment appetite responses for all participants are shown in Figure 4.3. The amount of fullness and thirst participants felt before each meal following supplementation was not different over time or between treatments ($p>0.10$). The amount of food participants felt they could eat, perceived fullness, nausea and thirst in the immediate hours following supplementation was not different over time or between treatments ($p>0.10$). The amount of food participants felt they could eat before each meal following supplementation was not different over time or between treatments for the first 9 weeks. However, there were main effects for time and time x treatment ($p<0.05$), which post hoc testing revealed as a main effect of time at 10-12 weeks vs 1-3 and 4-6 weeks ($p<0.05$), this was driven by a decline in WP vs 4-6 weeks which resulted in PB participants to feel as if they could eat more than WP ($p<0.05$) at 10-12 weeks. The level of hunger participants felt before the first meal following supplementation was not different over time or between treatments for the first 9 weeks. However, there was a main effects for time x treatment ($p<0.05$), which post hoc testing revealed as an increase in hunger PB at 10-12 weeks vs 4-6 weeks ($p<0.05$) and a trend vs 1-3 and 7-9 weeks ($p<0.08$). Interestingly, correlative analysis demonstrated a positive correlation between the change in arm lean mass and the level of perceived hunger at weeks 9-12 (Appendix Figure A.4.1. $r=0.36$, $p=0.014$). The amount of nausea participants felt before each meal following supplementation was not different over time or between treatments for the first 9 weeks. However, there were main effects for time ($p<0.05$), and a trend for time x treatment ($p<0.08$), which post hoc testing revealed as a treatment effect of time for greater levels of nausea at 10-12 weeks vs 1-3 for MDP. The level of perceived hunger

in the immediate hours following supplementation/consumption was not different over time or between treatments ($p<0.05$). However, there was a main effects for time x treatment ($p<0.05$), which post hoc testing revealed as a treatment effect of time for decreased levels of hunger at 4-9 weeks vs 1-3 weeks for MDP ($p<0.05$).

Figure 4.3. Appetite questionnaire responses



Appetite questionnaire responses from the visual analog scale (0-10cm) addressing quantity of food desired (A,F), perceived hunger (B, G), fullness (C, H), thirst (D, I), and nausea (E, J) in the immediate hours after ingestion of the supplement (A-E) and right before the first meal consuming after ingesting the supplement (F-J weekly by treatment during 12 weeks resistance exercise-training with nutritional supplementation. Protein blend (PB) or whey protein (WP) or maltodextrin placebo (MDP). Data are mean ± SEM, n=18 (WP), 20 (PB) & 13 (MDP).

Treatment, Nutritional Intake Log and Appetite Questionnaire Compliance.

Treatment compliance was similar for all treatments with 92.3% (range: 80.5-100%, median: 93.0%), 87.2% (range: 56.5-100%, median: 91.7%) and 88.1% (range: 64-100%, median: 91.8%) for PB, WP and MDP respectively. There were no differences between treatments for treatment compliance ($p>0.10$). Appetite questionnaire compliance was low, driven by several non-compliant participants in each group as follows; WP (n=3), PB (n=1) and MDP (n=9, n=4 completers, n=5 dropouts). Thus appetite questionnaire compliance was similar for PB (86.7%) and WP (79.6%), but significantly lower in MDP (61.5%) vs PB ($p=0.031$). Dietary log compliance was better, but a treatment difference was present ($p=0.037$). Dietary log compliance was higher ($p<0.05$) for PB (98.5±1.5%) than WP (85.2±4,8%) and MDP (83.3±6.7%).

Participant Assessment

There were no treatment differences on the personal trainers' expert evaluation of their trainee's body type, training status, training history, 6 & 12-week response, and motivation ($p>0.10$).

Serum Testosterone

Serum testosterone (Table 4.4) was similar at baseline between treatments and did not change in the WP and PB groups during treatment ($p>0.10$). A trend was evident for serum testosterone to increase the morning after an exercise bout at mid in MDP ($P=0.079$).

Table 4.4. Serum testosterone concentration (ng/dL) by treatment before (Pre), after 6 weeks (mid) and 12 weeks (Post) resistance exercise training1

	PB (N=21)	WP (N=22)	MDP (N=23)
Pre	375.5 ± 21.3	394.0 ± 21.0	404.6 ± 19.9
Mid	405.5 ± 21.0	410.7 ± 21.6	454.1 ± 20.4 #
Post	385.0 ± 21.0	390.4 ± 22.2	439.0 ± 21.0

Data are mean ± SEM. concentration (ng/dL) Protein blend (PB), whey protein (WP) and Maltodextrin Placebo. Mid was mid-week, in the morning after an exercise session. Post was 72h after the last exercise session. # Different than Pre p = 0.08.

Weight lifted & 1-RM strength

The total average weight lifted for WP, PB and MDP was not different (data not shown). At pre 1-RM strength was not different between treatments for any exercise. Yet all treatments demonstrated similar (p>0.10) absolute change improvements (Table 4.5) in strength at 3, 6, 9 and 12 weeks of training (p<0.05) for average gym strength. A similar pattern was revealed with each exercise individually (data for CR, IP, KC and SR not shown). However, there was a minor trend (p=0.073) for a random TRT difference in the absolute change (WP>MDP) with KE at 6 weeks, driven by 2 outliers in WP.

Table 4.5. Absolute change (kg) in 1 repetition maximum (1-RM) testing on select exercises by treatment at 3, 6, 9 and 12 weeks resistance exercise-training[1]

TRT	Time Period				ANCOVA
	3 wk	6 wk	9 wk	12 wk	
Δ Squat 1RM					
PB	20.6 (14.5,26.7)	42.2 (33.3,51.1)	62.7 (49.3,76.1)	92.7 (72.2,113.3)	
WP	25.1 (18.8,31.4)	40.2 (31.0,49.5)	59.0 (45.0,73.3)	100.7 (79.3,122.2)	T
MDP	18.3 (12.3,24.4)	40.4 (31.1,49.7)	60.6 (46.3,75.0)	91.3 (69.2,113.4)	
Δ Knee extension					
PB	17.0 (11.1,23.0)	31.1 (24.6,37.6)	45.1 (36.9,53.3)	61.6 (53.2,70.1)	T, 6wk:
WP	22.0 (15.7,28.2)	40.7 (33.8,47.5)	52.4 (43.6, 61.3)	65.5 (56.3,74.6)	WP>MDP p
MDP	18.2 (12.3,24.1)	30.0 (23.0,36.6)	40.6 (32.0,49.4)	63.1 (53.9,72.2)	= 0.073
Δ Chest press					
PB	12.5 (9.1,15.9)	20.5 (15.9,25.1)	26.6 (21.3,32.0)	35.6 (30.0,42.0)	
WP	13.9 (10.3,17.4)	22.8 (18.0,27.6)	28.2 (22.4,34.0)	34.0 (29.5,41.7)	T
MDP	12.5 (9.0,15.9)	20.9 (16.1,25.7)	29.0 (23.1,34.8)	37.4 (30.6,44.2)	
Δ Average					
PB	12.5 (10.6,14.4)	21.4 (18.8,24.1)	31.1 (26.0,36.2)	41.0 (35.0,46.5)	
WP	14.1 (12.2,16.1)	23.1 (20.3,26.0)	32.8 (27.3,38.3)	46.8 (40.3,53.3)	T
MDP	12.7 (10.8,14.5)	22.0 (19.1,25.0)	31.0 (25.3,36.3)	43.1 (37.0,50.0)	

[1]Data are ANCOVA estimates as mean ± 95% CI, n=22 (WP), 23 (PB) & 23 (MDP). ANCOVA T = main effect of time. Average represents the average 1RM increase from all the exercises trained.

Dynamometry

At pre isometric and isokinetic peak torque for flexion and extension was not different between treatments. Absolute values are shown in Appendix Table A.4.2. For isometric knee extension, only WP significantly increased at Mid, which resulted in a trend for a main TRT difference ($p=0.092$). However, all treatments increased similarly from Pre to Post (Table 4.6). For isometric knee flexion, only PB significantly increased at Mid, which resulted in a trend for PB ($p=0.097$) and PRO ($p=0.057$) to be greater than MDP. All treatments similarly displayed a slight, but significant increase from Pre to Post (Table 4.6).

For isokinetic knee extension peak torque, only WP increased Pre to Mid, however MDP did not change at any point, but PB and WP similarly increased Pre to Post (Table 4.6). This resulted in an effect of WP (17 newton-meters: 0.7,34), PB (14 newton-meters: -1,30) and thus PRO (16 newton-meters: 4,28) vs MDP. For isokinetic knee flexion peak torque none of the treatments increased demonstrated a change except for WP from Pre to Post (Table 4.6) and there were no TRT differences at any time point.

Thigh Muscle Thickness

Thigh muscle thickness (Figure 4.4, Appendix Table A.4.3) increased from baseline, in all treatments, at each time point ($p<0.05$), but there was no change from Mid to Post in any treatment. There were no effects with PRO vs MDP at any time point ($p>0.10$).

Table 4.6. Change values of isometric and isokinetic torque, (N-M) by treatment before (Pre) and 12 weeks (Post) resistance exercise-training with nutritional supplementation1[1]

TRT	Change values		ANCOVA
	Pre to Mid	Pre to Post	
Isometric KE			
PB	11 (-3,24)	**32 (15,49)**	
WP	**30 (15,44)**	**37 (18,55)**	Pre to Mid: TRT 0.092,
MDP	10 (-4,25)	**28 (10,47)**	
Isometric KF			
PB	**12 (4,19)**	**11 (2,20)**	
WP	7 (-1,16)	**12 (2,23)**	Pre to Mid: PB>MDP 0.097, PRO> MDP 0.057
MDP	-1 (-8,8)	**11 (1,21)**	
Isokinetic KE			
PB	3 (-5,13)	**18 (9,27)**	Pre to Post: TRT 0.031, WP>MDP 0.038, PB>MDP 0.083, PRO>MDP 0.009
WP	**15 (6,25)**	**21 (11,30)**	
MDP	6 (-4,15)	3 (-7,13)	
Isokinetic KF			
PB	5 (-3,13)	5 (-4,14)	
WP	5 (-3,13)	**15 (6,25)**	
MDP	1 (-7,9)	3 (-7,12)	

[1]Data are mean ± 95% CI, n=22 (WP), 23 (PB) & 23 (MDP). N-M = newton meters. Knee extension = KE. Knee Flexion = KF.

Figure 4.4. Knee extensor muscle thickness

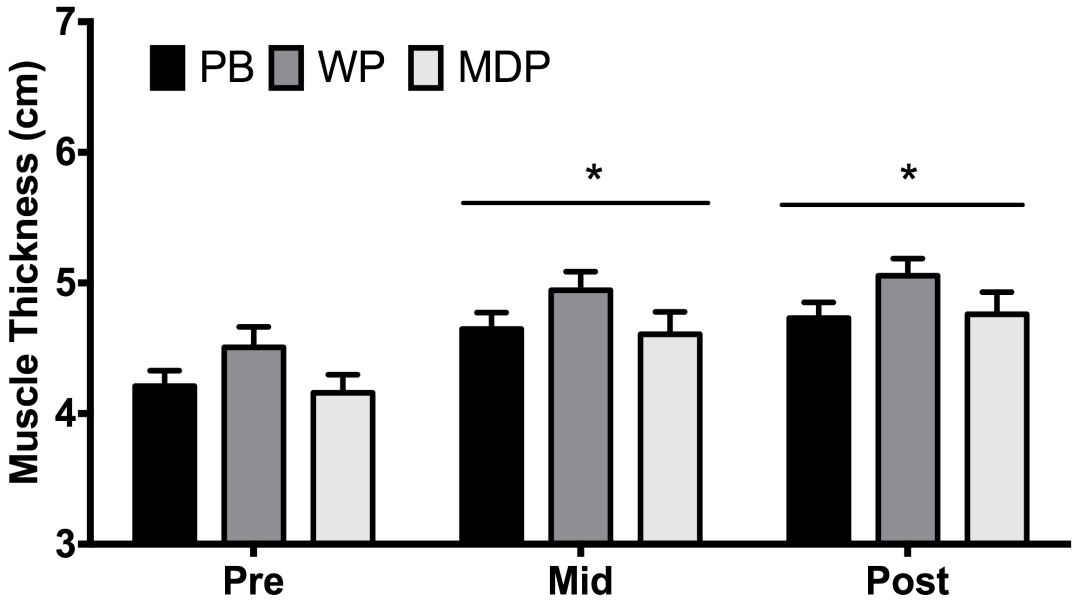


Fig 4.4. Sum of *vastus lateralis* and *vastus intermedius* muscle thickness by treatment before (Pre), 6 weeks (Mid) and 12 weeks (Post) resistance exercise-training with nutritional supplementation, Protein blend (PB) or whey protein (WP) or maltodextrin placebo (MDP). n=22 (WP), 21 (PB) & 22 (MDP). Data are mean ± SEM.

Body Composition

Resistance exercise training altered body composition. Body weight increased Pre to Mid in PB and Pre to Post in WP and PB ($p<0.05$), whereas it remained stable in MDP. The absolute values of lean mass measures were not different ($p>0.10$) between treatments at Pre (Table 4.7, Appendix Table A.4.4). There were no main effects for treatment or any of the lean mass measures ($p>0.10$). A main effect of time was evident for total, arm, leg, appendicular and trunk lean mass ($p<0.05$). Universal increases ($p<0.05$) from Pre to Mid and Pre to Post drove these effects in all treatments (Table 4.7). Interestingly, only PB further increased ($p<0.05$) whole body, arm, and trunk lean mass from Mid to Post (Table 4.7). ANCOVA point estimates of Pre to Post treatment difference for whole body lean mass (Figure 4.5, Table 4.7) indicated that the PB exhibited a trend for a greater change than MDP (0.92 kg: -0.12,1.95). This was not demonstrated with WP vs. MDP (0.46 kg: -0.63, 1.55) and PB was not different from WP (0.45 kg: -0.48, 1.49). When examining the percent frequency of responses above the a priori 1.5kg change threshold expected for a carbohydrate placebo response to resistance exercise-training (Inset in Figure 4.5) both WP (78%) and PB (86%) were greater than MDP (50%) ($p<0.05$). As such, this resulted in trend for PRO over MDP from Pre to Post (0.69kg: -0.08, 1.46). ANCOVA point estimates of Pre to Post treatment difference for arm lean mass (Table 4.7, (Figure 4.6)) indicated that PB exhibited a trend for a greater change than MDP (171 g: -20,358). There were no other TRT differences with ANCOVA point estimates. Change in lean mass did not correlate with changes in strength, however, absolute values of lean lass correlated with changes in strength (data not shown). The Pre to Post change in lean mass was not associated with change in

protein intake (data not shown), however, the absolute protein intake at all time-points was significantly associated, but with low levels of fitness to absolute levels of lean mass at all time-points ($r=0.30$-0.35, $p<0.03$). As internal validation of our methods, Pre to Post whole body lean mass change was positively correlated with muscle thickness change (Appendix Figure A.4.2; $r=0.47$, $p<0.001$).

Table 4.7. Absolute Pre values of lean mass by treatment and change value from before (Pre) to 6 weeks (Mid), Mid to after 12 weeks (Post) and Pre to Post resistance exercise-training with nutritional supplementation1[1]

TRT		Time Period		
	Pre	Pre to Mid	Mid to Post	Pre to Post
Lean mass, *kg*		Δ, g		
PB	56.6 ± 1.5	**1948 (1421,2276)**	**946 (411,1480)**	**2875 (2298,3452)***
WP	57.6 ± 1.5	**1632 (1076,2188)**	537 (-108,1182)	**2420 (1780,3060)**
MDP	55.2 ± 1.5	**1790 (1231,2349)**	293 (-486,1072)	**1959 (1318,2599)**
Arm lean mass, *kg*		Δ, *g*		
PB	7.1 ± 0.2	**393 (298,487)**	**183 (40,324)**	**576 (471,681)***
WP	7.2 ± 0.3	**324 (225,423)**	125 (-29,281)	**461 (345,577)**
MDP	7.0 ± 0.3	**340 (240,439)**	65 (-90,220)	**405 (290,521)**
Leg lean mass, *kg*		Δ, *g*		
PB	19.2 ± 0.4	**1014 (736,1292)**	94 (-302,490)	**1075 (789,1362)**
WP	20.3 ± 0.7	**750 (454,1046)**	93 (-339,525)	**934 (614,1255)**
MDP	18.8 ± 0.7	**792 (498,1088)**	78 (-354,510)	**802 (485,1121)**
Appendicular lean mass, *kg*		Δ, *g*		
PB	26.2 ± 0.3	**1407 (1092,1723)**	279 (-166,724)	**1658 (1322,1994)**
WP	27.5 ± 0.9	**1066 (732,1401)**	219 (-267,704)	**1373 (999,1748)**
MDP	25.8 ± 0.9	**1140 (806,1474)**	142 (-343,628)	**1222 (850,1595)**
Trunk lean mass, *kg*		Δ, *g*		
PB	26.7 ± 0.7	**480 (90,871)**	**806 (181,1430)**	**1317 (800,1834)**
WP	26.5 ± 0.8	**630 (221,1039)**	352 (-318,1022)	**1047 (492,1603)**
MDP	25.9 ± 0.7	**510 (98,923)**	252 (-432,936)	**676 (97,1255)**

[1]Data are mean ± SE or mean (lower, upper) 95% CI, n=22 (WP), 22 (PB) & 24 (MDP). Labeled time periods without a common letter had overall means (not shown) that differ, P<0.05). ANCOVA calculated TRT point estimates as mean (lower, upper) 95% CI. * = P < 0.1 vs MDP at that time period.

Figure 4.5. The absolute change in whole body lean mass

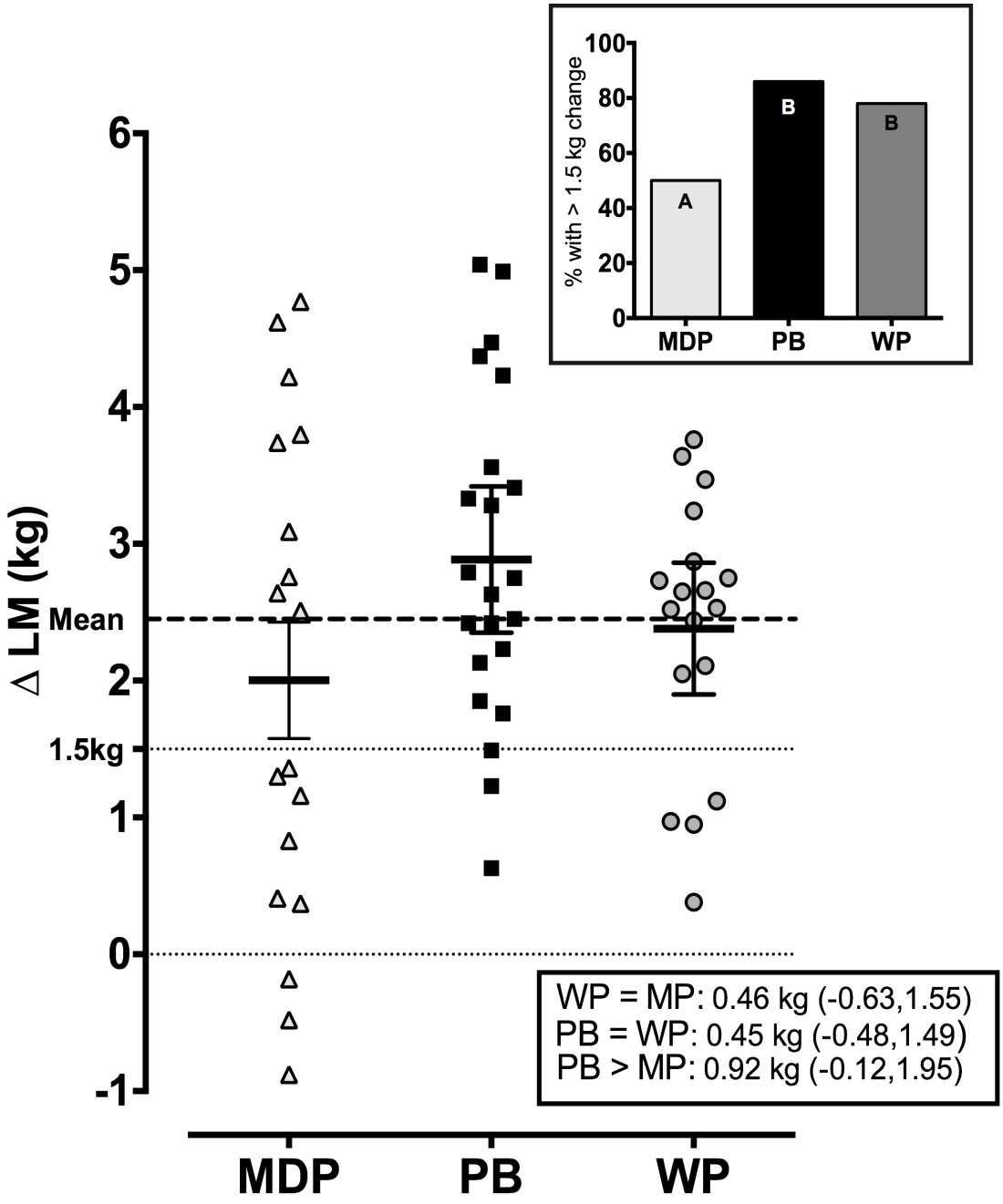


Fig 4.5. The absolute change in whole body lean mass by treatment from pre to post (12 weeks) resistance exercise-training with nutritional supplementation. Protein blend (PB) or whey protein (WP) or maltodextrin placebo (MDP). Data are individual responses with black bars as mean ± SEM, n=18 (WP), 22 (PB) & 18 (MDP). All treatments were greater than 0 ($p<0.05$). PB > MDP ($p=0.093$). Inset represents the percent frequency of responses above the a priori 1.5kg change threshold expected for a placebo response to resistance exercise-training. For inset, bars without a common letter differ ($p<0.05$).

Figure 4.6. The absolute change in arm lean mass

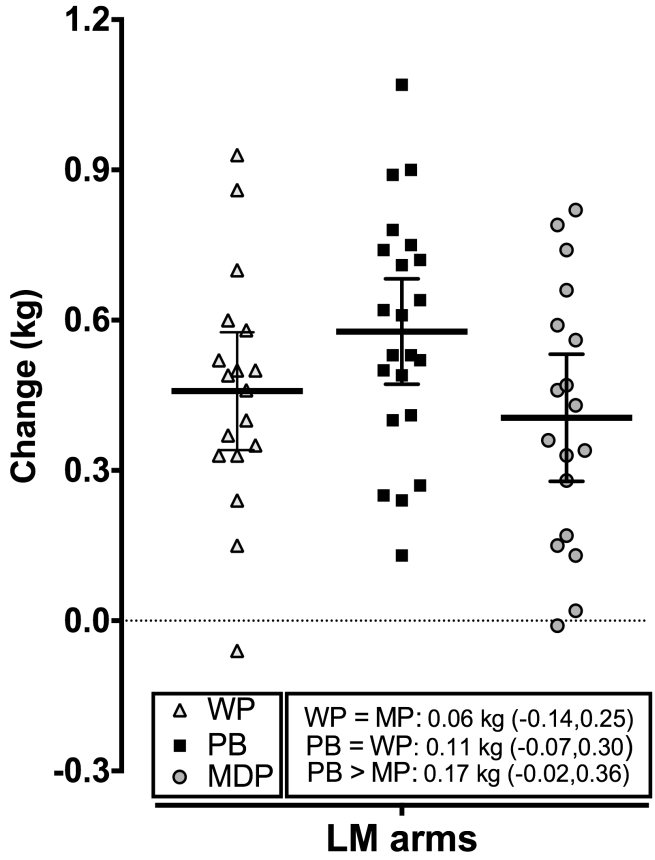


Fig. 4.6. The absolute change in arm lean mass by treatment from pre to post (12 weeks) resistance exercise-training with nutritional supplementation. Protein blend (PB) or whey protein (WP) or maltodextrin placebo (MDP). Data are individual responses with black bars as mean ± SEM, n=18 (WP), 22 (PB) & 18 (MDP). All treatments were greater than 0 ($p<0.05$). PB > MDP ($p=0.073$).

The absolute values of fat mass and percent body fat were not different between treatments at baseline (Table 4.8, Supplemental Table 5). There were no main effects for treatment or any of the fat mass measures. A main effect of time was evident for % body fat, total fat, and trunk fat mass ($p<0.05$) (Supplemental Table 5). Universal decreases from Pre to Mid and Pre to Post drove the effect for a decrease in % body fat (Table 7). ANCOVA point estimates of treatment differences did not detect any divergence by treatment. Total fat mass for WP and MDP decreased at Pre to Mid and Pre to Post

(Table 7), yet PB exhibited a non-significant tendency to decrease at Pre to Mid or Pre to Post and was not different from any other TRT at any time point (p>0.10) (Table 7). There were no TRT differences with ANCOVA point estimates. Trunk fat mass exhibited a weak tendency to decrease with PB (p=0.085) and WP (p=0.107), yet MDP significantly decreased (p=0.046) from Pre to Mid. Via the mixed model, only WP and MDP demonstrated a decrease from Pre to Post (p<0.05) (Table 6). There were no TRT differences with ANCOVA point estimates.

Table 4.8. Absolute Pre values of body fat and bone content and density by treatment and change value from before (Pre) to 6 weeks (Mid), Mid to after 12 weeks (Post) and Pre to Post resistance exercise-training with nutritional supplementation1[1]

TRT		Time Period		
	Pre	Pre to Mid	Mid to Post	Pre to Post
% Fat		Δ		
PB	23.7 ± 1.3	**-0.9 (-1.5,-0.4)**	-0.4 (-1.3,0.5)	**-1.5 (-2.2,-0.7)**
WP	25.9 ± 1.2	**-1.2 (-1.7,-0.5)**	-0.8 (-1.8,0.2)	**-1.8 (-2.7,-1.0)**
MDP	24.2 ± 1.6	**-1.0 (-1.6,-0.5)**	-0.7 (-1.7,0.3)	**-1.8 (-2.6,-0.9)**
Fat mass, *kg*		Δ, *g*		
PB	18.0 ± 1.3	-385 (-877,107)	-115 (--987,757)	-606 (-1388,174)
WP	20.5 ± 1.3	**-544 (-1065,-24)**	-569 (-1504,366)	**-1052 (-1895,-211)**
MDP	18.4 ± 1.7	**-590 (-1107,-73)**	-592 (-1548,364)	**-1240 (-2100,-379)**
Fat mass trunk, *kg*		Δ, *g*		
PB	9.3 ± 0.9	-310 (-655,43)	17 (-576,610)	-359 (-870,150)
WP	10.8 ± 0.9	-301 (-670,67)	-359 (-996,277)	**-634 (-1182,-85)**
MDP	9.7 ± 1.0	**-373 (-740,-6)**	-436 (-1087,214)	**-832 (-1394,-270)**
BMC, *g*		Δ, *g*		
PB	3172 ± 80	**13 (2,24)**	2 (-14,18)	**14 (4,25)**
WP	3194 ± 104	**15 (3,27)**	-1 (-15,18)	**15 (4,26)**
MDP	3114 ± 91	10 (-0,22)	8 (-9,26)	2 (-10,13)
BMD, *g/cm²*		Δ		
PB	1.316 ± 0.020	**-0.026 (-0.041,-0.010)**	**0.026 (0.005,0.048)**	0.002 (-0.014,0.017)
WP	1.327 ± 0.028	-0.015 (-0.032,0.003)	0.006 (-0.016,0.029)	-0.009 (-0.026,0.008)
MDP	1.306 ± 0.027	-0.007 (-0.024,0.009)	-0.006 (-0.030,0.016)	-0.012 (-0.030,0.005)

[1]Data are mean ± SE or mean (lower, upper) 95% CI, n=22 (WP), 23 (PB) & 23 (MDP). ANCOVA, Analysis of Covariance. TRT, main effect for TRT; T, Main Effect for time. ANCOVA calculated TRT point estimates as mean (lower, upper) 95% CI. BMD = Bone mineral density, BMC = Bone mineral content.

The absolute values of bone mineral content (BMC) and bone mineral density (BMD) were not different between treatments at baseline (Table 4.8, AppendixTable A.4.4). There were no main effects for treatment for BMC and BMD. However, a main effect of time was evident for BMC ($p<0.000$) but not BMD. This was driven by increases ($p<0.05$) in WP and PB from Pre to Mid and Pre to Post, which resulted in a trend for an effect of PRO from Pre to Post ($p= 0.069$) vs MDP (Table 4.8). ANCOVA point estimates indicated no other TRT differences for BMD or BMC.

Blood Amino Acid Concentrations

Blood amino acid concentrations where not different by treatment and tended to show effects of time (data not shown). Phenylalanine was not affected by time or treatment ($p>0.10$). All the BCAA's where elevated at mid in every treatment ($p<0.05$). The sum of all the BCAA's revealed an increase at Post in PB and MDP ($p<0.05$). Valine demonstrated an increase at Post in PB ($p<0.05$) and a trend in MDP ($p=0.08$). A trend was detected for an increase in WP at Post ($p=0.08$). Only MDP demonstrated increased Leucine at Post ($p< 0.05$). Leucine was not correlated ($p>0.10$), at any time point, with lean mass change.

DISCUSSION

There has been an inability to demonstrate a consistent effect of protein/AA supplementation to enhance resistance exercise training outcomes compared to placebo [324, 348, 349, 353]. The current theories posit that protein type may be a modulating factor behind this inconsistency. Almost all the selected protein types investigated have been single protein sources and types, with no comparison of a blended protein supplement against whey protein and also isocaloric placebo. We previously demonstrated the effectiveness of a soy-dairy protein blend (PB) in prompting lean mass growth in response to one bout of high intensity [189, 225]. Thereby, we tested this novel and promising protein combination against whey protein (WP) and isocaloric maltodextrin placebo (MDP) supplementation during 3 months of RET in young healthy males. All treatments improved lean mass, muscle thickness and strength, as would be expected during a progressive resistance training program. However, we demonstrate a trend that daily PB supplementation was more effective than carbohydrate placebo by 0.92 kg: (-0.12, 1.95) in enhancing lean mass gain, in young men, (**Figure 3**) over 3 months of RET. Interestingly, all treatments improved to a similar extent during the first 6 weeks, yet the PB continued to improve over the remaining 6 weeks for whole body, arm, and trunk lean mass. We could not discern consistent statistical differences in leg lean mass or muscle thickness between treatments suggesting that a large proportion of the lean mass enhancement occurred in the upper body. In fact, we are able to demonstrate a strong trend for PB to exhibit a greater change than MDP (171 g: -20,358) for arm lean mass (**Supplemental Figure 5**Interestingly, this change in arm lean mass

was positively correlated with pre-meal perceived hunger during the last 3 weeks of exercise training.

Although the group mean for the change in lean mass in our WP group, ~2.3 kg, is almost identical to the amount shown via meta-analysis of resistance exercise training and whey protein supplementation [355], our whey protein treatment did not statistically demonstrate an effect over placebo 0.58kg (-0.46,1.62), which in itself is not an uncommon finding [334, 335, 385]. In this case, we believe this lack of an effect was largely due to the heterogeneity of responses in the maltodextrin placebo treatment (**Figure 3**). This observation in the placebo participants supports the concept that some individuals are high responders to RET regardless of nutritional intervention [317, 372], as we suggest here. This intricacy and the idea that some individuals do not respond to RET regardless of added nutritional variance [372] may be the most likely reason for the inconsistency for an effect of protein supplements in the literature. To test the consistency of changes in lean mass, we determined the percent frequency of responses for each treatment above an a priori 1.5kg change threshold expected for a placebo response to resistance exercise-training (**Inset in Figure 3**). This analysis revealed that both the PB and WP treatments exhibited consistently more responses (86 and 78% respectively) above this threshold than MDP (50%), suggesting that these protein supplements were reliably effective in enhancing lean mass gain. Also, after combining the protein treatments, a strong trend for an effect of PRO vs MDP was observed (0.69kg: -0.08, 1.46) similar to that shown for untrained young adults via unadjusted meta-analysis [324]. However, the absolute change in daily protein intake did not correlate with changes in lean mass. These data add further support to the concept that increasing

absolute protein intake above normal intakes (1.2-1.3 g/kg/d) does not add further enhancement to lean mass during resistance exercise training [373, 483]. Rather, the timing or distribution of protein throughout the day is likely to play a more pivotal role in the regulation of lean mass.

The enhancement in lean mass with PB or WP did not translate to improved strength at the time of our assessment. This finding is well aligned with similar reports in the literature demonstrating improvements in DEXA lean mass [333, 344, 396] and/or muscle CSA [53, 78, 271, 340, 344, 368, 484, 485] against an isocaloric placebo without a concurrent enhancement in strength. In fact, enhancement in strength during RET is not a universal finding with protein supplementation [349], and is non-existent when comparing supplement types [333, 339, 342-344, 375, 379, 385, 486]. We are aware of data from only one investigator, which has not been duplicated, describing a rather tight relationship with changes in muscle strength and mass during protein supplementation and RET [270, 363, 452]. It has been repeatedly demonstrated that accrual of lean/muscle mass is not well coupled to changes in strength early on in a strength training program regardless of supplementation, even after substantial hypertrophy [487]. Further exercise training may be needed to build neuromuscular efficiency/coordination. Yet, this may require investigations beyond 21 [78] or 36 [333] weeks of RET. Also, it could be argued that the strength measures were non-specific to the assessment of muscle/lean mass, but we conducted both repetition maximum and maximum voluntary contraction testing, which likely would have captured an effect.

The increases in lean mass demonstrated in young subjects during resistance exercise training are always assumed to be muscle, yet the effect of protein

supplementation on DXA lean mass has only been tested at the whole body level [324], and rarely on appendicular lean mass, which is a better representation of muscle mass. Only a small proportion of studies have included data describing regional changes in lean mass during RET and PRO/AA supplementation [329, 488]. It is possible that changes in lean mass may not reflect contractile protein accrual and may partially explain why changes in lean mass are infrequently coupled to changes in muscle strength.

In lieu of these observations, it is surprising that we demonstrated an effect of protein, regardless of type, to enhance isokinetic knee extension torque. This finding may be reflective of a protein supplement induced enhancement of MHC IIa fibers seen with RET and protein supplementation [485], suggesting future analysis of fiber types is warranted. Even though strength is rarely enhanced with protein supplementation, the additional lean mass acquired with PB supplementation may confer additional health benefits, especially in aging or clinical populations [430].

Interestingly, BMC only improved in the protein-supplemented treatments. However, these changes are minimal and reside within the error of the measurement. Importantly, these data provide further support to the concept that protein supplementation, and protein type (whey vs protein blend) in particular, does not impair bone health during chronic resistance training [489].

All treatments improved body composition and decreased % body fat, but PB participants did not statistically decrease total body fat. Several participants randomized to the PB group were already very lean at Pre and actually displayed slight increases in fat mass (N=7), yet they remained leaner than the mean values for MDP and WP at Post. Although the WP and MDP groups lost fat mass and PB did not, the Post means for the

PB group were 1-2 kg lower (non-significant) than WP and MDP. This may partially explain why the PB demonstrated higher hunger and satiety responses at 9-12 week, yet the energy and macronutrient intake was not different across treatments or over time.

This is the first study, to our knowledge, examining the hunger and satiety responses during RET with protein supplementation. Our hypothesis was that the divergent digestion patterns and amino acid contents specific to whey protein and the protein blend could have distinct effects and that the gut would adapt to accommodate the chronic supplementation. We found similar responses during 3-week blocks, which surprisingly represented changes in exercise intensity, with the last 3 weeks being the most strenuous period (4x8 reps at 80%1-RM). The overall hunger and satiety responses were similar between groups in the immediate hours following supplementation. The responses were also very similar between groups immediately before the first meal following supplementation with a slight exception during the last 3 weeks of exercise training. WP responses of perceived food they could consume decreased over time and the PB maintained, which resulted a treatment difference between the two groups at 10-12 weeks. This finding may likely reflect the observation the WP group consumed ~400 total kcals more (non-significant, $p=0.2$) than PB at Post and may have felt that they could eat less because they were full from eating more. Another interesting finding was that the perceived hunger immediately before the first meal following supplementation increased during the last 3 weeks during supplementation in PB. The reasons for this response are unknown but may reflect an interaction between the change in exercise intensity and slower gastric emptying inherent in the blend of proteins [490]. Even more intriguing was that the change in arm lean mass, which was greatest in PB and was

correlated (r=0.36, p=0.014) with pre-meal perceived hunger during the last 3 weeks of exercise training. Some individuals who have difficulty gaining lean mass require high-calorie protein supplements and exercise training in order to maximize lean mass growth, however, future research should determine if the challenges with consuming additional energy can be attenuated by consumption of a protein blend.

Previous research has indicated that post-training blood leucine levels reflect changes in whole body lean mass during resistance training and whey protein supplementation [333]. With our data, none of the amino acids were correlated ($p>0.10$), at any time point, with lean mass change. However, there was a general trend for greater levels of amino acids post-training, which is likely a factor of the increase in lean mass serving as a reservoir of amino acids.

Although, meta-analysis has demonstrated that soy protein does not alter testosterone profile [491, 492], this idea still remains a common misconception. As further support of the evidence, we found no changes in serum testosterone with a soy-dairy protein blend or whey protein during resistance exercise training.

Our findings with the PB suggest a promising strategy for older adults to maximize muscle growth. Thus, older adults are less likely to decrease habitual macronutrient intake, as they commonly do during RET supplement studies on WP [336]. This may be one reason why the spread of protein between placebo and treatment needed to find an effect of PRO [348] has not easily demonstrated in older adults [336]. By combining PB supplementation during resistance training with an even spread of protein during the day [374], older participants may be able to maximize muscle growth. Although, this extra lean mass may not translate to improved strength/function, these

outcomes would improve with exercise training. More importantly, the additional lean mass may serve as a "buffer" during critical catabolic events (sickness, falls etc.), thus slowing the development of sarcopenia and loss of muscle mass essential to maintain strength/function.

Limitations

A potential limitation to our mid testing results is that they were taken the morning following an exercise-training day. This time point may represent an acute assessment in the trained condition. The changes in lean mass and muscle thickness may be an overestimation at this time point due to a transient increase in muscle water [289]. Also, knee extension strength at this time was depressed, indicating some overreaching may have been occurring during this phase of the exercise progression.

CONCLUSION

We previously demonstrated that soy-dairy protein blend supplementation prolongs muscle protein synthesis and muscle protein net balance [189, 225]. We followed up with these acute findings by demonstrating here that protein blend supplementation is an effective strategy to enhance lean mass growth during resistance exercise training in young adults. Although, there were no differences in strength increases between treatments, the protein blend was able to demonstrate greater trends for increases in whole body and arm lean mass compared to maltodextrin placebo. In addition, only the protein blend was able to continue accruing lean mass over the last 6 weeks of exercise training and supplementation. These improvements occurred without any changes in serum testosterone. The additional lean mass may serve as an amino acid buffer against periods of sickness and disuse, such that essential muscle contractile protein can be maintained. This is a promising strategy to enhance lean mass growth during resistance exercise training in older adults, who are a greater need for preservation of lean mass during the aging process.

CHAPTER 5

Effect of Protein Supplementation Type during Resistance Exercise Training on Fiber-Type Specific Myofiber Growth, Satellite Cells and Myonuclei

INTRODUCTION

Many reports from the results of acute molecular and metabolic investigations demonstrate the additive anabolic effect of protein/amino acid supplementation following an acute resistance exercise (RE) session [43, 47, 52, 54, 56, 64, 65, 232, 240, 253], yet there is less certainty whether chronic protein supplementation during RET enhances muscle growth and strength as compared to RE training without protein supplementation [324, 349, 353]. Overall, the evidence suggests that protein supplementation may confer enhancement of myofiber growth, at least for some individuals. We have demonstrated that a protein blend of soy, whey and casien is just as effective in stimulating muscle protein synthesis when compared to whey protein alone [189, 225].

The prevailing theory for contraction-induced myofiber growth posits that acute elevations in protein synthesis accumulate muscle protein, to expand the myofiber. This expansion strains the myonuclear domain, the area of a myofiber maintained by one myonucleus to regulate essential cell function. Several stimuli during this process activate satellite cells, which have several functions, including self-renewal, maintenance of the myofiber environment, repair/remodeling of myofibers and need to undergo terminal differentiation and fusion to current myofibers as myonuceli, (i.e. myonuclear

addition) to facilitate additional hypertrophy. The literature has been dominated by reports about how supplemental protein may influence the early muscle growth response (i.e. muscle protein synthesis), yet very little is understood regarding the effect of protein type and/or the influence of protein supplementation during chronic resistance exercise training on further mediation of muscle growth and adaptation through expansion of the satellite cell pool and via myonuclear addition.

In 2006, Olsen et al. first demonstrated that chronic resistance exercise training (RET) with protein supplementation may provide a slight enhancement of the satellite cell pool compared to RET alone [435]. Recently, Farup and colleagues have expanded upon these solitary findings by demonstrating that this effect is muscle fiber-type-specific, as reported in results from both acute [210] and chronic investigations [382]. These findings in human skeletal muscle studies have been sustained by basic scientific and pre-clinical approaches demonstrating enhanced myogenic proliferation via mTOR signaling [205, 206] prompted through nutrient provision, in particular the leucine metabolite HMB [204, 205]. Farup et al. conducted well designed studies to assess the interaction of contraction mode (concentric vs. eccentric) and protein supplementation on myofiber growth, and expansion of the satellite cell and the myonuclear pool [382]. However, no study has determined the effect of protein supplementation during traditional RET, with concurrent concentric and eccentric muscle action, on expansion of the satellite cell (SC) pool and myonuclear addition at the fiber-type-specific level. Using a large cohort of young men, our goal was to expand these findings by determining the role of protein supplementation type, protein blend (PB) vs whey protein (WP), on fiber-type-specific myofiber growth and SC/myonuclei accrual, as compared to a

maltodextrin placebo (MDP). Since the PB and WP contain similar leucine levels, 2 and 2.3g, respectively, our hypothesis is that a high quality protein supplement (soy-dairy PB) will enhance myofiber growth and satellite cell and myonuclei content over isocaloric MDP, and will elicit comparable effects to WP supplementation during RET.

MATERIALS AND METHODS

Participants

We recruited healthy male participants for this double-blind, randomized clinical trial. Participant characteristics are shown in Table 5.1.

Table 5.1. Baseline participant characteristics[1]

	PB (N=22)	WP (N=15)	MDP (N=17)
Characteristics			
Age, *years*	24.1 ± 0.6	24.6 ± 1.0	25.2 ± 1.1
Height, *cm*	178.6 ± 1.5	180.0 ± 2.1	176 ± 1.6
Weight, *kg*	77.5 ± 2.3	83.5 ± 3.4	76.3 ± 1.3
BMI, kg/m^2	24.3 ± 0.6	25.7 ± 0.9	24.5 ± 0.8
Muscle Thickness, *cm*			
Vastus Lateralis	2.37 ± 0.10	2.49 ± 0.11	2.30 ± 0.09
DEXA Lean Mass, *kg*			
Whole Body	56.2 ± 1.3	58.9 ± 2.3	55.4 ± 1.7
Leg	19.0 ± 0.4	20.7 ± 1.0	18.7 ± 0.8

[1]Data are mean ± SEM. Protein blend (PB), whey protein (WP) and maltodextrin placebo (MDP).

The participants were recruited through locally posted flyers, newspaper advertisements, and by word of mouth. After initial contact, prospective participants filled out a pre-screening questionnaire to determine eligibility and availability to participate. Individuals who could potentially participate were screened in the morning after an overnight fast at the Institute for Translational Sciences-Clinical Research Center (ITS-CRC) at the University of Texas Medical Branch (UTMB). The screening day

included 3-day food diary analysis, strength testing, a clinical history, physical exam, resting ECG, and laboratory tests (complete blood count with differential, liver and kidney function tests, coagulation profile, fasting blood glucose, hepatitis B and C screening, an HIV test, thyroid stimulating hormone level, lipid profile, urinalysis, and drug screening). Participants with clinical signs of malnutrition, those who were on anabolic steroids or corticosteroids in the past 6 months, current tobacco users, admitted vegan or vegetarians, individuals on a high-protein diet, high soy diet (>2 servings of soy per day), high dairy diet (> 6 servings of dairy per day), and those currently using protein supplements or having dairy allergies were excluded. The participants were healthy and recreationally active, but were not engaged in any regular exercise-training program (<2 sessions of high-intensity aerobic or resistance exercise/week) at the time of enrollment. All participants gave written informed consent before enrollment in the study. The study was approved by the UTMB Institutional Review Board, and is in compliance with the Declaration of Helsinki as revised in 1983. Of the 70 participants who underwent baseline testing, 2 withdrew before undergoing exercise training (WP: n = 1, PB: n = 1), 4 withdrew during the first 6 weeks (MDP: n = 3, WP: n = 1), and 6 withdrew during the last 12 weeks (MDP: n = 4, WP: n = 2). Of the 58 study completers, longitudinal, snap frozen muscle sections necessary for immunohistochemical analysis could not be obtained for 4 participants, thus all the data provided herein are from the 54 completers for whom we have data on the primary immunohistochemical outcomes (PB: n = 22, WP: n = 15, MDP: n = 17).

Study Design

Following enrollment, participants completed a 10-14 day pre-training, run-in period that consisted of the pre-training study day at UTMB and then 3 non-consecutive days of exercise familiarization and baseline 1-repetition max (1-RM) strength testing at the University of Texas Medical Branch Alumni Fieldhouse. At the run-in, participants were given a study binder containing, study information, food diary record instructions and supplement logs.

The pre-training study day included assessment of body composition, *vastus lateralis* muscle thickness, a muscle biopsy, serum collection, and strength testing via dynamometry. Two to three days later, the participants reported to the UTMB Alumni Fieldhouse for familiarization/testing before beginning 12 weeks of training. After 12 weeks of training, participants were re-tested exactly 3 days following the final exercise session of the training program. For the post-testing, participants reported to the ITS-CRC at the same time in the morning as for the pre-training study day to repeat the same laboratory tests and sample collection.

Resistance Exercise Training

Following familiarization and 1-repetition maximum (1-RM) strength testing, participants began a 12-week whole-body progressive resistance exercise-training (RET) program. All exercise-training sessions were performed at the UTMB Alumni Fieldhouse. Exercise sessions were performed on non-consecutive days, 3 times weekly, with 4 rest days per week, under supervision of qualified personal trainers. Participants were allowed to maintain their recreational physical activity, but instructed not to do any other strength training outside the study. RET was performed at an intensity of 60-80%

of 1-RM and consisted of 3-4 sets of 8-10 repetitions performed to failure for each exercise. In week 1, 3 sessions were conducted with 3 sets of 10 repetitions at 60% 1-RM. In weeks 2-8, 2 sessions per week were performed with an intensity of 70% 1-RM, where 3 sets of 10 repetitions were performed to momentary muscular failure. Each session consisted of whole-body resistance exercise that lasted ~60-70 min. To reduce the risk of injury and overtraining, one additional training session per week was conducted with 3 sets of 10 repetitions at 60% 1-RM with the goal of not reaching momentary muscular failure. These sessions were scheduled to occur as the training sessions immediately before and after the 1-RM training days. In weeks 9-12, 2 sessions per week were performed at an intensity of 80% 1-RM, where 4 sets of 8 repetitions were performed to momentary muscular failure. The 3rd session was performed at an intensity of 60% 1-RM, as before. Each session consisted of whole-body resistance exercise that lasted ~70-90 min. Resistance exercises included flat and incline chest press; leg press, curl and extension; seated pull-downs and rows; calf raises; and abdominal exercises. Participants rested for 1-2 minutes between exercises and individual sets. 1-RM was directly tested on the chest press, leg press, and the knee extension. 1-RM was estimated with 8-RM testing on the remaining exercises. Strength was re-tested at 3, 6 and 9 weeks so as each participants' strength increased, absolute training loads could be adjusted to maintain a relative training intensity between 60-80% 1-RM. 1-RM strength testing was performed again at the completion of the training program as the final exercise session. During and after each training session, the personal trainer recorded the sets, reps and total weight lifted along with other relevant notes, which were entered into a secure database. To allow for unforeseen life events, participants were given 13 weeks

following the familiarization period to complete 36 exercise sessions. This allowed for 100% exercise compliance.

Supplementation

Participants were randomized (20 per group) to the Placebo (MDP), Whey (WP) or Blend (PB) groups. The PB and WP groups were pooled to reflect protein supplementation (PRO) overall. Immediately following each workout, under direct observation of the study personnel, the participants ingested either the placebo beverage or one of the protein supplements to which they were assigned. On the four resting (non-exercise) days each week, the participants ingested the placebo or supplement one time between meals. Participants were instructed to refrain from any other food or macronutrient-containing beverage for 2 hours before or after exercise or supplementation.

Whey and protein blend samples were provided by DuPont Nutrition & Health (St. Louis, MO) and were independently tested for amino acid profile. The soy-dairy blend (PB) was composed of 25% soy protein isolate, 25% whey protein isolate, and 50% sodium caseinate. The whey (WP) treatment consisted of 100% whey protein isolate and carbohydrate placebo (MDP) was an isocaloric maltodextrin mixture. The dose for the two protein nutritional supplements was ~22 g protein/day. This dose has been chosen on the basis of the laboratory's preliminary data showing that this protein dose for all protein supplments will contain an amount of leucine sufficient to acutely maximize protein synthesis (i.e., ≧2 g of leucine). Therefore, the leucine was 2.00 g for the PB and 2.31 g for the WP. Supplements were separated into individual ready-made packets for daily consumption, and participants were given a 2-week supply. The personal trainer

collected the empty supplement packets from each subject every 2 weeks. Supplements and placebo were given in powder form and dissolved in 300 ml water to ensure a rapid and predictable absorption.

Pre and Post-testing Study Days

Participants reported to the ITS-CRC at UTMB in the morning following an overnight fast. They were instructed to refrain from any medication that effects muscle metabolism, and also caffeine, fish oil supplements, and alcohol for several days before testing. They were instructed to avoid strenuous or long-duration exercise for 3 days before arrival and to drink a liter of water the night before. After arrival on the unit, participants voided to ensure an empty bladder and bowel, and then lay supine for 30 min prior to assessment of body composition by DXA scan (dual-energy X-ray absorptiometry) (Hologic ADR 4500W, Bedford, MA). The same technician set the regions of interest for all the DXA scans.

To maintain a supine position, participants were transported to and from their CRC bed on a stretcher. After the DXA scan, ultrasound (Phillips HDI 5000) of the *vastus lateralis* (VL) was conducted while the participant lay in bed, as previously described [481]. Briefly, several B-Mode real time images of the VL were taken in the mid-sagittal position at 50 and 75% of the femur length (from the anterior superior iliac spine to the superior border of the pattella). The ultrasound head position, pre- and post-training, was placed relative to specific measured landmarks. The image that offered the sharpest contrast with the femur was chosen to ensure perpendicular placement of the scan head. LV muscle thickness was assessed as the average distance from the superficial aponeurosis to the deep aponeurosis at these two locations. Preliminary

testing, on the same individuals, revealed that the within-day and week-to-week coefficient of variation for measurements was 1.42±0.20 and 1.84±0.40%, respectively.

A percutaneous biopsy sample of the VL muscle was performed using a 5 mm Bergström biopsy needle [493]with suction, under sterile procedure and local anesthesia (1% lidocaine). The sample was aliquoted and snap-frozen in liquid nitrogen and stored at -80◦C for future analysis. Suitable longitudinal muscle cross-sections were carefully laid on Tissue Tek Optimal Cutting Temperature (OCT; Thermo Fisher Scientific, Rockford, IL) affixed to cork, submerged in liquid nitrogen-cooled isopentane, and then placed on dry ice until they could be stored at -80◦C until subsequent immunohistochemical analysis.

Peak torque and power of the knee extensors and knee flexors of the non-biopsied leg were subsequently determined by dynamometry (Biodex Medical, Shirley, NY) of the non-biopsied leg. Participants were previously familiarized with the test at the screening session. Briefly, participants were restrained in the dynamometer, with the anatomical access of the knee joint of their leg aligned with the mechanical axis of the dynamometer. Range of motion was measured from 90º to 10º (0º = full extension). After demonstration of proper technique and an explanation of the strength-test protocol, participants performed practice contractions to warm-up and re-familiarize themselves with the dynamometer. Thereafter, isometric peak torque (extension and flexion) was determined at a 60◦ angle of knee flexion over 3 maximal voluntary contractions (5 seconds long) with 90 seconds rest between attempts. Then, isometric peak torque and power (extension and flexion) were determined from 4 attempts at an angular velocity of

120◦/sec and was set relative to total body weight. The coefficient of variation for these repeated measures of strength is <4%.

Following the strength test, participants were fed a meal before leaving the unit. All testing was repeated on the post-testing day in the same order.

Nutritional Intakes

Participants were instructed to maintain their habitual diet and to log a 3-day food diary on 3 occasions: pre-testing, mid-testing and post-testing. On each occasion participants were given detailed instruction and were told to record their normal diet in the week before the testing day on two weekdays and one weekend day, with emphasis that one of the days was the day before testing. These records were entered into Nutrition Data System for Research 2012 to estimate energy intake and macro-nutrient composition.

Treatment Compliance

Treatment compliance was confirmed via supervision of post-exercise supplementation at the field house, tallying the number of returned and empty packets and with documentation of the self-reported supplement log.

RNA Isolation

RNA isolation, cDNA synthesis, and real-time qPCR were performed as we have previously described [481]. Total RNA was isolated by homogenizing 10-20 mg of tissue with a hand-held homogenizing dispenser (T10 Basic Ultra Turrax, IKA, Wilmington, NC) in 1 ml of Tri reagent. The RNA was separated into an aqueous phase using 0.2 ml of chloroform and subsequently precipitated from ~475µl of aqueous phase using 0.5 ml

of isopropanol. Total RNA was quantified by measuring the total volume of the aqueous phase as previously conducted [72]. RNA was washed twice with 1 ml of 75% ethanol, air-dried, and suspended in a known amount of nuclease-free water. RNA concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE).

Immunoblotting Analysis

Immunoblotting was performed as previously described [225]. In brief, 20-50mg of frozen muscle tissue was processed and assayed for total protein content. After further processing, each sample (50μg of total protein) was loaded in duplicate onto a 7.5% or 15% polyacrylamide gel (Criterion; Bio-Rad, Hercules, California) and subjected to electrophoresis at 150 V for 70 min. Following electrophoresis, proteins were transfered to a polyvinylidene difluoride membrane (Bio-Rad) which was then blocked in 5% non-fat dried milk. Membranes (blots) were then incubated with a single primary antibody overnight at 4°C. Rabbit polyclonal primary antibodies (Cell Signaling, Beverley, MA) used were the following: Akt (Ser308), mTOR (Ser2448), S6K1 (Thr389), 4E-BP1 (Thr37/46), ribosomal protein S6 (Ser240/244) and monoclonal alpha-tubulin (Sigma-Aldrich, St Louis, MO). Blots were incubated with secondary antibody (Amersham Biosciences) washed, and then a chemiluminescent solution (ECL plus, Amersham Biosciences) was applied. Optical density measurements were then immediately obtained with a digital imager (Bio-Rad) and densitometric analysis (Quantity One software, version 4.5.2; Bio-Rad) was performed. Following detection of phosphorylated proteins, blots were stripped of primary and secondary antibodies and then re-probed for

total protein, which was determined for each blot. Data were normalized to an internal control and expressed as phosphorylated and total protein or relative to alpha-tubulin.

Muscle Water and Protein Composition

Muscle water content and protein concentration analyses were conducted as previously described [494]. The wet weight of a muscle sample (~10 mg) was determined on a precision microbalance and subsequently freeze-dried for 72 hr. Muscle water content was calculated from the difference in dry and wet weight for each muscle sample and expressed as percentage of initial wet weight. Each muscle sample was then homogenized in 40 volumes of cold homogenization buffer (250 mM sucrose, 100 mM potassium chloride, 20 mM imidazole and 5 mM EDTA; pH 6.8) in a ground glass homogenizer. Samples were centrifuged at 21,000 g for 30 min at 4°C. The supernatant was taken as the sarcoplasmic protein fraction and the remaining pellet was re-suspended in 40 volumes of the same buffer with gentle sonication on ice and taken as the myofibrillar fraction. Aliquots of the homogenate (total protein), sarcoplasmic and myofibrillar protein fractions were then measured for protein concentration, in triplicate, using the bicinchoninic acid assay (Thermo Scientific, Rockford, IL) with bovine serum albumin used as the protein standard [495]. The amount of protein in each of the three fractions was normalized to the wet weight and dry weight of the muscle.

Immunohistochemistry

Immunohistochemical techniques were conducted as previously described [496]. Samples were removed from the cork at -25°C in a ThermoFisher Cryostat (Fisher Scientific HM 525X) where they were cut in 7 μm cross-sections. Pre and post samples

for the same subject were placed on the same slides Fisherbrand Superfrost®/Plus microscope slides (Fisher Scientific, USA). Two slides were generated per subject, one for analysis of myofiber myosin heavy chain (MHC) typing and cross-sectional area (CSA) and the other for fiber-type-specific satellite cells and myonuclei. Following cutting, a hydrophobic marker (Vector, H-4000, Burlingame, CA) separated the sections, which were dried at room temperature (RT) and then stored at -20°C until analysis.

Myofiber MHC type and CSA was determined as following. Sections were rehydrated in phosphate buffered saline (PBS) for 2 x 5 minutes at RT. Slides were incubated for at least 1 h at RT and then overnight at 4˚c with primary antibodies, mouse anti-myosin heavy chain (MHC) type I (BA.D5 IgG2b, 1:50, Developmental Studies Hybridoma Bank, Iowa City, IA) in 1:1 ratio of supernatant with mouse anti-MHC IIa (SC.71 IgG1, Developmental Studies Hybridoma Bank) and mouse anti-MHC IIx (6H1 IgM, Developmental Studies Hybridoma Bank). Slides were rinsed 3 times for 5 min each with PBS followed by 1 hour incubation with secondary antibodies diluted in PBS, Alexa Fluor 488 conjugated goat anti-mouse IgG1 (for MHC IIa: 1:500, #A21121, Invitrogen, Carlsbad, CA), Alexa Fluor 647 conjugated goat anti-mouse IgG2b (for MHC I: 1:250, #A21242, Invitrogen) and Alexa Fluor 594 goat anti-mouse IgM (for MHC IIx: 1:250, #A21044, Invitrogen) at RT in the dark. Slides were rinsed 3 x 5 minutes each with PBS, before and after a 5 minute post-fix in methanol. Slides were mounted with fluorescent mounting media (Vector, H-4000) and dried before imaging. Staining procedures resulted in MHC IIa staining green, MHC I staining purple, and MHC type IIx staining red (Figure. 5.1).

Figure 5.1. Representative immunohistochemical image for identification of myosin heavy chain fiber typing and cross-sectional area

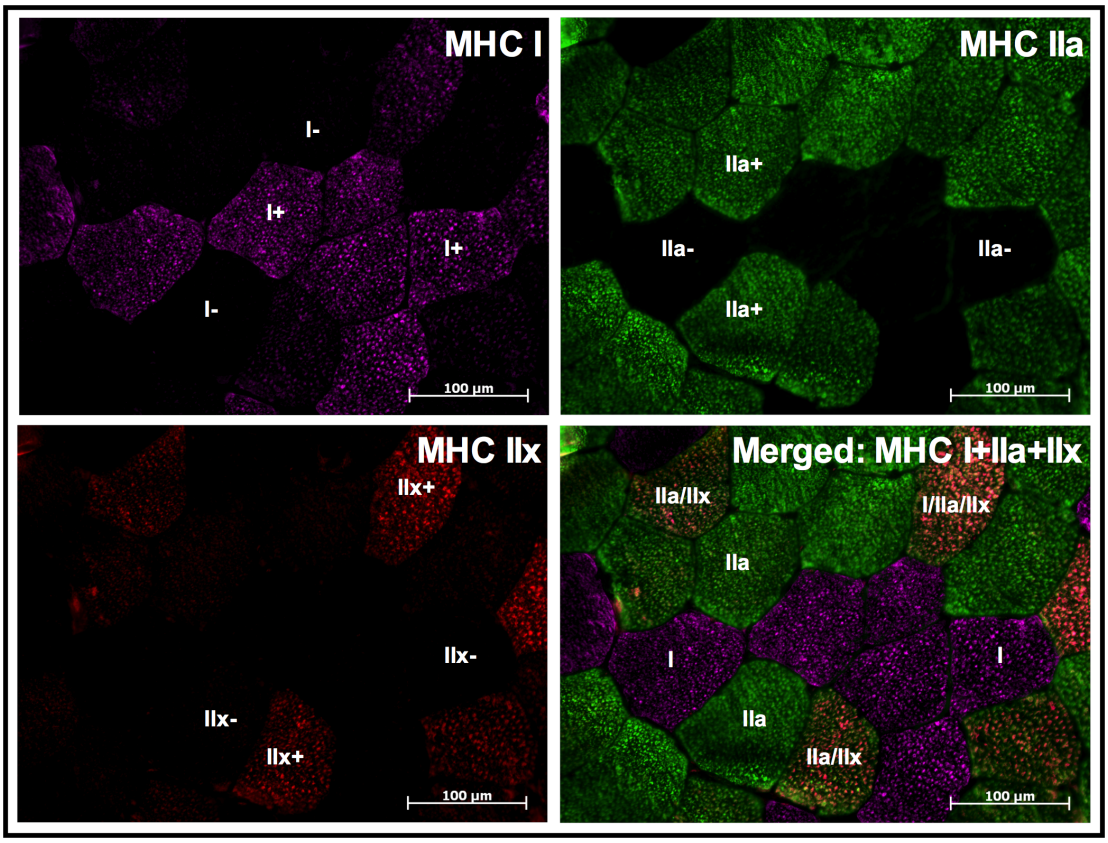


Fig 5.1. Representative immunohistochemical image for identification of myosin heavy chain fiber typing and cross-sectional area quantification in *vastus lateralis*. MHC I stained purple (top left), MHC IIa stained green (top right), and MHC type IIx stained red (bottom right) and merged image (bottom left).

Images for fiber typing were captured at 100x magnification using a fluorescence microscope (Axio Imager.M1m, Carl Zeiss, Toronto, Ontario, Canada) and AxioCam MRm camera (Carl Zeiss). Image processing and analysis was done using AxioVision 4.8.2 software. For each image, the number of muscle fibers for pure MHC type I, IIa, IIx and hybrid type I, I/IIa, I/IIx, IIa/IIx and I/IIa/IIx fibers were counted, and cross sectional areas (CSA) for MHC type I, IIa, IIa/IIx and I/IIa/IIx fibers were measured. Fibers with frequencies less than 1-2% (pure IIx and hybrid I/IIa and I/IIx) were removed from

further analysis. Hybrid denotes all hybrid groups combined. T2s is all MHC II (IIa+IIa/IIx) combined. About 250 muscle fibers were analyzed for fiber type distribution and ~200 for CSA in each sample (Appendix Table A.5.1).

Fiber-type-specific satellite cells and myonuclei were determined as follows. Sections were fixed in ice cold acetone for 3 minutes followed by three 3-minute rinses in PBS. Sections were incubated for at least an hour at RT and then overnight at 4°C with primary antibodies against MHC I (BA.D5 IgG2b, 1:50, Developmental Studies Hybridoma Bank) and Laminin (L9393, 1:200, Sigma-Aldrich, St. Louis, MO). On day 2, three 5-minute washes in PBS preceeded a 7 min H_2O_2 treatment (3% in PBS) to block endogenous peroxidases. After three 3- minute rinses in PBS, sections were incubated for 1 hour with the secondary antibodies, Alexa Fluor 647 conjugated goat anti-mouse IgG2b (for MHC I: 1:250, #A21242, Invitrogen) and Alexa Fluor 594 goat anti-rat IgG1 (for laminin: 1:500, #A11034, Invitrogen) diluted in PBS at RT in the dark. After three 3-minute rinses in PBS, sections were blocked for 1 hour in 2.5% normal horse serum (NHS) (Vector, S-2012) at RT. Sections were incubated for at least an hour at RT and then overnight at 4°C with a primary antibody against mouse anti-Pax7 (1:100, Developmental Studies Hybridoma Bank). On day 3 of staining, sections were rinsed 4 x 5 min with PBS before and after 1 hour incubation with goat anti-mouse IgG biotin –SP-conjugated (1:1000) (Jackson Immuno Research, Cat #115-065-205) in 2.5% NHS (for Pax7) at RT. Sections were exposed to a 1 hour incubation of Streptavidin-horseradish peroxidase conjugate (1:100) in PBS, washed, 3 x 5min in PBS, and incubated for 20 min in Alexa Fluor 488 (1:200, Tyramide signal amplification kit, #T20932, Invitrogen) in amplification diluents. Following three 5-min washes in PBS, sections were mounted in

4',6-diamidino-2-phenylindole (DAPI) containing medium mounting media (Vector, H-1200) and allowed to air dry. This staining protocol, of muscle fiber-type specific identification resulted in DAPI positive nuclei (staining blue), Pax7+ cells (staining yellow), MHC I (staining purple), MHC II (Black - negative staining) and laminin basement membrane (staining red) (Figure 5.2, next page).

Myonuclei were manually counted in images captured at 100x magnification using AxioVision 4.8.2 software to determine the number of myonuclei per fiber. A nucleus was identified as a myonucleus if it met one of the following criteria: 1) it was clearly located within the laminin boundary; 2) it was on the boundary facing inside the fiber; or 3) greater than 50% of the area fell inside the laminin boundary. Rapid, repeated manual switching back-and-forth between single channel laminin images and merged laminin/DAPI images was used to determine the location of a nucleus as inside or outside of the laminin boundary. Following counting of myonuclei within an image, fiber number was quantified manually to express the number of myonuclei per fiber specific to each fiber type (MHC I or II). Pax7+ nuclei/myofiber, % SC, myonuclei per fiber, and myonuclear domain (fiber area per myonuclei) were determined from > 200 cross sectional muscle fibers at each time point (Appendix Table A.5.2), as Mackey et al. [497] recommend that counting from a minimum of 125 muscle fibers is needed to obtain reliable data for satellite cell content.

Figure 5.2. Representative immunohistochemical image for fiber-type specific identification of Pax7 positive satellite cells and myonuclei

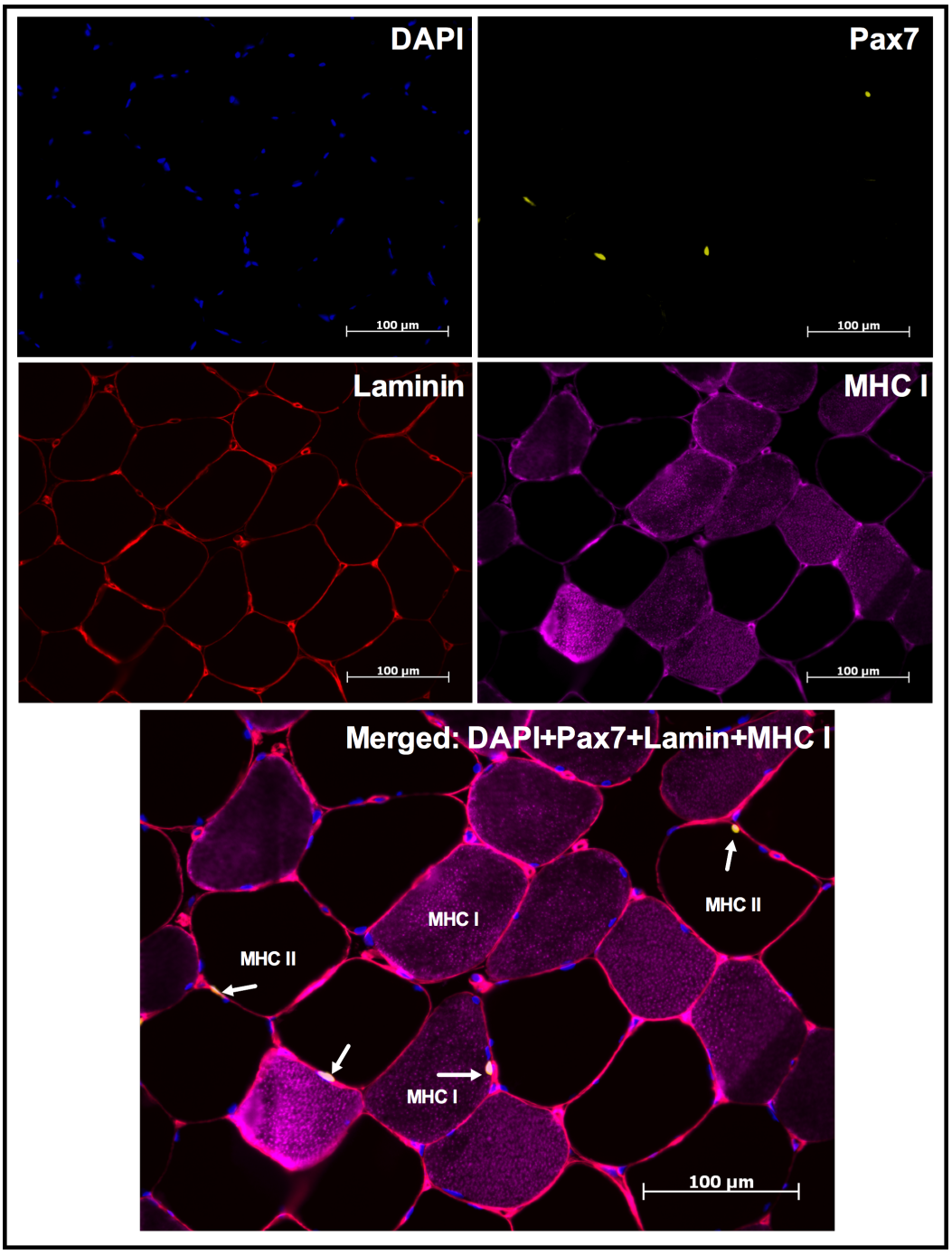


Fig 5.2. Representative immunohistochemical image for fiber-type specific identification of Pax7 positive satellite cells and myonuclei. DAPI positive nuclei stained blue (top left), Pax7+ cells stained yellow (top right), laminin basement membrane stained red (middle left), MHC I stained purple and MHC II black - negative staining (middle right) and merged image with arrows highlighting Pax7+ myonuclei (bottom).

Statistical Analysis

Values are the raw values or model-corrected estimates expressed as Mean ± SEM or Mean ± 95% CI. Data were transformed using the Box-Cox set of transformations to stabilize the variance and make the data approximately normally distributed. To test differences between treatments, the data were modeled using an ANCOVA model with baseline (pre) values as a covariate. Contrasts were used to test the difference between treatments, along with a Tukey adjustment for multiple comparisons. Each of the post-baseline time-points was analyzed separately, to allow for changes in variance at each time point. The data was then combined and analyzed using a mixed factors model, with time and group as fixed effects and differing within-group variances at each time point. If within each time point group variances differed significantly, transformations of the response were used to stabilize the variance. In the ANOVA Mixed Model subjects were set as a random effect, and treatment (PB, WP and MDP), and time (baseline [pre] and 12 weeks [post] as appropriate) were treated as fixed effects. To test the effect of protein supplementation we pooled the protein treatments WP and PB as PRO. An additional model was conducted with treatment effects of PRO and MP only. The fiber numbers analyzed and the MHC relative frequency were only examined in the mixed model. Treatment effects for change in mean myofiber CSAs and myonuclear number were tested with ANCOVA. CSA bin analysis satellite cell, myonucli and myonuclear domain changes and treatment differences were tested through ANCOVA of the absolute change from Pre to Post. Significance was set at $p<0.05$ with trends at $0.05 < p < 0.1$. All calculations were done in R 3.1.1, with the exception of

Pearson correlations, which were calculated with Graph Pad Prizm 6.0f for Mac (La Jolla, California USA). All figures were generated with the same program.

RESULTS

Subject Characteristics

Descriptive characteristics for all participants are shown in Table 5.1 (above). There were no differences between groups at baseline for any variable ($p>0.10$).

Treatment Compliance

Compliance was similar for all treatments with 92.3% (range: 80.5-100%), 90.8% (range: 77.7-100%) and 90.2% (range: 65.5-100%) for PB, WP and MDP respectively.

Habitual Energy and Macronutrient Intake

The average habitual (non-supplemented) nutritional intakes for all participants are shown in Table 5.2. There were no differences between groups at baseline or across time in for any outcome ($p>0.10$).

Table 5.2. Habitual energy and macronutrient intake by treatment before (Pre), 6 weeks (Mid) and 12 weeks (Post) resistance exercise training with nutritional supplementation

TRT	Time Period		
	Pre	Mid	Post
Energy, *MJ*			
PB	10.13 ± 0.93	10.30 ± 0.59	9.51 ± 0.68
WP	9.67 ± 0.27	10.30 ± 0.97	11.41 ± 1.02
MDP	9.51 ± 0.61	8.98 ± 0.56	9.10 ± 0.70
Protein intake, *g/kg/d*			
PB	1.33 ± 0.06	1.40 ± 0.11	1.27 ± 0.08
WP	1.27 ± 0.12	1.22 ± 0.17	1.48 ± 0.17
MDP	1.27 ± 0.11	1.17 ± 0.11	1.21 ± 0.14
Carbohydrate intake, *g/kg/d*			
PB	3.58 ± 0.35	3.71 ± 0.24	3.39 ± 0.25
WP	3.31 ± 0.24	3.38 ± 0.24	3.44 ± 0.35
MDP	3.36 ± 0.23	3.22 ± 0.15	3.41 ± 0.27
Fat intake, *g/kg/d*			
PB	1.19 ± 0.11	1.26 ± 0.12	1.10 ± 0.09
WP	1.19 ± 0.15	1.12 ± 0.15	1.38 ± 0.19
MDP	1.27 ± 0.13	1.14 ± 0.13	1.16 ± 0.12

[1]Data are mean ± SEM. Protein blend (PB), whey protein (WP) and maltodextrin Placebo (MDP). TRT = treatment.

Lean Mass, VL Muscle Thickness and Leg Anthropometry

Muscle hypertrophy was observed at the whole muscle level. Percent change in DXA whole-body lean mass was increased with all treatments ($p<0.05$); however, there was a strong trend for PB supplementation to show more of an increase than MDP ($p=0.056$) (Figure 5.3). Combined results from treatment with the two protein supplements (PRO) also showed a significant effect ($p=0.050$) compared to MDP (Figure 5.3). DXA Leg lean mass was increased with all treatments ($p<0.05$) (Figure 5.3) and not different by treatment. Thigh circumference and *Vastus Lateralis* muscle thickness were increased similarly with all treatments ($p<0.05$) (Figure 5.4, Appendix Table A.5.3). Leg volume was only increased with PB and WP supplementation, but not MDP ($p<0.05$) (Figure 5.4, Appendix Table A.5.3).

Figure 5.3. The percent change in whole body lean mass and leg lean mass

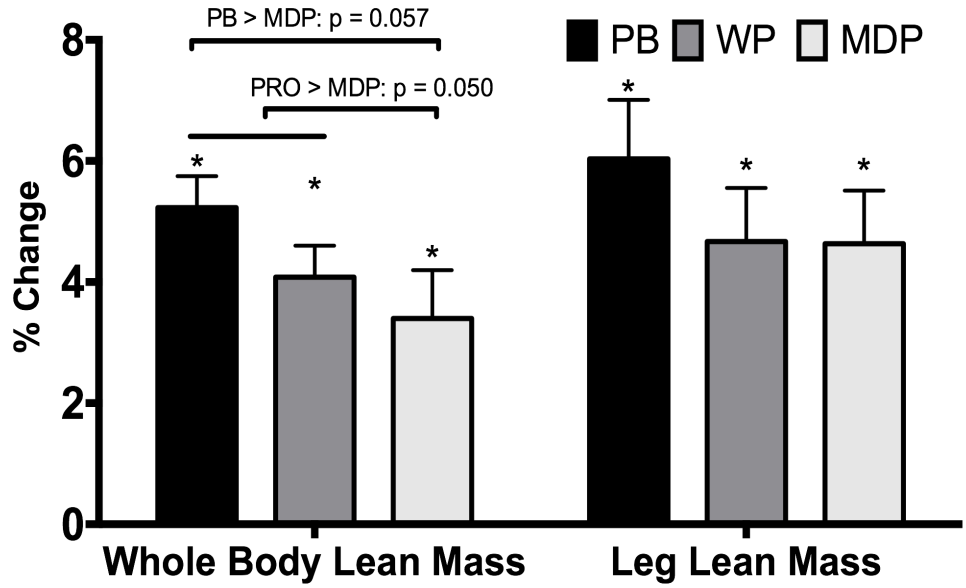


Fig. 5.3. The percent change in whole body lean mass and leg lean mass by treatment from pre to post (12 weeks) resistance exercise-training with nutritional supplementation. Protein blend (PB) or whey protein (WP) or maltodextrin placebo (MDP). Data are mean ± SEM, n=15 (WP), 22 (PB) & 17 (MDP). Significant change * ($p<0.05$). PB > MDP ($p=0.057$). PB+WP (PRO) > MDP ($p=0.050$).

Figure 5.4. The percent change in *vastus lateralis* muscle thickness, thigh circumference and leg volume

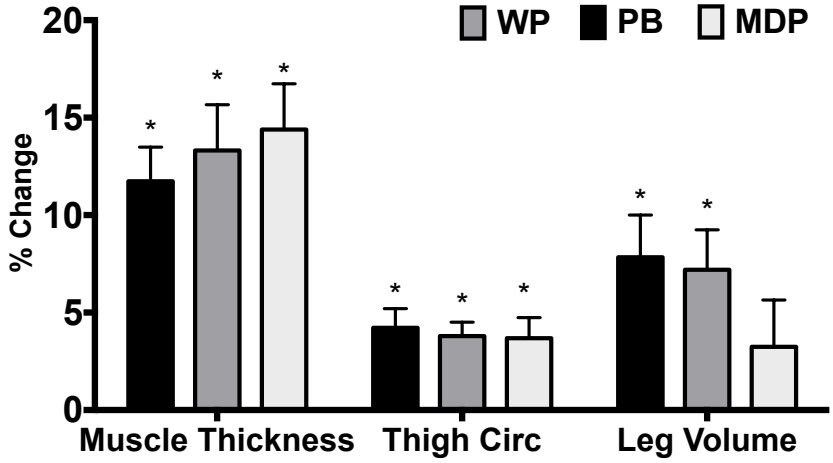


Fig. 5.4. The percent change in *vastus lateralis* muscle thickness, thigh circumference and leg volume (liters) by treatment from pre to post (12 weeks) resistance exercise-training with nutritional supplementation. Protein blend (PB) or whey protein (WP) or maltodextrin placebo (MDP). Data are mean ± SEM, n=15 (WP), 22 (PB) & 17 (MDP). Significant change * ($p<0.05$).

Isometric Strength and Isokinetic Strength and Power

At baseline, isometric and isokinetic peak torque (relative to body weight) and power for flexion and extension were not different between treatments (Appendix Table A.5.4). Isometric knee extension torque increased similarly in all treatment groups ($p<0.05$) (Figure 5.5, Appendix Table A.5.4). Isometric and isokinetic knee flexion torque did not change via the Mixed Model ($p>0.10$), but ANCOVA analysis demonstrated a significant increase in isometric knee flexion torque in subjects treated with MDP ($p<0.05$), PRO ($p<0.05$) and a trend with WP treatment ($p=0.066$). Isokinetic knee extension torque, and extension power did not change in subjects treated with MDP, but treatment with PB and WP similarly resulted in an effect of protein (PRO) was present compared to MDP for torque ($p=0.017$) and power ($p<0.001$). Also, for isokinetic knee extension torque the change in subjects treated with WP was greater than the change after treatment with MDP ($p=0.019$), whereas for knee extension power both protein treatments individually, resulted in greater changes vs treatment with MDP ($p<0.020$). Isokinetic knee flexion power demonstrated an effect of exercise training that was event in treatment with PRO ($p<0.05$), but was not different by treatment or in treatment with MDP ($p>0.10$).

Figure 5.5. The absolute change in isometric and isokinetic peak torque and isokinetic power for knee extension (Ext) and flexion (Flex)

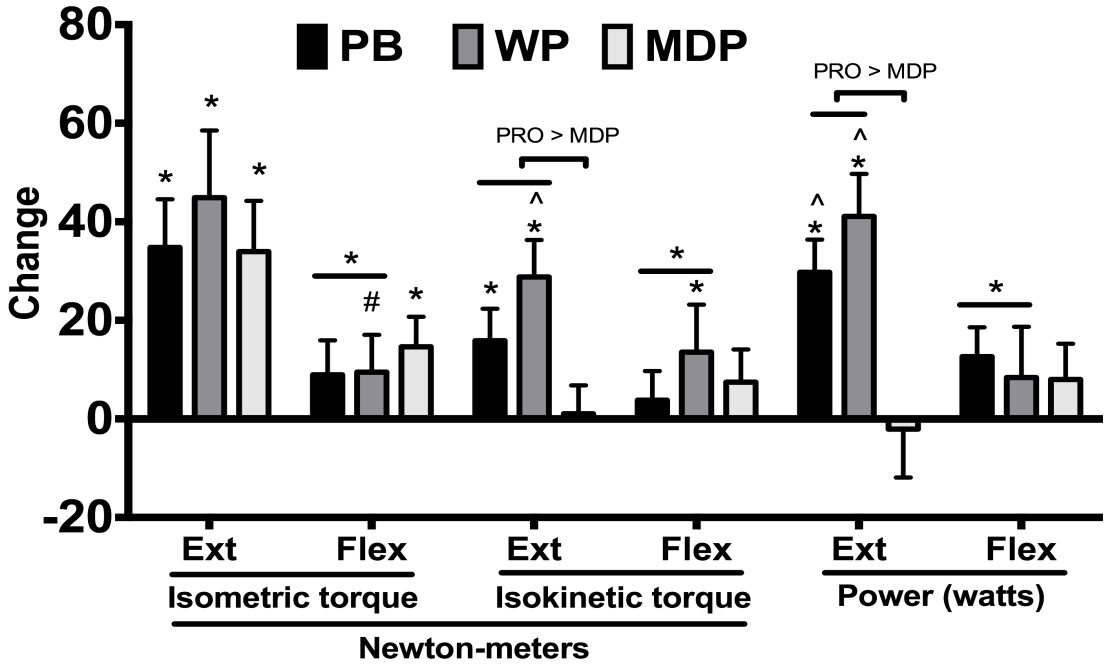


Fig. 5.5 The absolute change in isometric and isokinetic peak torque and isokinetic power for knee extension (Ext) and flexion (Flex) by treatment from pre to post (12 weeks) resistance exercise-training with nutritional supplementation. Protein blend (PB) or whey protein (WP) or maltodextrin placebo (MDP). Data are mean ± SEM, n=15 (WP), 22 (PB), 17 (MDP) 37 (PRO). Torque was newton-meters relative to body weight. Isokinetic force at a specific velocity (Power) was measured as watts. * ($p<0.05$), # ($p<0.1$) for the change from pre. ^ ($p<0.05$) vs change with MDP.

Muscle RNA Concentration

A proxy for translational capacity, *vastus lateralis* RNA concentration (Appendix Table A.5.5), was increased with resistance exercise training and did not differ by treatment ($p>0.10$).

Muscle mTORC1 Signaling

The pre- and post-training basal signaling of mTORC1-associated signaling proteins was relatively unchanged with exercise training and did not differ by treatment (Appendix Table A.5.6) ($p>0.10$). However, there were a few minor exceptions.

Phosphorylated mTOR was significantly increased following MDP treatment ($p<0.05$). Also, treatment with PRO demonstrated an increased level of total eEF2 ($p=0.056$), mTOR ($p=0.049$) and a trend for Akt ($p=0.070$). When normalized to alpha-tubulin no change was observed in the total protein levels. Only phosphorylated 4EBP1 was increased in treatment with PRO ($p=0.016$), yet a trend was observed for treatment with MDP ($p=0.098$).

Muscle Water and Protein Concentration

There was a decrease in percent vastus lateralis muscle water content (Table 5.3) that was presented as trend for a time effect ($p=0.066$) and an interaction ($p=0.048$), which was driven by a decrease in in subjects treated with PB ($p=0.034$) and PRO ($p=0.008$) from pre- to post-training. Total and crude estimates of myofibrillar vastus lateralis protein (Table 5.3) were not different by treatment and did not change over time when expressed as wet and dry weight. Sarcoplasmic protein (Table 3) expressed as wet weight increased 7.7% in treatment with PB ($p=0.034$), but did not change in WP or MDP treatment. This change was displayed as a trend to differ vs the change in treatment with WP ($p=0.094$) and MDP ($p=0.076$). When expressed as dry weight this effect was removed and did not change in WP or MDP ($p>0.100$).

Table 5.3. Water content and protein concentration of the *vastus lateralis* muscle by treatment before (Pre) and at 12 weeks (Post) resistance exercise-training with nutritional supplementation.

	PB		**WP**		**MDP**	
	Pre	Post	Pre	Post	Pre	Post
% muscle water	78.52 ± 0.27	**77.55 ± 0.28**	78.21 ± 0.35	**77.62 ± 0.33**	77.76 ± 0.22	77.98 ± 0.35
Total, wet	175.1 ± 5.1	178.0 ± 7.6	185.6 ± 10.2	185.9 ± 3.6	171.8 ± 9.2	179.0 ± 6.0
Sarcoplasmic, wet	45.3 ± 1.1	**48.7 ± 1.7&**	47.9 ± 1.9	44.4 ± 1.2	44.4 ± 1.4	43.9 ± 1.4
Myofibrillar, wet	129.8 ± 4.7	129.4 ± 6.7	130.8 ± 8.9	132.1 ± 4.1	127.3 ± 8.6	132.6 ± 6.3
Total, dry	817.3 ± 22.4	791.2 ± 31.3	845.0 ± 49.9	844.0 ± 21.2	775.6 ± 36.1	800.6 ± 30.0
Sarcoplasmic, dry	211.6 ± 5.2	215.9 ± 6.4	217.1 ± 8.8	201.6 ± 5.0	201.1 ± 4.9	198.6 ± 6.9
Myofibrillar, dry	605.6 ± 20.9	575.2 ± 28.4	593.9 ± 39.4	601.0 ±21.3	574.5 ± 34.9	601.8 ± 29.3

[1]Data are mean ± SEM, n=15 (WP), 22 (PB) & 17 (MDP). **Boldface,** ANCOVA difference from Pre ($p<0.05$). &, TRT difference from MDP and WP ($p<0.10$). Wet = µg protein / mg muscle wet weight. Dry = µg protein / mg muscle dry weight.

Vastus Lateralis MHC Fiber Type Composition

The pre- and post-training MHC fiber-type composition (Figure 5.6, Table 5.4) changes demonstrated a reduction in hybrid fibers, mainly I/IIa/IIx fibers, in all treatments ($p<0.05$). The reduction in hybrid fibers resulted in a shift toward more pure MHC IIa fibers that was significant for PB and PRO treatments ($p<0.05$). MHC type I, and IIa/IIx frequencies remained unchanged ($p>0.10$).

Figure 5.6. Myosin heavy chain composition (MHC) in the *vastus lateralis*

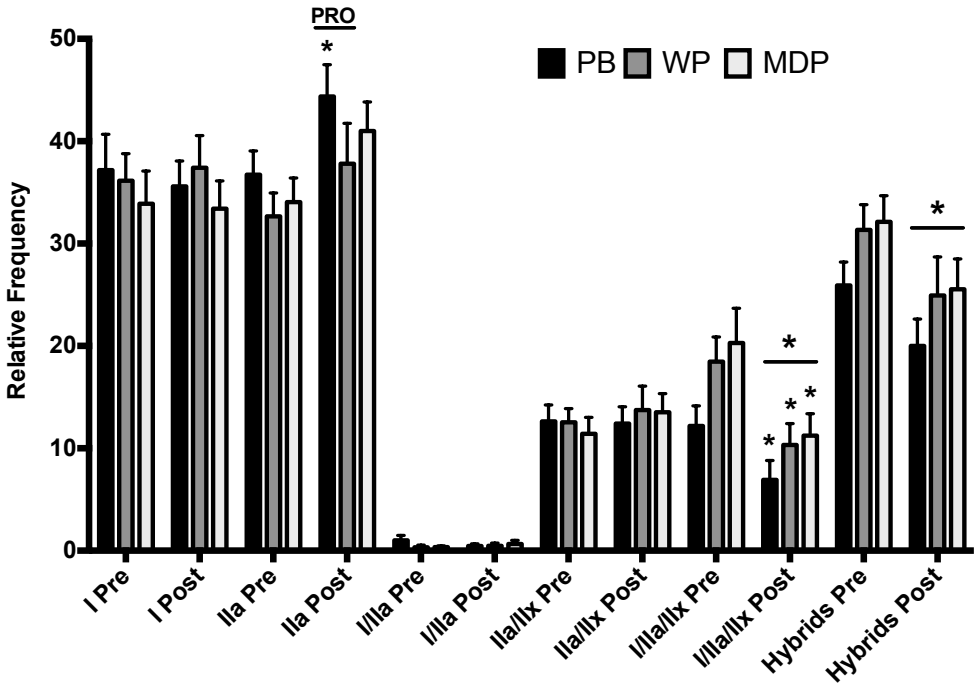


Fig. 5.6. Myosin heavy chain composition (MHC) in the *vastus lateralis* expressed as relative frequency. PRO indicates an effect of the pooled protein groups to increase over pre. Significant change vs. pre * ($p<0.05$). Bar indicates an exercise effect.

Table 5.4. Pre to post-training absolute change for fiber-typing and myofiber CSA immunohistochemical analysis

	Change				
Treatment	PB	WP	PRO	MDP	PRO vs MDP
MHC Typing (relative frequency)					
I	-0.9 (-5.1,3.4)	1.4 (-3.7,6.6)	0.3 ± 1.7	-1.6 (-6.4,3.3)	1.8 (-4.0,7.7)
IIa	**8.5 (2.9,14.1)**	4.2 (-2.5,10.9)	**6.4 ± 2.2**	**6.6 (0.3,12.9)**	-0.3 (-7.9,7.4)
I/IIa	-0.3 (-0.8,0.2)	-0.0 (-0.6,0.5)	-0.2 ± 0.2	-0.1 (-0.4,0.6)	-0.3 (-0.9,0.3
IIx	-	-	-	-	-
IIa/IIx	0.1 (-3.3,3.5)	1.4 (-2.7,5.6)	0.8 ± 1.3	1.5 (-2.4,5.4)	-0.7 (-5.5,4.0)
I/IIx	-	-	-	-	-
I/IIa/IIx **T PRO**	**-7.3 (-10.1,-4.4)**	**-7.2 (-10.6,-3.9)**	**-7.2 ± 1.1**	**-7.3 (-10.5,-4.1)**	0.1 (-3.8,3.9)
IIa+IIa/Iix **T**	**8.6 (4.7,12.6)**	**5.7 (1.0,10.4)**	**7.2 ± 1.5**	**8.3 (3.9,12.7)**	-1.1 (6.5,4.3)
Hybrids **PRO**	**-7.1 (-12.0,-2.2)**	-5.7 (-11.5,0.1)	**-6.4 ± 1.9**	**-5.6 (-11.1,-0.1)**	-0.8 (-7.5,5.9)
CSA					
I	**551 (184,919)**	**547 (105,989)**	**548 ± 143**	**755 (336,1174)**	-206 (-721,308)
IIa	**1006 (560,1453)**	**991 (455,1529)**	**992 ± 174**	**875 (362,1388)**	125 (-506,755)
I/IIa	-	-	-	-	-
IIx	-	-	-	-	-
IIa/IIx	**880 (325,1435)**	**1060 (412,1708)**	**970 ± 213**	**1080 (442,1719)**	-111 (-891,669)
I/IIx	-	-	-	-	
I/IIa/IIx	**880 (43.2,1717)**	**1262 (419,2105)**	**1071 ± 292**	751 (-32,1535)	320 (-665,1305)
IIa+IIa/IIx **PRO**	**1058 (609,1506)**	**975 (437,1513)**	**1016 ± 175**	**970 (453,1485)**	46 (-588,681)
Hybrids	**1027 (526,1528)**	**1186 (601,1770)**	**1107 ± 192**	**1040 (464,1616)**	67 (-636,769)
All	**898 (498,1307)**	**821 (326,1316)**	**859 ± 160**	**892 (419,1366)**	-33 (-617,551)

[1]Data are mean ± 95%CI or SEM, n=15 (WP), 22 (PB) & 17 (MDP). boldface $p<0.05$, underlined $p<0.10$ vs pre for that treatment.
T = $p<0.05$ for an overall change over time, PRO = $p<0.05$ for an overall change after treatment in the protein supplements from pre.

Vastus Lateralis Myofiber Cross-sectional Area

Vastus Lateralis myofiber cross-sectional area means (Figure 5.7, Table 5.4) were increased following resistance exercise training. Mean fiber area of all fiber types was increased ~800-900 um^2 following resistance exercise training ($p<0.05$). However, there was no effect of treatment ($p=0.967$). Mean MHC I CSA was increased ~500 um^2 after WP and PB treatment and ~750 um^2 after consumption of MDP following resistance exercise training ($p<0.05$). There was also no effect of treatment ($p=0.721$). The contrast test of individual treatment changes revealed significant increases after treatment with WP and MDP ($p<0.05$) and a trend for and increase ($p=0.083$) following treatment with WP. Mean MHC IIa and MHC IIa/IIx CSA was increased ~900-1100 um^2 following resistance exercise training ($p<0.05$) with no effect of treatment ($p=0.921$ for MHC IIa, $p=0.866$ for MHC IIa/IIx). The contrast test of individual treatment changes revealed significant increases in all groups ($p<0.05$). Mean I/IIa/IIx CSA was increased ~900-1300 um^2 following resistance exercise training ($p<0.05$) with no effect of treatment ($p=0.661$). The contrast test of individual treatment changes revealed a significant increase in WP ($p<0.05$) and only a trend for MDP to increase ($p=0.064$). Mean fiber area of all hybrid fiber types was increased ~1000-1100 um^2 following resistance exercise training ($p<0.05$) with no effect of treatment ($p=0.906$). PRO (PB+WP) treatment displayed significant increases in all fiber types ($p<0.05$). No significant effect of PRO vs MDP treatment was observed in any fiber type ($p>0.423$).

Figure 5.7. Fiber-type specific and mean (MFA) *vastus lateralis* cross-sectional area

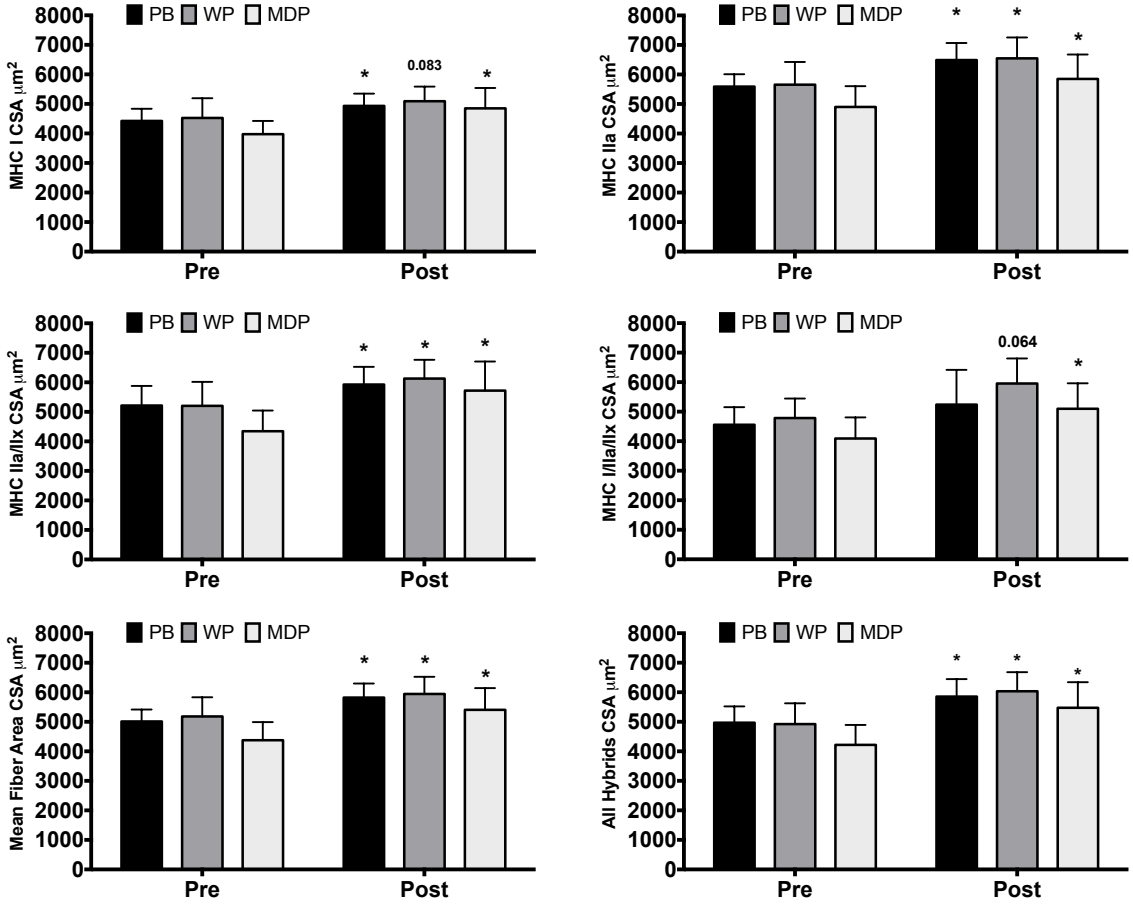


Fig. 5.7. Fiber-type specific and mean (MFA) *vastus lateralis* cross-sectional area by treatment from pre to post (12 weeks) resistance exercise-training with nutritional supplementation. Protein blend (PB) or whey protein (WP) or maltodextrin placebo (MDP). Data are mean ± SEM, n=15 (WP), 22 (PB) & 17 (MDP). Units are um^2. Significant change * ($p<0.05$).

Analysis of cross-sectional area (CSA) relative frequency distribution (Appendix Figure A.5.1) demonstrated that all treatments displayed myofiber growth (rightward shift). However there were slight trends for differences between groups not observed with CSA means shown in Figure 5.7. MDP treatment displayed a greater frequency of smaller fibers in the following bins at pre for MFA and Hybrids (2000 μm^2, 2500 μm^2), MHC I (2000 μm^2) and MHC II (2000 μm^2, 3000 μm^2, 3500 μm^2, 4000 μm^2) vs PRO treatment ($p<0.10$). MDP treatment also displayed a slightly greater change in the

frequency of MHC I bins (10500 μm^2, 11000 μm^2 and 11500 μm^2) vs PRO ($p<0.10$) treatment. Also, an effect of treatment was observed at MHC I bin 10500 μm2 for MDP treatment to have a greater change in the relative frequency than after PB treatment ($p<0.05$).

PRO (PB+WP) groups demonstrated a greater frequency of larger myofibers in the following bins at pre for MFA (6000 μm^2, 6500 μm^2), Hybrids (6000 μm^2), and MHC II (6000 μm^2, 7000 μm^2) vs MDP ($p<0.10$). PRO (PB+WP) treatment resulted in a slightly greater change in the frequency of larger MFA and MHC II myofibers in bins (7500 μm^2, 8000 μm^2 and 8500 μm^2 and 10000 μm^2) vs MDP ($p<0.10$). An effect of treatment was observed at MFA bin 8000 μm^2 for treatment with WP and PB to have a greater change in the relative frequency of these larger myofibers than following MDP treatment ($p<0.05$). An effect of treatment was observed at MHC II bin 10000 μm2 for PB treatment to have a greater change in the relative frequency than following MDP treatment ($p<0.10$). This effect for the protein groups to have a greater change in the frequency of larger fibers is seen in Figure 5.8 where CSA bins were expanded to reflect changes greater than 6000, 7000, 7500 and 8000 μm^2 and 1000 to 5000 μm^2. Only treatment with protein, PB and WP, resulted in a significant change in the frequency of larger MHC IIa myofibers. When examining these larger CSA bins very weak trends ($p=0.098$-0.194) were observes for an effect of protein (PRO) treatments vs MDP treatment.

Figure 5.8. Change in the relative frequency of *vastus lateralis* MHC II myofibers by select cross-sectional area bins

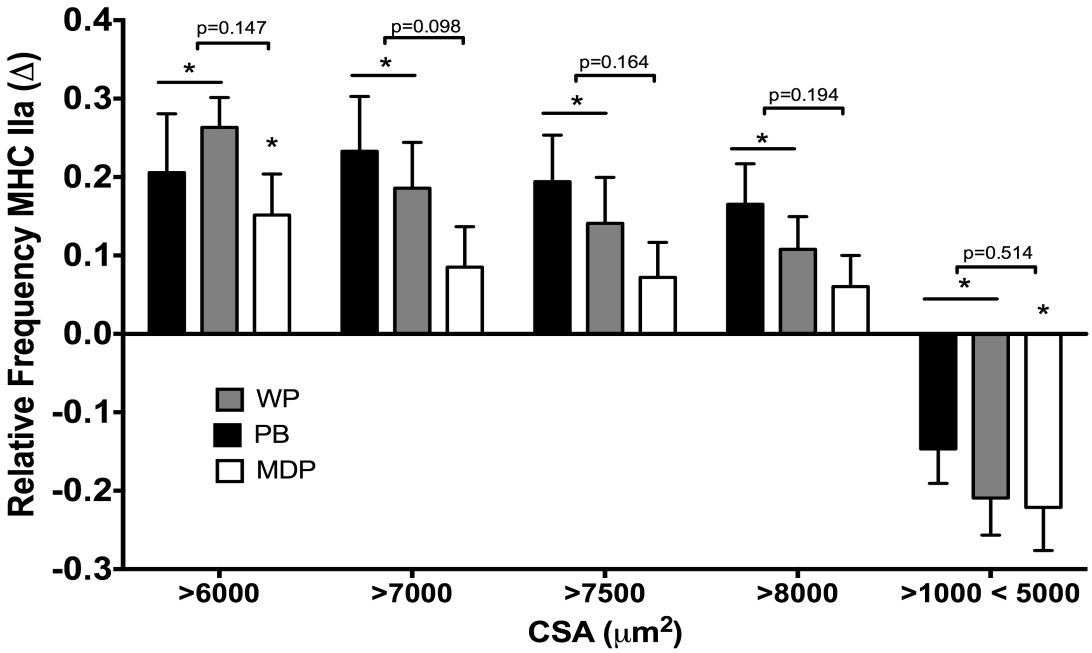


Fig. 5.8 Protein blend (PB) or whey protein (WP) or maltodextrin placebo (MDP). Data are mean ± SEM, n=15 (WP), 22 (PB) & 17 (MDP). ANCOVA between PRO (PB+WP) and MDP * $p<0.05$ vs 0.

Vastus Lateralis Satellite Cell Content

Vastus Lateralis myofiber Pax7+ satellite cell content was doubled following resistance exercise training (Figure 5.9, Table 5.5). Mean fiber satellite cell content (SC/fiber), proportion (% SC/myonuclei) and domain (SC/mm2) increased following resistance exercise training ($p<0.05$) with no effect of treatment ($p>0.588$). This increase was driven primarily by changes in MHC II myofibers. MHC II satellite cell content (SC/fiber), proportion (% SC/myonuclei) and domain (SC/mm2) increased following resistance exercise training ($p<0.05$) and there was no effect of treatment ($p>0.575$). MHC I satellite cell content (SC/fiber) displayed a strong trend ($p=0.059$) to increase following WP and PB treatment, which drove an increase with PRO treatment ($p<0.05$)

and a trend for an effect of PRO treatment vs MDP treatment (p=0.073). MHC I satellite cell proportion (% SC/myonuclei) and domain (SC/mm2) was unchanged following resistance exercise training (p>0.100), but SC domain (SC/mm2) also displayed a trend for an effect of PRO treatment vs MDP treatment (p=0.072).

Figure 5.9. *Vastus lateralis* Fiber-type specific satellite cell content, myonuclei and myonuclear domain

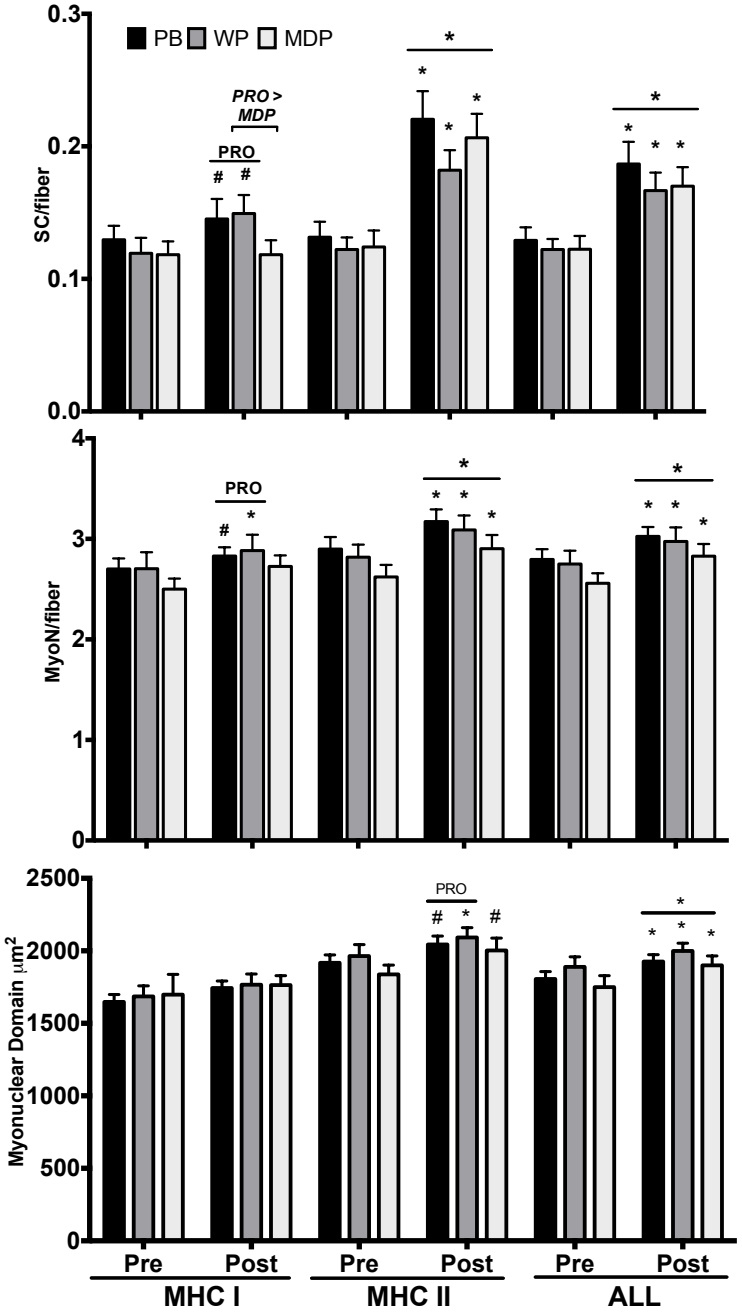


Fig. 5.9 *Vastus lateralis* fiber-type specific satellite cell content, myonuclei and myonuclear domain by treatment from pre to post (12 weeks) resistance exercise-training with nutritional supplementation. Protein blend (PB), whey protein (WP) or maltodextrin placebo (MDP). Data are mean ± SEM, n=15 (WP), 22 (PB), 17 (MDP) & 37 (PRO). Units are um^2. * ($p<0.05$), # ($p<0.10$) vs pre within that group, main effect of exercise is denoted as a bar across all three treatments. PRO ($p<0.05$) for change in pooled protein group vs pre. PRO > MDP & ($p=0.073$).

Table 5.5 Pre- to post-training absolute change for pax7 satellite cell immunohistochemical analysis

	Change				
Treatment	PB	WP	PRO	MDP	PRO vs MDP
$PAX7^+$ Satellite Cells/Fiber					
I	0.025 (-0.001,0.051)	0.031 (-0.001,0.061)	**0.028 ± 0.010**	0.004 (-0.033,0.024)	0.032 (-0.003,0.067)
II **T**	**0.097 (0.067,0.127)**	**0.067 (0.032,0.102)**	0.082 ± 0.011	**0.082 (0.050,0.114)**	-0.000 (-0.040,0.039)
All **T**	**0.066 (0.038,0.093)**	**0.049 (0.016,0.081)**	**0.057 ± 0.010**	**0.046 (0.017,0.076)**	0.010 (-0.026,0.047)
% $PAX7^+$ Satellite Cells/Myonuculei					
I	0.7 (-0.4,1.8)	0.7 (-0.8,2.2)	0.6 ± 0.5	-0.3 (-1.4,0.8)	0.9 (-0.5,2.5)
II **T**	**2.3 (1.1,3.4)**	**2.0 (0.4,3.5)**	**2.1 ± 0.4**	**2.2 (0.9,3.3)**	-0.0 (-1.5,1.5)
All	**1.6 (0.8,2.4)**	**1.4 (0.5,2.4)**	**1.5 ± 0.3**	**1.3 (0.4,2.1)**	0.2 (-0.8,1.3)
$PAX7^+$ Satellite Cells/mm^2					
I **PRO**	1.5 (-3.5,6.6)	4.1 (-2.0,10.2)	2.8 ± 2.0	-3.4 (-8.9,2.1)	6.2 (-0.6,13.0)
II **PRO**	**10.8 (5.6,16.0)**	**6.9 (0.7,13.1)**	**8.9 ± 2.0**	**10.8 (5.1,16.5)**	-1.9 (-8.9,5.0)
All	**6.6 (2.1,11.1)**	5.4 (-0.0,10.8)	**6.0 ± 1.7**	**5.3 (0.4,10.2)**	0.7 (-5.3,6.8)

[1]Data are mean ± 95%CI, n=15 (WP), 22 (PB) & 17 (MDP). boldface $p<0.05$, underlined $p<0.10$ vs Pre for that treatment. T = $p<0.05$ for an overall change over time. PRO = $P<0.05$ for an overall change in the PRO treatments from pre.

Vastus Lateralis Myonuclei Content and Myonuclear Domain

Vastus Lateralis myofiber myonuclear content and domain (Figure 5.9, Table 5.6) were altered by resistance exercise training. Mean myonuclei content (MyoN/fiber) increased following resistance exercise training ($p<0.05$) with no effect of treatment ($p=0.743$). This increase was driven primarily by changes in MHC II fibers, which increased ($p<0.05$) irrespective of treatment ($p=0.623$) or protein type ($p=0.378$). These increases were significant ($p<0.001$) for each treatment. MHC I myonuclei content was not statistically increased ($p=0.140$) following resistance exercise training ($p<0.05$) with no effect of supplement treatment ($p=0.811$). However, ANCOVA changes revealed an increase following treatment with PRO ($p=0.007$) that was significant after WP ($p=0.035$) and a trend with PB treatment ($p=0.073$), while no increase was seen with MDP treatment ($p>0.10$).

Myonuclear domain (Figure 5.9, Table 5.6) demonstrated a slight increase following resistance exercise training ($p<0.05$) with no effect of supplement treatment ($p=0.849$). By pooling fiber types an increase was observed for every treatment ($p<0.05$). This increase was likely due to greater statistical power by grouping all myofibers. This effect was absent in MHC I fibers, but there was a trend ($p=0.081$) in MHC II fibers to increase for only PRO. ANCOVA revealed an increase in PRO ($p=0.008$) that was significant in WP ($p=0.043$), yet revealed only trends for PB ($p=0.075$) and MDP ($p=0.066$).

Table 5.6. Pre- to post-training absolute change for myonuclei immunohistochemical analysis

	Change				
Treatment	PB	WP	PRO	MDP	PRO vs MDP
Myonuclei/Fiber					
I	0.18 (-0.02,0.37)	**0.25 (0.19,0.49)**	**0.22 ± 0.08**	0.15 (-0.06,0.37)	0.06 (-0.20,0.33)
II	**0.37 (0.17,0.57)**	**0.32 (0.09,0.55)**	**0.34 ± 0.08**	**0.23 (0.01,0.44)**	0.12 (-0.14,0.38)
All	**0.31 (0.13,0.50)**	**0.28 (0.06,0.50)**	**0.30 ± 0.07**	**0.21 (0.00,0.41)**	0.09 (-0.16,0.34)
Myonuclear Domain					
I	68 (-26,162)	32 (-80,143)	50 ± 36	82 (-19,185)	-33 (-158,92)
II	111 (-12,233)	**153 (5,300)**	**132 ± 48**	125 (-9,260)	6 (-160,173)
All	**97 (2,191)**	**138 (24,253)**	**117 ± 37**	**105 (1,208)**	13 (-115,141)

[1]Data are mean ± 95% CI, n=15 (WP), 22 (PB), 17 (MDP) & 37 (PRO). boldface $p<0.05$, underlined $p<0.10$ vs Pre for that treatment. T = $p<0.05$ for an overall change over time. PRO ($p<0.05$) for an overall change in the PRO treatments from pre.

Correlational Analysis

Correlations are visually represented in appendix B. Myonuceli number per fiber was highly correlated with fiber size at each time point and in all fiber types (r=0.743-0.826, p<0.000). Myofiber number per fiber change was well correlated to CSA change in MHC I (r=0.643, p<0.000), MHC II (r=0.573, p<0.000) and all (r=0.676, p<0.000) fiber types. Myofiber number per fiber change was well correlated to satellite cells per fiber change in all (r=0.545, p<0.000) fiber types. Type 1 Myofiber number per fiber change was weakly correlated to post- training testosterone levels (r=0.330, p=0.018).

The myonuclear domain change was inversely correlated with myonuclear domain at Pre (r=-0.706, p<0.000) and myonuceli number per fiber change (r=-0.409, p=0.003), yet positively correlated with MFA cross-sectional area (CSA) change (r=0.438, p=0.002). The MHC II myonuclear domain change was inversely correlated with MHC II myonuclear domain at Pre (r=-0.515, p<0.000), MHC II myonuclear number per fiber at Pre (r=-0.466, p<0.001) and MHC II satellite cell domain change (r=-0.409, p=0.003). The MHC II myonuclear domain change was positively correlated with MHC II CSA change (r=-0.417, p=0.003). *Vastus lateralis* muscle thickness change was positively correlated with total muscle RNA concentration change (r=0.392, p=0.003).

Whole body lean mass pre was correlated with MFA pre (r=0.518, p<0.000). Whole body lean mass post was correlated with MFA post (r=0.505, p<0.000). However, MFA change did not correlate with whole body lean mass change (r=0.028, p=0.847) or leg lean mass change (r=-0.115, p=0.428). Yet, MFA change did weakly correlate with average strength change (r=0.381, p=0.007) and leg volume % (r=0.308, p=0.040). MFA change did weakly correlate with all satellite cells per fiber change (r=0.382, p=0.006),

which was driven by a correlation with MHC I satellite cells per fiber change (r=0.411, p=0.003), but not MHC II satellite cells per fiber change (r=0.203, p=0.158). MHC I satellite cells per fiber change was correlated with MHC I CSA change (r=331, p=0.019), but MHC II satellite cells per fiber change was not correlated with MHC II CSA change (r=181, p=0.209).

DISCUSSION

This is the first study examining the role of protein supplementation and protein supplementation type on fiber-type specific adaptations of myofiber growth, satellite cells and myonuceli during traditional progressive resistance training of combined shortening and lengthening contractions. We demonstrated a greater increase in whole body lean mass in the soy-dairy protein blend (PB) vs placebo. However the increases in leg lean mass, vastus lateralis muscle thickness, vastus lateralis cross-sectional area means and thigh circumference were similar between protein types and placebo suggesting that the additional lean mass was accrued in other locations (arms or trunk) and/or that leg hypertrophy had peaked for all treatments after 3 months of RET with our protocol. Indeed, we previously demonstrated that the majority of the lean mass and thigh muscle thickness increases occurred within 6 weeks of exercise training (unpublished observations), but unfortunately, we were not able to take an additional biopsy at the mid-point in this study. The greater overall lean mass following treatment with the soy-diary protein blend is likely due to the prolonged delivery of amino acids to lean tissue, thus prolonging post-exercise muscle protein synthesis and net balance [189, 225].

Our whey protein treatment demonstrated similar adaptations when compared to maltodextrin placebo. Contrary to popular dogma, it is not unusual to observe no effect of protein supplementation, in particular whey protein, over placebo on lean mass or myofiber CSA. A recent meta-analysis determined that protein supplementation during resistance exercise training in young adults will produce greater increases in vastus lateralis CSA, ~250 μm^2, yet that analysis only included data from 4 studies. We are aware of only 3 studies demonstrating greater changes in vastus lateralis myofiber CSA

[340, 344, 485] or 2 studies with magnetic resonance imaging (MRI) [78, 484] comparing protein versus carbohydrate placebo. In one of the vastus lateralis myofiber CSA studies, the placebo group started with higher CSA and did not experience hypertrophy following resistance exercise training [340], while the other two studies demonstrated this effect only in MHC II fibers [344, 485]. In comparison, 5 other studies demonstrated equivalent increases in vastus lateralis myofiber CSA in protein supplemented groups [whey protein (n=3), milk (n=1) or EAA (n=1)] and carbohydrate placebo groups [53, 271, 337, 435, 452]. In addition, studies utilizing MRI of the biceps [334] or ultrasound [53, 338, 339, 498] of the thigh muscles have clearly shown the same pattern; no effect of protein supplementation (whey) to enhance vastus lateralis muscle hypertrophy. Given these findings, it is no surprise that protein supplementation in the studies mentioned, has been shown to enhance strength adaptations in only one study [452], which was actually a study that demonstrated identical changes in myofiber CSA between the protein supplemented and carbohydrate placebo groups. The remainder of the studies demonstrated identical increases in strength in the protein supplemented and carbohydrate placebo groups [53, 78, 271, 334, 337, 340, 344, 435, 484, 485] as we demonstrate here. These data further illustrate the minimal effectiveness of protein supplementation to enhance thigh, in particular, vastus lateralis muscle strength and hypertrophy during resistance exercise training.

Table 5.7 Summary of all studies with a placebo group directly assessing muscle size (hypertrophy) during RE

Author, Year	Subjects	Groups	Protein/AA	Muscle	Mass Measure	Duration	fCSA	DXA Δ	Strength Δ	Note
Andersen 2005	22M Sed	PRO vs CHO	25g mix	Quad	fCSA	3/wk, 14wks	PRO > CHO	-	=	PLA high pre
Bird 2006	32M UT	EAA,EAA+CHO,PLA, CHO	6gEAA, 6% CHO	VL	fCSA	2x/wk, 12wk	EAA+CHO>EAA=CHO > PLA	3,4.1,1.8,3 (kg)	=	PRO =< CHO, PLA high pre
Olsen 2006	32M	CrM+CHO, PRO+CHO, CHO, cntl	20g	VL	fCSA	3x/wk, 16wk	PRO =< CHO	-	=	PRO =< CHO
Cribb 2007	33M Rec BB	CrM CHO, CrM Whey, Whey, CHO	1.5 g/kg bw/d	VL	fCSA	3x/wk, 11wk	PRO = CHO	5.5,5,3.9,1.1%	PRO > PLA	PRO = CHO
Hartman 2007	56M UT	Milk, soy, PLA	18gx2	VL	fCSA	5 d/wk, 12 wk	Milk> Soy=CHO T2 not T1	6.2>4.4 =3.7	=	PRO > PLA
Hulmi 2009	29M UT	PRO, PLA vs Cntl	15gx2	VL	fCSA, UT	2x/wk, 21 wk	PRO = PLA for fCSA & UT	4.1,3.8, 0.6 %	=	PRO =< PLA
Hulmi 2010	31M UT	PRO, PLA vs Cntl	15gx2	VL, QF	MRI	2x/wk, 21 wk	PRO > PLA for VL not QF	3.6,3.3,-0.4%	=	PRO > PLA
Vieillevoye 2010	29M UT	EAA+CHO vs CHO	15g	Gast	UT	4x/wk, 12wk	PRO >= CHO	3.3,2.3%	>=	PRO = CHO
Herda 2013	106M UT	WPC+Leu, WPC, CHO, vs PLA	20g (+7g Leu) 2x	Quad	pQCT	3d/wk, 8wk	PRO = CHO = PLA	–	=	PRO = CHO = PLA
Farup 2014	22 Rec	Con & Ecc WP+CHO vs CHO	20g	VL	fCSA	2-3x/wk, 12wk	PRO CON > CHO, PRO ECC = CON CHO	-	=	PRO > CHO, sort of
Farup 2014	23 Rec	Con & Ecc WP+CHO vs CHO	20g	VL	MRI	2-3x/wk, 12wk	PRO > PLA: 5% > 2.8%	-	=	PRO > CHO, sort of
Erskine 2014	33M UT	PRO vs PLA	20gx2	BB	UT, MRI	3x/wk, 12wk	PRO = PLA	-	=	PRO = PLA, well controled
Babult 2014	68M	PRO isolate, Casein vs CHO	10gx3	VL	UT	3x/wk, 10wk	PRO = CHO	-	=	PRO = CHO
Babult 2015	161M	Pea, Whey, CHO	25gx2	BB	UT	3x/wk, 12wk	Pea >= CHO = Whey	-	=	PRO = CHO
Mitchell 2015	16UT M	PRO	14g	VL	fCSA	3x/wk, 12wk	PRO = PLA	-	=	PRO = PLA

Sed, Sedentary; UT, untrained, BB, body builders; Rec, recreationally active; M, Men; Con, concentric, ECC, eccentric PRO, protein; PLA, placebo; CHO, carbohydrate; EAA, essential amino acids; CrM, creatine monohydrate; cntl, control; bw, body weight; VL, vastus lateralis; QF, quadriceps femoris; gast, gastrocnemius; BB, bicep bracii; fCSA, myofiber CSA; magnetic resonance imaging, MRI; UT, ultrasound; red= no effect of protein, blue=effect of protein.

Our data provide further support for the concept that whey protein is not consistently a superior type of protein supplement compared to other protein sources, as commonly promoted. In fact, the soy-dairy protein blend tended to promote greater change in lean body mass than maltodextrin placebo and we found no significant difference between the effects of soy-dairy protein blend and whey protein. This is in agreement with several recent studies, which show that as long as leucine content is sufficient (>2g for young adults) and the protein is readily digestible, there will be no difference in the overall adaptations to resistance exercise training by type of protein supplementation [338, 339, 342, 375, 385, 486].

Analysis of cross-sectional area (CSA) means, the predominant method utilized in these types of clinical trials, can obscure subtle changes in myofiber hypertrophy. Recently, Farup et al. completed an elegant study comparing the effect of whey protein supplementation on isolated lengthening or shortening contractions of skeletal muscle [485]. They demonstrated that myofiber CSA was enhanced in MHC II fibers with whey protein supplementation during shortening, but not lengthening contractions. They followed up with this finding by demonstrating a tendency ($p<0.10$) for protein supplementation to result in a shift toward a greater frequency of larger myofibers (>8000 μm^2) and a lower frequency of smaller fibers (>1000 <5000 μm^2) post-training, compared to post-training whey-supplemented eccentric training. Although we did not observe a difference in the CSA means between the protein supplemented and carbohydrate placebo groups, we similarly demonstrated that protein supplementation displayed a slightly greater change ($p<0.10$) in the frequency of MFA and MHC II bins (7500 μm^2, 8000 μm^2 and 8500 μm^2 and 10000 μm^2) vs the maltodextrin placebo. This suggests that

protein supplementation may play a role in expanding MHC II size during resistance exercise training. However, we stress that this effect is minimal, and given the low statistical confidence seen in these examples, we believe this effect is limited to a sub-population of myofibers/individuals that is likely an example of responder/non-responder clustering. The functional relevance of this finding is unknown; however, we were able to demonstrate improved isokinetic torque and power in the protein supplemented groups only, suggesting a possible role for the changes in these MHC II fibers with protein supplementation.

Other investigators, using much smaller samples sizes, have demonstrated enhanced increases in myofibrillar protein concentrations during RET with protein, amino acid and/or creatine supplementation [270, 343, 363, 455]. The method we utilized has repeatedly demonstrated that muscle protein, in particular the contractile protein concentration, is remarkably fixed, even in periods of pronounced atrophy [499, 500] or hypertrophy [500-502]. This concept is supported by classical work demonstrating that the volume density of myofibrils does not change following heavy resistance training [503, 504], but hypertrophy increases the total muscle volume and thus expands the absolute contractile protein volume or content. We did observe an increase in the sarcoplasmic protein content with the PB supplement, yet this was partially explained by a slight decrease in muscle water content. If this effect occurred in other muscles, this finding may provide some insight as to why the protein blend induced the greatest increases in whole body lean mass. Sarcoplasmic proteins are relevant to muscle health and function as they direct anaerobic ATP production, intracellular transport, and several other necessary enzyme functions [505]. It appears that the contractile protein

concentration is fixed during chronic resistance training, but the content increases with hypertrophy, and that protein blend supplementation may enhance sarcoplasmic protein concentration.

Olsen et al. first demonstrated that chronic resistance exercise training (RET) with protein supplementation may provide a slight enhancement of the satellite cell pool compared to RET alone [435]. Based on basic science and pre-clinical findings, we anticipated that protein supplementation would enhance satellite cell activity and content through mTORC1 [206, 506] and particularly on MHC II fibers [204, 507]. Instead, we demonstrated similar increases in satellite cell content between treatment groups, which were driven primarily through increases in MHC II fibers. However, we did demonstrate a significant increase in satellite cell number per fiber, for MHC I fibers, with protein supplementation, but not with a maltodextrin placebo. This resulted in a trend for an effect of protein ($p=0.073$) over maltodextrin placebo, which was also seen when expressed as SC/mm2. Interestingly, MHC I, but not MHC II, satellite cell number per myofiber change was correlated with CSA change. Farup and colleagues demonstrated similar findings after 3 months of RET with protein supplementation in MHC I, but not MHC II fibers, suggesting that protein supplementation may provide greater expansion of the SC pool in this fiber type to slightly promote myofiber growth. The specific relevance of this finding is unknown and warrants further investigation. MHC II fibers are thought to be most responsive to heavy strength training [195], yet this training was whole body, high-intensity training, which likely recruited all fiber types. We also discovered that those who had lower initial satellite cell content in MHC I fibers, experienced the greatest change in MHC I satellite cells per fiber ($r=-0.529$, $p<0.000$) and

MHC I myonuclei per fiber (r=-0.387, p=0.006). However this effect was absent in MHC II fibers. These data may suggest that myonuclear addition was a primary fate of satellite cells in MHC I fibers. Our data in this large cohort of young men, and also demonstrated elsewhere is in contrast with a previous report in the literature suggesting that a higher pre-training satellite cell content is a characteristic of high-responders to RET [508]. However, we could not discern a similar pattern in this sample of young men.

Myonuclear accretion occurred with RET, as has been previously demonstrated [194], but was not different by treatment. A significant increase was seen with PB treatment, but only trends with WP and MDP. Others have suggested that CSA changes greater than ~15% are needed to occur before changes in myonuceli number per fiber are witnessed [194, 509]. Here we demonstrated 15-20%, ~20% and 20-30% increases in CSA of MHC I, II and hybrid fibers, respectively, suggesting that our larger sample size included enough participants with substantial changes in CSA to detect changes in myonuclear number with RET. Myonuceli number per fiber was highly correlated with fiber size at each time point and in all fiber types (r=0.743-0.826, p<0.000), illustrating remarkable control of the myonuclear domain, as others have shown [510-514].

Even with such tight coupling of myonuclear number to myofiber size we were able to witness, in our study, a slight but consistent and significant expansion of the myonuclear domain, ~100 μm^2, after 3 months of RET. In fact, a significant, inverse relationship (r=-0.706, p<0.001) was demonstrated, indicating that those with smaller initial myonuclear domain experienced the greatest change in myonuclear domain over the course of the training. This effect was most evident in MHC II fibers, highlighting

their remarkable plasticity to this contractile stimulus. This expanded myonuclear domain was likely a catalyst behind the level of myofiber growth observed during the exercise training. Indeed, these changes in myonuclear domain were correlated with changes in CSA. Maintenance of this expanded domain was likely assisted by the increased total RNA content (translational capacity), and it is also possible that the size of existing myonuceli was also increased, as demonstrated by Cabric et al. in human skeletal muscle following 3 weeks of electrical stimulation [515]. This would suggest enhanced transcriptional capacity in each myonucleus. We anticipated that an expansion of the myonuclear domain and an increased translational capacity would coincide with greater levels of mTORC1 signaling following RET. Although, we did not witness this, we did observe a tendency for greater levels of total mTOR protein, which does reflect the expanded translational capacity demonstrated with total RNA content.

Certainly, many studies, including many from our laboratory, have clearly demonstrated a robust effect of protein/amino acids to stimulate the early response of muscle growth [50, 356]. The question persists as to why these effects are not as readily discovered in physiological outcomes following chronic exposure to the stimulus [353]. They key, we believe, is a concept overlooked in the modern paradigm to unravel complex molecular mechanisms, physiologic adaptation. Farup and colleagues demonstrated that whey protein supplementation following eccentric exercise accelerated the satellite cell response compared to consumption of carbohydrate as a placebo [507], however, by 168 hr post-exercise [382] and after 12 weeks of training [382] the satellite cell pool expansion was identical between their treatment groups. the satellite cell pool

expansion was identical between their treatment groups. For novice exercisers, most of the satellite cell pool activity occurs at 2 weeks of RET [516]. Also, some evidence suggests that the majority of the satellite cell pool expansion occurs early, 1-4 wk into RET, during dietary supplementation [435]. These data suggest that protein supplementation may provide an enhancement early during the start of exercise training, but additional protein is unlikely to confer added benefit as adaptation occurs. Interestingly, this time frame is also when the most myofiber damage and remodeling is likely to occur. Although attractive as this hypothesis is, it has not yet been clearly proven [354]. The results of some studies have indicated that protein metabolism becomes more efficient after resistance training (80, 81), which provides further support to the concept that as long as a well-balanced diet is maintained, increases in muscle hypertrophy and strength will not suffer during resistance exercise training [353, 354].

Limitations

A limitation to this study is that several samples from the WP group were not suitable for immunohistochemical analysis and as a result the sample size of that group was lower than the size of other treatments. It is possible that we were slightly underpowered in our ability to determine certain exercise effects (myonuclear domain or number); however, statistical analysis clearly demonstrated an absence of treatment differences in most outcomes suggesting that sample size was not an issue in delineating treatment effects. It was not feasible for us to sample at earlier time points throughout the training, although this may have provided greater insight regarding the effect of protein supplementation. Also this would have allowed us to better examine the preferential order of changes in, satellite cell content, myonuclear domain and myonuclear addition throughout the resistance exercise training. Also, although many of

the inferences were made using correlational analyses, which do not discern cause and effect, a major strength of this study is that this large cohort makes correlational analysis possible and opens the door for generating additional research questions.

CONCLUSION

Daily supplementation of a soy-diary protein blend, but not whey protein during 3 months of chronic resistance exercise training accrued more lean mass than with maltodextrin placebo at the whole body level. However, when we focused our analysis on the *vastus lateralis* in the thigh, we observed nearly identical increases in muscle strength, hypertrophy (whole muscle and myofiber-type specific), MHC II satellite cell content, and overall myonuclear addition. When results from the soy-diary protein blend and whey protein treatments were pooled, very modest effects of protein supplementation existed to enhance MHC I satellite cell content, isokinetic torque and power, and a slight expansion of a greater proportion of larger MHC II fibers over placebo after resistance exercise training. These data further illustrate the minimal effectiveness of protein supplementation in enhancing thigh muscle, in particular, *vastus lateralis* muscle, adaptation following chronic resistance exercise training in young men. However, supplementation of the soy-dairy protein blend is likely to expand lean mass in other muscle groups.

CHAPTER 6

Conclusions

Dietary protein is often touted as the macronutrient of the strong, powerful and successful [517, 518]. Indeed, ever since ancient times, those who exhibited such qualities boasted the greatest quantity and quality of food. Modern marketing has built a complex, billion dollar, sports-nutrition industry [519] off such enduring beliefs – often promoting the best protein supplements to enhance the consumer's goals [520]. However, research determining the effectiveness of additional protein supplementation for successfully enhancing strength, power and muscle mass has not produced the same certainty behind these fundamental beliefs.

Skeletal muscle is a highly adaptive tissue, sensitive to nutritional and contractile modulation. Reports in the literature clearly reveal the robust effects of exercise and ingestion of supplementary amino acids/protein in enhancing the growth response muscle protein in the immediate hours following these respective stimuli (exercise/amino acids) [50]. The research findings thus far suggest that ~20-25g of high quality protein, which contains ~8-10g EAA, which maximally stimulates muscle protein synthesis (MPS) [300]. Further, it appears that protein/amino acids provide an additive or enhancing effect to exercise on stimulating muscle protein synthesis [54, 69, 108, 253]. It is clear that crystalline amino acids have a potent effect on post-exercise MPS [40, 41, 54, 252, 253, 255, 258]. Intact protein ingestion in the form of soy, casein, whey, egg or beef also increases post-exercise MPS [43, 65, 190, 218, 232, 233, 238, 241, 245, 246, 249, 291]. Since proteins differ by amino acid composition (overall protein quality) and digestion

rate (i.e., fast, intermediate, or slow) their source and processing, one can ask which protein source or type is most beneficial for stimulating this post-exercise growth response in muscle protein?

It was though that the rapid aminoacidemia (from fast digestion) and higher branch-chain amino acid content, primarily leucine, in whey protein, compared to other high quality proteins [292], made it the superior choice [234, 238, 245, 305, 306]. However, we proposed that by creating a blend of the three primary protein sources described in numerous studies (whey, casein and soy: all of which can stimulate post-exercise MPS [43, 65, 218, 232, 238, 241, 245, 246, 291]), we would produce a high quality and unique protein supplement. This supplement would, in theory, have multiple amino acid release profiles to minimize indispensable loss of amino acids and to maximize protein retention, while containing an adequate content of leucine, which seems to be important for initiating the additive effect of amino acids on MPS. Also, this supplement would contain high levels of some other amino acids, glutamine and arginine, which may not be needed with healthy individuals, but could provide other essential roles in clinical nutrition [521].

Therefore, as described in Chapter 2, we tested this blend against an isolated dairy protein, whey, and demonstrated that it was effective. This blend clearly promoted muscle protein synthesis and mTORC1 anabolic signaling during post-exercise recovery. We concluded that our data, and that of others [246], further support the use of a blended protein supplement following resistance exercise to stimulate muscle protein synthesis. Interestingly, although we observed distinctly different post-ingestion amino acid profiles, we saw similar increases in post-exercise MPS between the blend and whey,

suggesting that a slightly different aminoacidemia stimulus could lead to a similar end result (MPS).

We followed up on these findings, as described in Chapter 3, by doing a more in-depth analysis on a subset of subjects from this first clinical trial. Using a stable isotopic tracer infusion and femoral catheterization, we discovered that the increase in the net balance of post-exercise phenylalanine across the leg (an indicator of muscle protein anabolism) was prolonged following ingestion of a protein blend compared to whey protein. We also reported that ingesting a protein blend or whey protein enhances the rate of amino acid transport into muscle, increases the mRNA expression of select amino acid transporters (LAT1/SLC7A5, CD98/SLC3A2, SNAT2/SLC38A2, PAT1/SLC36A1, CAT1/SLC7A1), and increases post-exercise myofibrillar protein synthesis. These results provide further support for the efficacy of ingesting a protein blend to increase and prolong post-exercise muscle protein anabolism. These acute investigations were interesting from a mechanistic point of view, but we determined that further research was necessary to determine the efficacy of protein blend supplementation on muscle growth and strength during chronic resistance exercise training.

We designed a clinical trial (described in Chapters 4 & 5) to test the effectiveness of a soy-dairy protein blend in enhancing the adaptations of chronic resistance exercise training. Our goal was to randomize 60 young men into 3 groups receiving daily supplementation of 22g grams of whey protein, a soy-dairy protein blend, or a maltodextrin placebo during 12 weeks of whole body progressive resistance training. As described in Chapter 4, we first focused on whole body changes in primary outcomes of body composition (lean mass in particular) and strength. As described in Chapter 5, we

then focused on the thigh muscle, in particular the *vastus laterialis* myofiber, by examining muscle thickness, muscle composition, mTORC1 anabolic signaling, myofiber type composition, cross-sectional area, satellite cell content, and myonuceli.

We followed up with our acute findings by demonstrating that protein blend supplementation is an effective strategy to promote increases in whole body lean mass growth during resistance exercise training in young adults. The protein blend demonstrated a trend for greater increases in whole body and arm lean mass compared to the maltodextrin placebo. In addition, only treatment with the protein blend was able to continue the accrual of lean mass over the last 6 weeks of exercise training and supplementation. It is possible that the prolonged post-exercise net balance following protein blend ingestion contributed to the trend for an enhancement in lean mass. Similar increases in muscle hypertrophy (muscle thickness) following our protein treatments are in agreement with the similar increases in myofibriallar protein synthesis we demonstrated in the acute study. It appears that the increases in contractile tissue were similar between treatments as a result of the chronic loading. Accordingly, there were no differences in strength increases between treatments.

Daily supplementation with a soy-diary protein blend, but not whey protein, during 3 months of chronic resistance exercise training resulted in the accrual of more lean mass than maltodextrin placebo ingestion at the whole body level. However, when we focused our analysis to the thigh muscle, *vastus lateralis* (Chapter 5), we observed nearly identical increases in muscle strength, hypertrophy (whole muscle and myofiber-type specific), MHC II satellite cell content and overall myonuclear addition. When the soy-diary protein blend and whey protein treatments were pooled to represent overall

protein supplementation, very modest effects of protein supplementation existed to enhance MHC I satellite cell content, isokinetic torque and torque at a set velocity and a slight increase in the proportion of larger MHC IIa myofibers, compared to the maltodextrin placebo after resistance exercise training. These data further illustrate the minimal effectiveness of protein supplementation in enhancing thigh muscles, in particular *vastus lateralis* muscle adaptation, following chronic resistance exercise training in young men. However, given our whole body lean mass findings, supplementation of the soy-dairy protein blend is likely to expand lean mass in other muscle groups.

Many acute experiments indicate that protein supplementation causes a robust increase in MPS and mTORC1 signaling in the immediate hours following the stimulus. However, these effects are not necessarily reflected in physiological outcomes following chronic exposure to the stimuli. This suggests that sometime over the course of the exercise training adaption, the muscle growth potential lessens as individuals move closer to their genetically set "hypertrophic limits". Subsequently, an additional anabolic, non-pharmaceutical stimulus, such as protein supplementation, may not be able to surpass such a hypothetical limitation. Indeed, studies have indicated that protein metabolism becomes more efficient after resistance training [360, 361] providing further support to the concept that as long as a well-balanced diet is maintained, increases in muscle hypertrophy and strength will not suffer during resistance exercise training [353, 354].. However, the current evidence does not exclude the possibility that protein supplementation may be able to reduce the time frame for maximizing hypertrophy.

However, the research described in this dissertation was not designed to answer that particular question.

In summary, althought protein blend supplementation provides only minor enhancement to the effect of resistance exercise in young adult males it may be a promising nutritional strategy to enhance lean mass growth during resistance exercise training in older adults, who have a greater need for preservation of lean mass during the aging process. The additional lean mass may also serve as an amino acid buffer against periods of sickness and disuse, such that essential muscle contractile protein can be maintained for optimal function. Future applications of protein blend supplementation to promote or maintain muscle mass should include studies related to aging and also studies in muscle-wasting clinical populations, such as cancer patients, where the use of blended protein has demonstrated positive effects [454].

Glossary

FSR – Fractional synthetic rate

FBR – Fractional breakdown rate

PB – Protein Blend

WP – Whey Protein

MDP – Maltodextrin placebo

1RM – One repetition maximum

BMI – Body mass index

ICG – indocyanine green

mTORC1 – Mammalian target of rapamycin complex 1

mTORC2 – Mammalian target of rapamycin complex 2

ERK1/2 – Extracellular-related kinase 1/2

MAPK – Mitogen activated protein kinase

Akt – Protein kinase B

S6K1 – p70 ribosomal S6 kinase 1

4E-BP1 – Eukaryotic initiation factor 4E binding protein 1

rpS6 – Ribosomal protein S6

eIF4E – Eukaryotic initiation factor 4E

Mnk1 – Mitogen activated protein kinase interacting-kinase 1

RSK1 – p90 ribosomal S6 kinase 1

eEF2 – Eukaryotic elongation factor 2

eEF2K – Eukaryotic elongation factor 2 kinase

AMPK – Adenosine monophsophate – activated protein kinase

FAK – Focal adhesion kinase

FoxO3a – Forkhead box 3a

FoxO1 – Forkhead box 1

FoxO4 – Forkhead box 4

SLC – Solute-linked carrier

SNAT2/SLC38A2 – System A amino acid transporter

CAT1/SLC7A1 – cationic amino acid transporter

LAT1/SLC7A5 – System L amino acid transporter

CD98/SLC3A2 – cluster of differentiation 98

PAT1/SLC36A - proton-assisted transporter 1

HIF1α – Hypoxia inducible factor 1α

eIF2Bε – Eukaryotic initiation factor 2Bε

IL-6 – Interleukin 6

HSP70 – Heat shock protein 70

IGF-1 – Insulin-like growth factor 1

REDD1 – Regulated in development and DNA damage responses 1

REDD2 – Regulated in development and DNA damage responses 2

GAPDH – Glyceraldehyde 3-phosphate dehydrogenase

β2M – Beta 2 microglobulin

UPS – Ubiquitin proteasome system

MuRF1/TRIM63 – Muscle really interesting novel gene (RING) finger 1

MAFbx/Atrogin-1/FX032 – Muscle atrophy F-box

MSTN – myostatin

CDK2 – cyclin dependent kinase 2

ACTB – actin

RPL13A – ribosomal protein L13a

LC3 – Microtubule-associated protein light chain 3

DAPI – 4',6-diamidino-2-phenylindole

PAX7 – Paired box protein 7

MFA – mean fiber area

CSA – cross-sectional area

GABARAP – Gamma-aminobutyric acid receptor-associated protein

GCN2 – general control nonderepressible 2

VPS34 – Vacuolar protein sorting 34

Atg12 – Autophagy-related gene 12

Atg7 – Autophagy-related gene 7

p38 – p38 mitogen-activated protein kinase

Beclin-1 – Autophagy-related gene 6

GβL – G protein β-subunit-like protein

TCTP – Translationally controlled tumor protein

Raptor – Regulatory associated protein of mTOR

Rictor – Rapamycin-insensitive companion of mTOR

Rheb – Ras-homologue enriched in brain

MAP4K3 – Mitogen activated protein kinase kinase kinase kinase-3

TSC2 – Tuberous sclerosis complex 2

PRAS40 – Proline-rich Akt substrate-40

PAM – Protein associated with Myc

ATF4 – activating transcription factor 4

PA – Phosphatidic acid

Ser – Serine

Thr – Threonine

Tyr – Tyrosine

cDNA – copy deoxyribonucleic acid,

tRNA – Transfer ribonucleic acid

mRNA – Messenger ribonucleic acid

EAA - Essential amino acids

BCAA – Branch-chain amino acids

OGTT – Oral glucose tolerance test

ECG – Electrocardiogram

HIV – Human immunodeficiency virus

AIDs – Acquired immune deficiency syndrome

IgG – Immunoglobulin G

AU – Arbitrary units

VAS – visual analog scale

CR – Calf raise

IP – incline press

KC – knee curl

SR – seated raise

KE – knee extension

KF – knee flexion

LP – leg press

ITS-CRC – Institute for Translational Sciences Clinical Research Center

RDA – recommended daily allowance

DXA – Dual-energy X-ray absorptiometry

PRO – protein

AA – amino acids

BMC – bone mineral content

BMD – bone mineral density

SC – satellite cells

MyoN – Myonuclei

MHC – myosin heavy chain

MYOG – myogennin

MYOD – Myoblast determination protein 1

SYBR – cyber green

GH – Growth hormone

BFR – Blood flow restriction

Ctrl – Control

RPM – Revolutions per minute

RT-PCR – Reverse transcription polymerase chain reaction

RET – Resistance Exercise Training

MPS – Muscle protein synthesis

MPB – Muscle Protein Breakdown

TTR – Tracer to tracee ratio

GCMS – Gas chromatography mass spectrometry

SDS-PAGE – Sodium dodecyl sulfate polyacrylamide gel electrophoresis

PVDF – Polyvinylidene fluoride

NFDM – Non-fat dry milk

TBS – Tris buffered saline

DTT – Dithiolthreitol

PMSF – Phenlymethylsulfonyl fluoride

SBTI – Soybean tripson inhibitor

ANOVA – Analysis of variance

ANCOVA – Analysis of covariance

SE – Standard error of the mean

Ep – Increment in protein-bound phenylalanine enrichment

t – Time

E_M – Phenylalanine enrichments in the free intracellular pool

Appendix A

Figure A.2.1. Blood and Muscle Intracellular Enrichments.

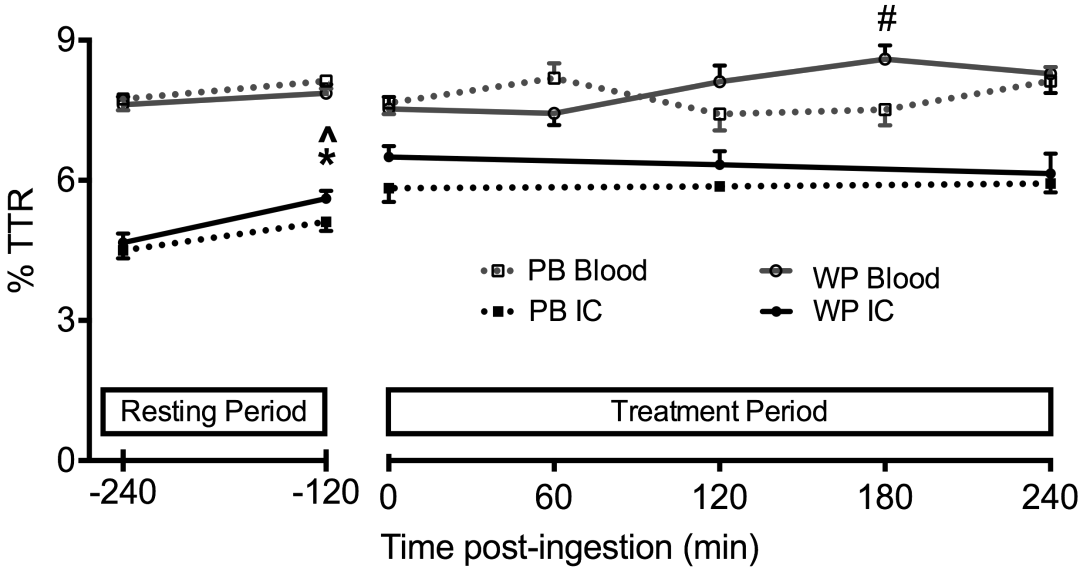
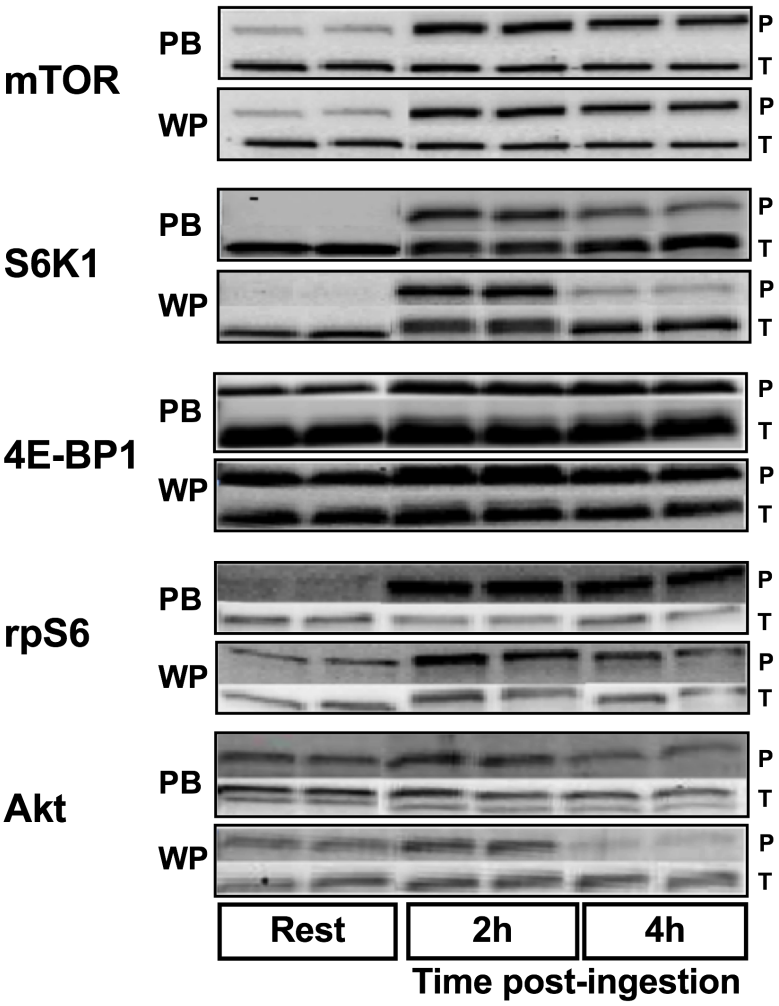


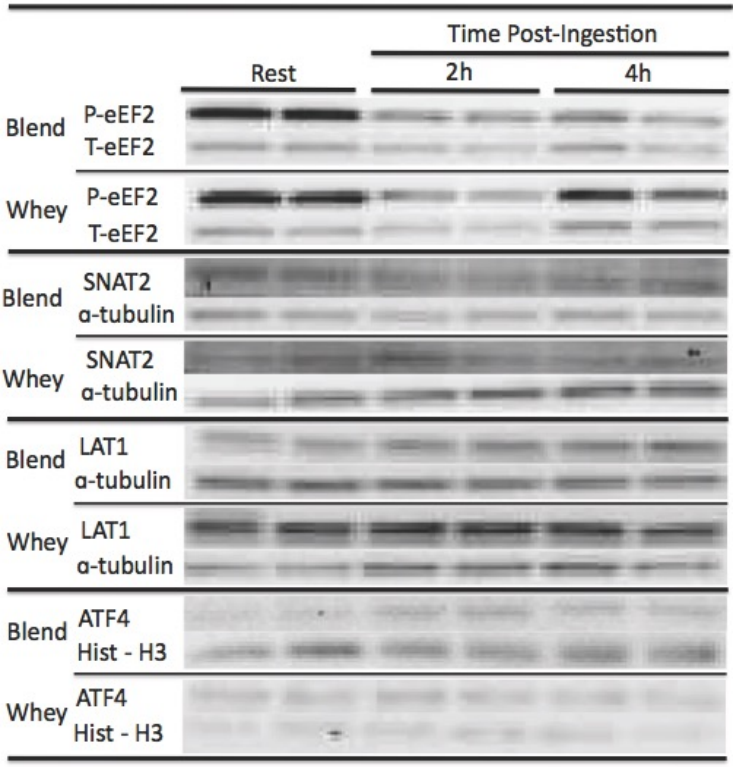
Fig. A.2.1 Blood and intracellular (IC) muscle 13C6 phenylalanine enrichment as % tracer to trace ratio (TTR) in young adults at rest during the post-exercise recovery period following ingestion of the protein blend (PB) or whey protein (WP) 1 h after completion of resistance exercise. Data are mean ± SEM, n=9 (WP) or 10 (PB). Data are presented at Rest (-240 and -120 min) and post-ingestion treatment (0, 60,120,180 and 240 min) periods. *Difference across time for that time period, $P < 0.05$; #Different from PB at that time, $P < 0.05$, ^Different from PB at that time, $P = 0.07$.

Figure A.2.2. mTORC1 signaling representative blots.



Representative western-blot images of synthesis-associated signaling proteins in young adults during the post-exercise recovery period following ingestion of the protein blend (PB) or whey protein (WP) 1 h after completion of resistance exercise. P = phosphorylated protein and T = total protein. Images are shown in duplicate.

Figure A.3.1. Representative immunoblots of protein expression of LAT1, SNAT2, ATF4 and eEF2



Appendix Fig A.3.1. Representative immunoblots of protein expression of LAT1, SNAT2, ATF4 and eEF2 at rest and in the hours post-ingestion for subjects given Whey and a Blend at 1h post-exercise. All samples were loaded in duplicate. Representative blots for the groups were found on separate blots, yet all samples were derived at the same time and processed in parallel.

Table A.4.1. Participant (non-supplemented) dietary intake by treatment before (Pre), 6 weeks (Mid) and 12 weeks (Post) resistance exercise-training with nutritional supplementation[1]

TRT	Time Period			ANCOVA
	Pre	Mid	Post	
Total Energy non-supplemented, *mJ*				
PB	10.13 ± 0.66	10.30 ± 0.66	9.51 ± 0.67	t: 0.923, trt: 0.222, t x trt: 0.820
WP	10.23 ± 0.74	10.41 ± 0.79	10.79 ± 0.81	
MDP	9.30 ± 0.75	8.96 ± 0.81	8.88 ± 0.81	
Protein Intake non-supplemented, *g/d*				
PB	101.3 ± 7.0	108.4 ± 7.0	100.5 ± 7.1	t: 0.755, trt: 0.386, t x trt: 0.596
WP	101.9 ± 7.1	104.0 ± 8.1	113.0 ± 8.3	
MDP	95.1 ± 7.0	95.1 ± 8.3	93.2 ± 8.3	
Protein Intake non-supplemented, *g/kg/d*				
PB	1.33 ± 0.10	1.27 ± 0.10	1.40 ± 0.10	t: 0.688, trt: 0.987, t x trt: 0.757
WP	1.29 ± 0.10	1.28 ± 0.11	1.36 ± 0.11	
MDP	1.27 ± 0.10	1.22 ± 0.11	1.23 ± 0.11	
Carbohydrate Intake non-supplemented, *g/d*				
PB	274.2 ± 18.3	290.0 ± 18.3	272.4 ± 18.6	t: 0.789, trt: 0.224, t x trt: 0.989
WP	283.3 ± 20.7	291.3 ± 22.0	284.3 ± 23.0	
MDP	245.8 ± 21.0	250.2 ± 23.0	252.0 ± 22.6	
Carbohydrate Intake non-supplemented, *g/kg/d*				
PB	3.58 ± 0.24	3.71 ± 0.24	3.42 ± 0.24	t: 0.919, trt: 0.491, t x trt: 0.900
WP	3.54 ± 0.27	3.52 ± 0.28	3.46 ± 0.29	
MDP	3.27 ± 0.27	3.16 ± 0.29	3.31 ± 0.29	
Animal Protein Intake non-supplemented, *g/d*				
PB	74.5 ± 6.5	75.1 ± 6.5	69.6 ± 6.6	t: 0.916, trt: 0.543, t x trt: 0.444
WP	75.1 ± 6.5	72.3 ± 7.7	82.0 ± 8.0	
MDP	69.5 ± 7.4	63.1 ± 8.0	65.0 ± 8.0	
Vegetable Protein Intake non-supplemented, *g/d*				
PB	32.5 ± 2.4	33.3 ± 2.4	32.0 ± 2.4	t: 0.930, trt: 0.149, t x trt: 0.911
WP	35.2 ± 2.7	35.0 ± 2.9	37.3 ± 3.0	
MDP	29.3 ± 2.7	30.9 ± 3.0	29.2 ± 3.0	

[1]Data are mean ± 95% CI, n=18 (WP), 22 (PB) & 18 (MDP).

Table A.4.2. Absolute values of isometric and isokinetic Torque, (N-M) by treatment before (Pre), 6 weeks (Mid) and 12 weeks (Post) resistance exercise-training with nutritional supplementation[1]

Treatment	Absolute Values			Main Effects
	Pre	Mid	Post	
Isometric KE				
PB	254 ± 9	275.4 ± 6.8	296.5 ± 8.5	t: 0.000, trt: 0.443, t x trt: 0.471
WP	271 ± 13	294.5 ± 7.1	301.3 ± 9.2	
MDP	268 ± 14	275.0 ± 7.3	292.7 ± 9.3	
Isometric KF				
PB	136 ± 5	156.4 ± 3.8	156.0 ± 4.6	t: 0.000, trt: 0.145, t x trt: 0.204
WP	156 ± 7	152.2 ± 4.1	157.2 ± 5.0	
MDP	143 ± 6	144.6 ± 4.1	157.0 ± 5.0	
Isokinetic KE				
PB	180 ± 5	191.5 ±4.6	206.0 ± 4.5	t: 0.000, trt: 0.389, t x trt: 0.086
WP	193 ± 10	203.4 ± 4.8	209.0 ± 4.9	
MDP	191 ± 8	193.7 ± 4.8	191.2 ± 4.9	
Isokinetic KF				
PB	109 ± 4	120.0 ± 3.8	119.7 ± 4.5	t: 0.014, trt: 0.358, t x trt: 0.369
WP	119 ± 6	120.0 ± 4.2	130.0 ± 4.7	
MDP	117 ± 6	116.0 ± 4.0	118.0 ± 4.8	

[1]Data are mean ± 95% CI, n=22 (WP), 23 (PB) & 23 (MDP). Knee extension = KE. Knee Flexion = KF. * P<0.05).

Table A.4.3. Participant thigh muscle thickness (MT) by treatment Pre to Mid , Mid to Post and Pre to Post for 6 weeks (Mid) and 12 weeks (Post) resistance exercise-training[1]

TRT	Time Period					
	Pre	Mid	Post	Main Effects	Pre to Mid	Pre to Post
MT, *cm*					%Δ	
PB	4.20 ± 0.14	**4.63 ± 0.14**	**4.70 ± 0.14**	t: 0.000,	**10.5 (6.2,14.8)**	**12.0 (8.4,15.1)**
WP	4.51 ± 0.13	**4.89 ± 0.14**	**5.07 ± 0.14**	trt: 0.160,	**10.2 (6.3,14.0)**	**15.4 (12.0,19.0)**
MDP	4.19 ± 0.13	**4.64 ± 0.13**	**4.73 ± 0.14**	t x trt 0.859	**10.4 (6.4,14.3)**	**12.0 (8.3,15.8)**

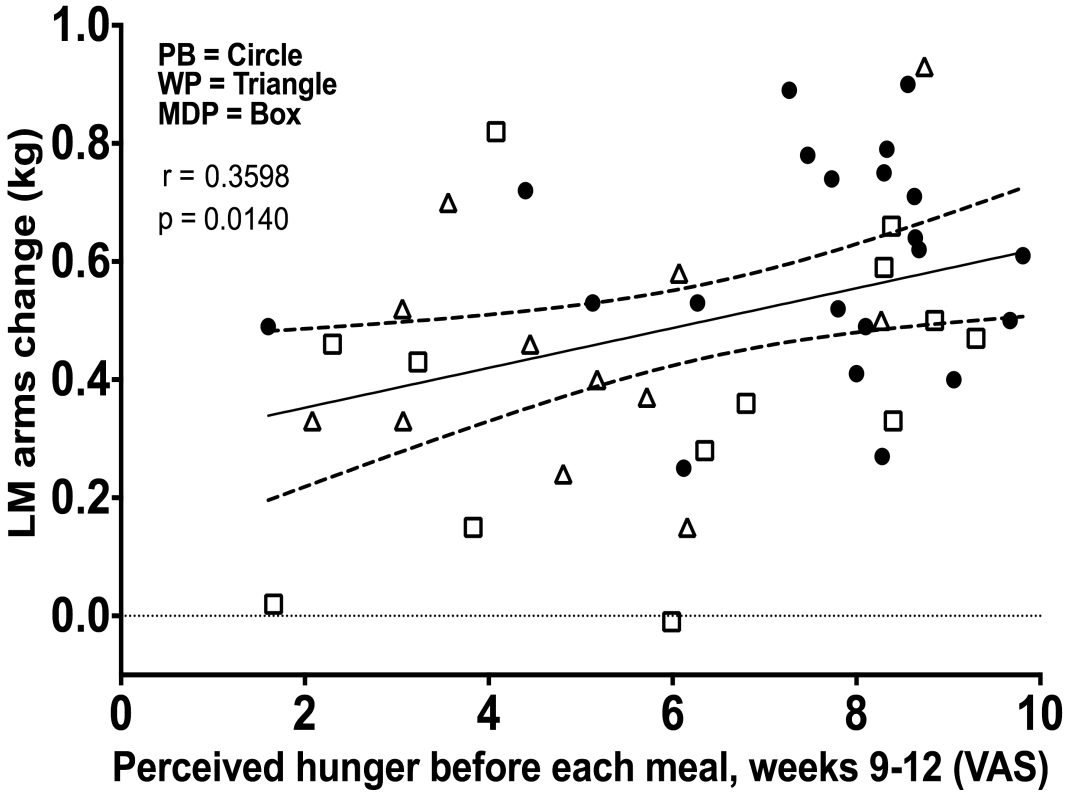
[1]Data are mean ± SEM or 95% CI, n=22 (WP), 23 (PB) & 23 (MDP). Boldface, P <0.05.

Table A.4.4. Absolute values of body composition by treatment before (Pre), 6 weeks (Mid) and 12 weeks (Post) resistance exercise-training with nutritional supplementation[1]

TRT	Time Period			Main Effects
	Pre	Mid	Post	
Lean mass, *kg*				
PB	56.6 ± 1.5	58.1 ± 1.4	59.1 ± 1.3	T: 0.000,
WP	57.6 ± 1.5	59.4 ± 1.9	60.0 ± 2.0	trt: 0.497,
MDP	55.2 ± 1.5	56.6 ± 1.7	57.4 ± 1.7	t x trt: 0.326
% Fat				
PB	23.7 ± 1.3	22.9 ± 1.3	22.1 ± 1.2	T: 0.000,
WP	25.9 ± 1.2	24.7 ± 1.4	23.6 ± 1.4	trt: 0.466,
MDP	24.2 ± 1.6	23.2 ± 1.8	23.2 ± 1.7	t x trt: 0.770
Fat mass, *kg*				
PB	18.0 ± 1.3	17.6 ± 1.4	16.8 ± 1.1	T: 0.000,
WP	20.5 ± 1.3	20.0 ± 1.5	19.0 ± 1.7	trt: 0.388,
MDP	18.4 ± 1.7	17.6 ± 1.7	17.8 ± 1.8	t x trt: 0.515
Fat mass trunk, *kg*				
PB	9.3 ± 0.9	9.1 ± 0.9	8.6 ± 0.7	T: 0.000,
WP	10.8 ± 0.9	10.5 ± 1.0	9.9 ± 1.0	trt: 0.492,
MDP	9.7 ± 1.0	9.3 ± 1.0	9.3 ± 1.2	t x trt: 0.416
Arm lean mass, *kg*				
PB	7.1 ± 0.2	7.4 ± 0.2	7.6 ± 0.2	T: 0.000,
WP	7.2 ± 0.3	7.6 ± 0.3	7.7 ± 0.3	trt: 0.828,
MDP	7.0 ± 0.3	7.3 ± 0.3	7.4 ± 0.3	t x trt: 0.183
Leg lean mass, *kg*				
PB	19.2 ± 0.4	20.0 ± 0.5	20.1 ± 0.5	T: 0.000,
WP	20.3 ± 0.7	21.1 ± 0.8	21.2 ± 0.8	trt: 0.193,
MDP	18.8 ± 0.7	19.4 ± 0.7	19.6 ± 0.7	t x trt: 0.650
Appendicular lean mass, *kg*				
PB	26.2 ± 0.3	27.4 ± 0.7	27.7 ± 0.6	T: 0.000,
WP	27.5 ± 0.9	28.6 ± 1.0	28.9 ± 1.1	trt: 0.325,
MDP	25.8 ± 0.9	26.7 ± 1.0	27.0 ± 1.0	t x trt: 0.358
Trunk lean mass, *kg*				
PB	26.8 ± 0.7	27.1 ± 0.7	27.6 ± 0.7	T: 0.000,
WP	26.5 ± 0.8	27.1 ± 0.9	27.5 ± 0.9	trt: 0.510,
MDP	25.9 ± 0.6	26.1 ± 0.8	26.3 ± 0.7	t x trt: 0.369
BMC, *g*				
PB	3172 ± 80	31434 ± 72	3134 ± 73	T: 0.000,
WP	3194 ± 104	3203 ± 114	3194 ± 113	trt: 0.698,
MDP	3114 ± 91	3074 ± 98	3036 ± 101	t x trt: 0.540
BMD, g/cm^2				
PB	1.316 ± 0.020	1.282 ± 0.020	1.310 ± 0.022	T: 0.000,
WP	1.327 ± 0.028	1.307 ± 0.032	1.305 ± 0.031	trt: 0.781,
MDP	1.306 ± 0.027	1.287 ± 0.032	1.264 ± 0.032	t x trt: 0.062

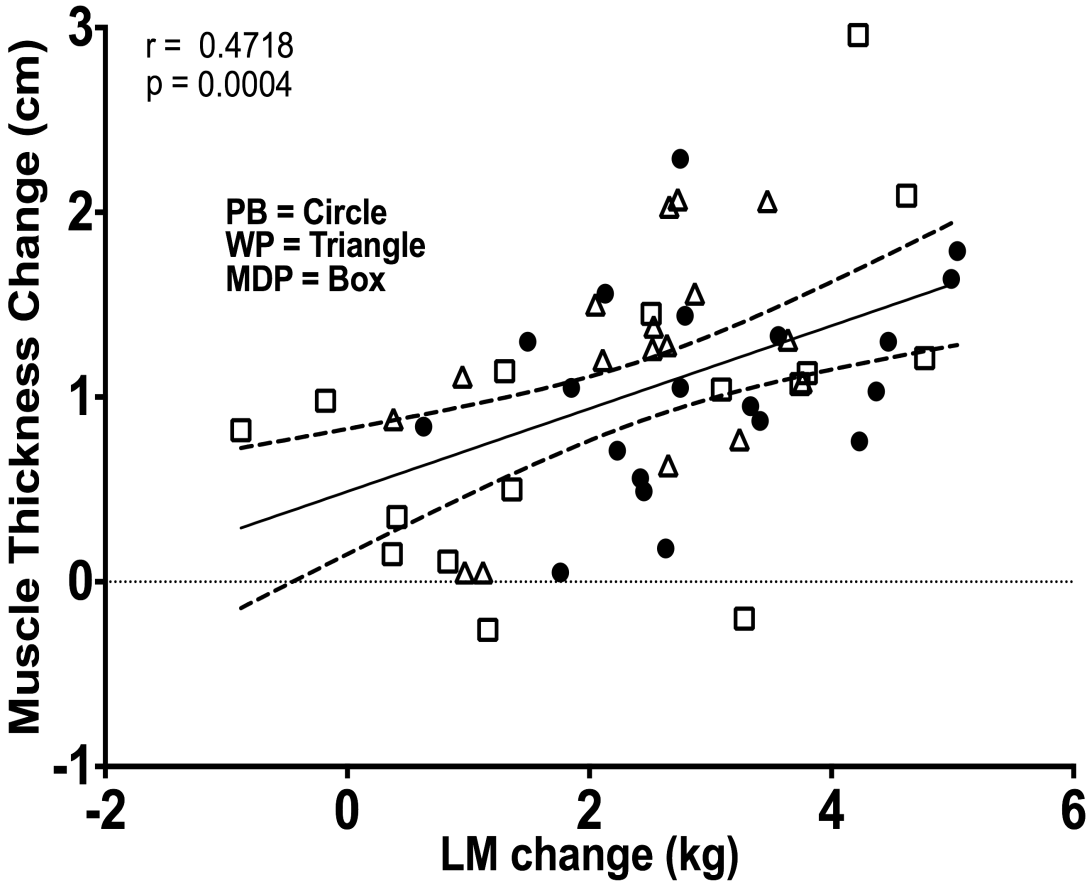
[1]Data are mean ± SEM CI, n=22 (WP), 23 (PB) & 23 (MDP).1Data are mean ± SE or mean (lower, upper) 95% CI, n=22 (WP), 23 (PB) & 23 (MDP). TRT, main effect for treatment; T, Main Effect for time. BMD = Bone mineral density, BMC = Bone mineral content

Figure A.4.1. Correlation between change in arm lean mass change from pre to post-training and appetite questionnaire responses



Correlation between change in arm lean mass change from pre to post-training and appetite questionnaire responses from the visual analog scale (0-10cm) addressing perceived right before the first meal consuming after ingesting the supplement by treatment during 12 weeks resistance exercise-training with nutritional supplementation. Protein blend (PB) or whey protein (WP) or maltodextrin placebo (MDP). Data are mean ± SEM, n=18 (WP), 20 (PB) & 13 (MDP).

Figure A.4.2. Correlation between change in arm lean mass change from pre to post-training and appetite questionnaire responses



Correlation between the pre to post-training change in thigh muscle thickness and the change in whole body lean mass by treatment during 12 weeks resistance exercise-training with nutritional supplementation. Protein blend (PB) or whey protein (WP) or maltodextrin.

Figure A.5.1. Pre and Post-Training Relative Frequency of Myofiber CSA by Treatment and Fiber Type

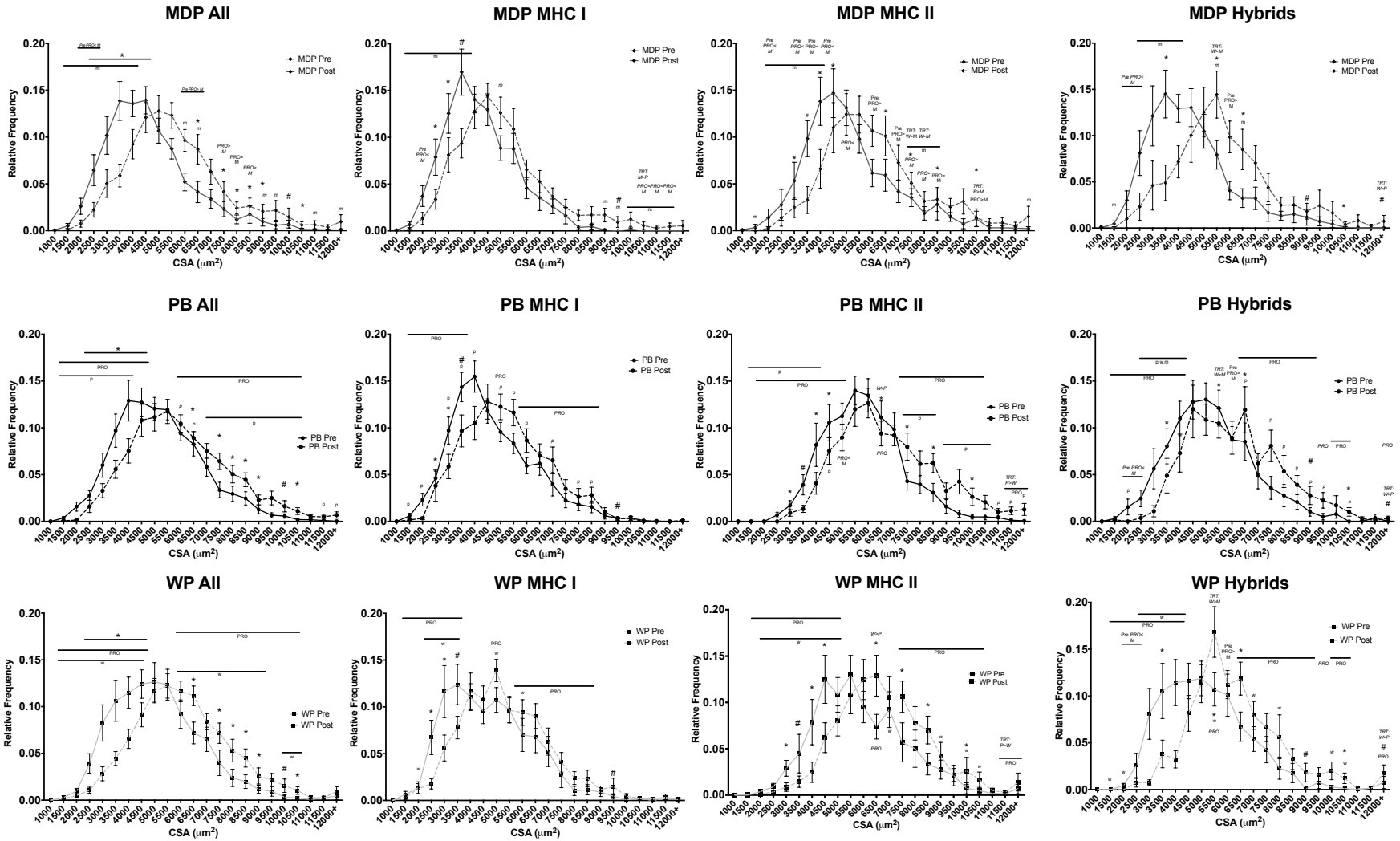


Fig. A.5.1 Pre and Post-Training Relative Frequency of Myofiber CSA by Treatment and Fiber Type during 12 weeks resistance exercise-training with nutritional supplementation. Protein blend (PB, N=22) or whey protein (WP, n=15) or maltodextrin placebo (MDP, n=17). Data are mean ± SEM. * $p<0.05$,# $p>0.05$ <0.10, main effect of training; p,w,m and PRO $p<0.05$ vs pre for ANCOVA change for the PB, WP, MDP and PB+WP, respectively; Italics $p>0.05$ <0.10.

Table A.5.1. Characteristics of Fiber Typing and Cross-sectional Area Immunohistochemical Analysis

	Pre			Post				
TRT	PB	WP	MDP	PB	WP	MDP	Total	Main Effects
MHC Fibers Counted								
I	104 ± 13	90 ± 8	90 ± 9	83 ± 7	98 ± 11	78 ± 8		t:0.218 trt:0.650 t x trt:0.178
IIa	98 ± 8	85 ± 11	90 ± 7	107 ± 11	103 ± 13	98 ± 10		t:0.053 trt:0.707 t x trt:0.745
I/IIa	3 ± 1	1 ± 1	1 ± 0	1 ± 1	1 ± 1	1 ± 1		-
IIx	-	-	-	-	-	-		-
IIa/IIx	35 ± 5	31 ± 4	32 ± 5	29 ± 4	35 ± 5	32 ± 5		t:0.742 trt:0.994 t x trt:0.501
I/IIx	-	-	-	-	-	-		-
I/IIa/IIx **T PRO**	31 ± 5	45 ± 7	55 ± 11	16 ± 48*	26 ± 6*	25 ± 5*		t:0.000 trt:0.079 t x trt:0.329
IIa+IIa/IIx **T**	134 ± 11	116 ± 12	122 ± 11	136 ± 10	137 ± 11	129 ± 10		t:0.065 trt:0.770 t x trt:0.348
Hybrids **PRO**	70 ± 7	77 ± 7	88 ± 10	46 ± 7*	63 ± 9	58 ± 8*		t:0.000 trt:0.174 t x trt:0.560
All	272 ± 15	252 ± 17	268 ± 16	236 ± 11*	264 ± 13	234 ± 13		t:0.042 trt:0.926 t x trt:0.077
Sum All	5,973	3,774	4,551	5,181	3,955	3,975	27,409	
CSA Fibers Counted								
I	69 ± 5	75 ± 5	69 ± 5	64 ± 4	73 ± 6	64 ± 6		t:0.182 trt:0.376 t x trt:0.964
IIa	65 ± 5	66 ± 9	69 ± 6	74 ± 7	70 ± 9	73 ± 7		t:0.109 trt:0.850 t x trt:0.865
I/IIa	-	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 0		-
IIx	-	-	-	-	-	-		-
IIa/IIx	23 ± 3	23 ± 3	27 ± 5	22 ± 4	29 ± 4	25 ± 3		t:0.791 trt:0.589 t x trt:0.467
I/IIx	-	-	-	-	-	-		-
I/IIa/IIx **T PRO**	25 ± 5	36 ± 6	45 ± 8	14 ± 4#	21 ± 5*	22 ± 4*		t:0.000 trt:0.050 t x trt:0.228
IIa+IIa/IIx **PRO**	89 ± 5	90 ± 9	96 ± 8	98 ± 8	103 ± 8*	98 ± 8		t:0.029 trt:0.955 t x trt:0.199
Hybrids	50 ± 5	60 ± 7	73 ± 7^	37 ± 6	52 ± 8	47 ± 5*		t:0.000 trt:0.034 t x trt:0.176
All	185 ± 8	201 ± 11	212 ± 10	172 ± 8	193 ± 10	181 ± 9*		t:0.003 trt:0.147 t x trt:0.286
Sum All	3,877	2,818	3,385	3,620	2,888	3,079	19,667	

[1]Data are mean ± SEM, n=15 (WP), 22 (PB) & 17 (MDP). * P<0.05, # P<0.10 vs Pre for that treatment. T = P<0.05 for an overall change from pre. PRO = P<0.05 for an overall change in the PRO trts from pre. ^ = p <0.05 vs PRO at Pre

Table A.5.2. Characteristics of Fiber-Type Specific Satellite Cell and Myonuclei Immunohistochemical Analysis [1]

	Pre			Post				
TRT	PB	WP	MDP	PB	WP	MDP	Total	Main Effects
Satellite Cell & Myonuclei Fibers Counted								
I	96 ± 7	103 ± 12	88 ± 6	85 ± 5	92 ± 9	93 ± 7		t:0.169 trt:0.678 t x trt:0.200
II **T**	122 ± 6	136 ± 7	141 ± 9	127 ± 8	116 ± 9	136 ± 10		t:0.162 trt:0.317 t x trt:0.113
All **T**	218 ± 7	239 ± 14	229 ± 11	211 ± 9	208 ± 10#	229 ± 7		t:0.072 trt:0.340 t x trt:0.202
Sum All	4,574	3,339	3,901	3,115	4,435	3,894	23,258	
$PAX7^+$ Satellite Cells Counted								
I **PRO**	12 ± 1	12 ± 2	10 ± 1	11 ± 1	13 ± 1*	11 ± 1*		t:0.834 trt:0.346 t x trt:0.801
II **PRO**	16 ± 2	17 ± 2	17 ± 1	27 ± 3	22 ± 3*	28 ± 4*		t:0.000 trt:0.552 t x trt:0.250
All	28 ± 2	29 ± 2	27 ± 2	38 ± 4	34 ± 3	39 ± 4		t:0.000 trt:0.865 t x trt:0.466
Sum All	589	405	468	807	515	665	3,449	
Myonuclei Counted								
I	255 ± 15	272 ± 31	219 ± 16	237 ± 14	256 ± 22	254 ± 19		t:0.993 trt:0.509 t x trt:0.092
II	346 ± 18	382 ± 24	361 ± 20	393 ± 23	350 ± 23	387 ± 27		t:0.363 trt:0.956 t x trt:0.127
All	601 ± 21	654 ± 45	580 ± 28	630 ± 25	606 ± 24	641 ± 23		t:0.517 trt:0.799 t x trt:0.144
Sum All	12,628	9,150	9,855	13,222	9,088	10,897	64,840	

[1]Data are mean ± SEM, n=15 (WP), 22 (PB) & 17 (MDP). * P<0.05, # P<0.10 vs Pre for that treatment. T = P<0.05 for an overall change from pre. PRO = P<0.05 for an overall change in the PRO trts from pre. ^ = p <0.05 vs PRO at Pre

Table A.5.3. Thigh circumference and leg volume by treatment before (Pre) and 12 weeks (Post) resistance exercise-training with nutritional supplementation [1]

Supplemental Table 3

	PB		WP		MDP	
	Pre	Post	Pre	Post	Pre	Post
Thigh Circumference, *cm*	50.15 ± 0.78	52.43 ± 0.66*	51.21 ± 1.15	52.60 ± 1.07*	49.43 ± 1.09	51.26 ± 1.29*
Leg Volume, *L*	10.09 ± 0.26	10.86 ± 0.30*	10.53 ± 0.48	11.38 ± 0.55*	9.92 ± 0.38	10.27 ± 0.49

[1]Data are mean ± SEM, n=15 (WP), 21 (PB) & 17 (MDP). * $p<0.05$ vs. pre for that TRT.

Table A.5.4. Isometric and isokinetic strength (relative to body weight) and power by treatment before (Pre) and 12 weeks (Post) resistance exercise-training with nutritional supplementation[1]

Supplemental Table 4

	PB		WP		MDP	
	Pre	Post	Pre	Post	Pre	Post
Isometric KE	331.9 ± 12.0	371.1 ± 11.5	316.1 ± 13.7	361.0 ± 15.0	331.0 ± 17.5	365.0 ± 17.7
Isometric KF	181.2 ± 6.7	192.6 ± 6.2	197.0 ± 9.2	206.4 ± 9.6	185.2 ± 7.5	198.9 ± 9.3
Isokinetic KE	237.5 ± 7.7	256.1 ± 9.0	226.0 ± 9.4	255.0 ± 10.0	244.9 ± 12.6	245.9 ± 12.9
Isokinetic KF	145.1 ± 6.6	151.4 ± 5.2	150.7 ± 9.8	164.2 ± 8.7	151.3 ± 8.1	152.7 ± 6.7
Power KE	243.4 ± 7.7	272.2 ± 10.2	257.1 ± 16.3	297.2 ± 16.0	256.0 ± 17.0	253.4 ± 15.1
Power KF	171.3 ± 6.7	184.3 ± 6.0	193.3 ± 10.7	201.7 ± 11.9	171.3 ± 11.2	176.9 ± 10.7

[1]Data are mean ± SEM, n=15 (WP), 22 (PB) & 17 (MDP). Knee extension = KE. Knee Flexion = KF. Strength = Torque (N-M). Torque was calculated relative to body weight. Power as Watts.

Table A.5.5. RNA concentration of the *vastus lateralis* by treatment before (Pre) and after 12 weeks (Post) resistance exercise training [1]

Supplemental Table 5

	PB (N=22)	WP (N=18)	MDP (N=20)
Pre	0.559 ± 0.013	0.570 ± 0.015	0.592 ± 0.014
Post	**0.614 ± 0.013**	0.629 ± 0.015	0.664 ± 0.014
Change	0.055 ± 0.018	0.059 ± 0.019	0.072 ± 0.019
Change 95% CI	(0.011,0.100)	(0.010,0.109)	(0.025,0.120)

[1]Data are mean ± SEM. Protein blend (PB), whey protein (WP) and Maltodextrin Placebo (MDP).

Table A.5.6. Western-blot analyses of mTORC1-associated signaling proteins in young men by treatment before (Pre) and 12 weeks (Post) resistance exercise-training with nutritional supplementation[1]

Supplemental Table 6

Time	Pre			Post		
TRT	PB	WP	MDP	PB	WP	MDP
Phosphorylated						
Akt Ser^{308}	0.122 ± 0.025	0.189 ± 0.035	0.138 ± 0.024	0.116 ± 0.023	0.224 ± 0.042*%	0.114 ± 0.022
mTORC1 Ser^{2448}	0.107 ± 0.020	0.094 ± 0.036	0.109 ± 0.026	0.144 ± 0.031	0.144 ± 0.031	0.231 ± 0.052#
4E-BP1 $Thr^{37/42}$	0.134 ± 0.019	0.212 ± 0.032	0.180 ± 0.029	0.110 ± 0.012	0.190 ± 0.025	0.150 ± 0.018
eEF2 Thr^{56} &	0.421 ± 0.033	0.457 ± 0.031	0.432 ± 0.037	0.426 ± 0.035	0.514 ± 0.044#	0.474 ± 0.045
Total						
Akt	0.218 ± 0.037	0.185 ± 0.025	0.226 ± 0.027	0.325 ± 0.086	0.296 ± 0.079	0.255 ± 0.027
mTORC1 %	0.194 ± 0.048	0.109 ± 0.036	0.119 ± 0.035	0.358 ± 0.102	0.271 ± 0.096	0.274 ± 0.057
p70S6K1	0.183 ± 0.028	0.215 ± 0.049	0.247 ± 0.048	0.207 ± 0.062	0.243 ± 0.061	0.237 ± 0.044
4E-BP1	0.199 ± 0.044	0.316 ± 0.065	0.157 ± 0.037	0.228 ± 0.064	0.363 ± 0.079	0.136 ± 0.027
eEF2	0.212 ± 0.027	0.295 ± 0.053	0.271 ± 0.049	0.274 ± 0.045	0.378 ± 0.051#	0.355 ± 0.040
Phosphorylated/alpha-tubulin						
Akt Ser^{308}	0.300 ± 0.054	0.381 ± 0.085	0.234 ± 0.52	0.350 ± 0.138	0.455 ± 0.149	0.159 ± 0.033
mTORC1 Ser^{2448}	0.457 ± 0.150	0.143 ± 0.030	0.209 ± 0.057	0.472 ± 0.133	0.300 ± 0.131	0.347 ± 0.073
4E-BP1 $Thr^{37/42}$ %, &	0.309 ± 0.038	0.399 ± 0.054	0.338 ± 0.063	0.216 ± 0.033	0.296 ± 0.042	0.221 ± 0.036#
eEF2 Thr^{56}	1.384 ± 0.243	1.236 ± 0.370	1.010 ± 0.249	1.317 ± 0.248	1.050 ± 0.237	0.748 ± 0.124
Total/alpha-tubulin						
Akt	0.633 ± 0.135	0.367 ± 0.071	0.417 ± 0.067	0.834 ± 0.228	0.431 ± 0.090	0.372 ± 0.054
mTORC1	0.710 ± 0.267	0.175 ± 0.043	0.155 ± 0.043	0.917 ± 0.270	0.380 ± 0.150	0.348 ± 0.086
p70S6K1	0.512 ± 0.089	0.378 ± 0.066	0.383 ± 0.045	0.560 ± 0.164	0.333 ± 0.051	0.304 ± 0.048
4E-BP1	0.433 ± 0.084	0.558 ± 0.096	0.269 ± 0.056	0.319 ± 0.055	0.512 ± 0.107	0.217 ± 0.047
eEF2	0.566 ± 0.095	0.517 ± 0.075	0.443 ± 0.062	0.614 ± 0.117	0.526 ± 0.047	0.497 ± 0.055

1Data are mean ± SEM, n=15 (WP), 22 (PB) & 17 (MDP). * P<0.05, # P<0.06 vs Pre for that treatment. # P<0.08. % Effect of PRO. & effect of time. Red highlight P<0.05 vs Pre via ANCOVA. Underlined, p < 0.10 vs rest for that TRT.

Appendix B

CHAPTER 5 CORRELATIONS

Figure B.5.1. Correlation between myonuclear number and cross-sectional areas (absolute and change data)

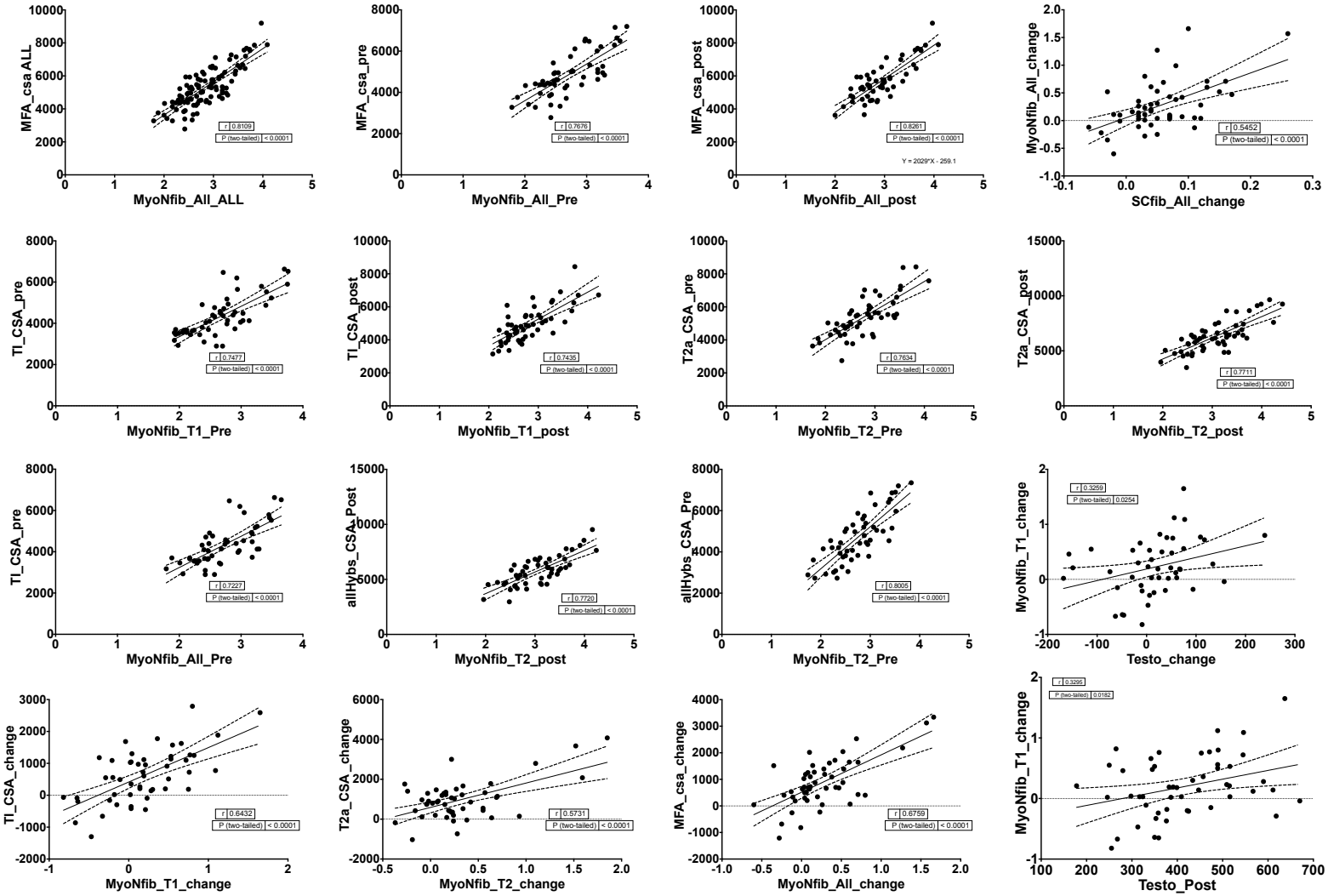


Figure B.5.2. Correlation between myonuclear domain and various outcomes

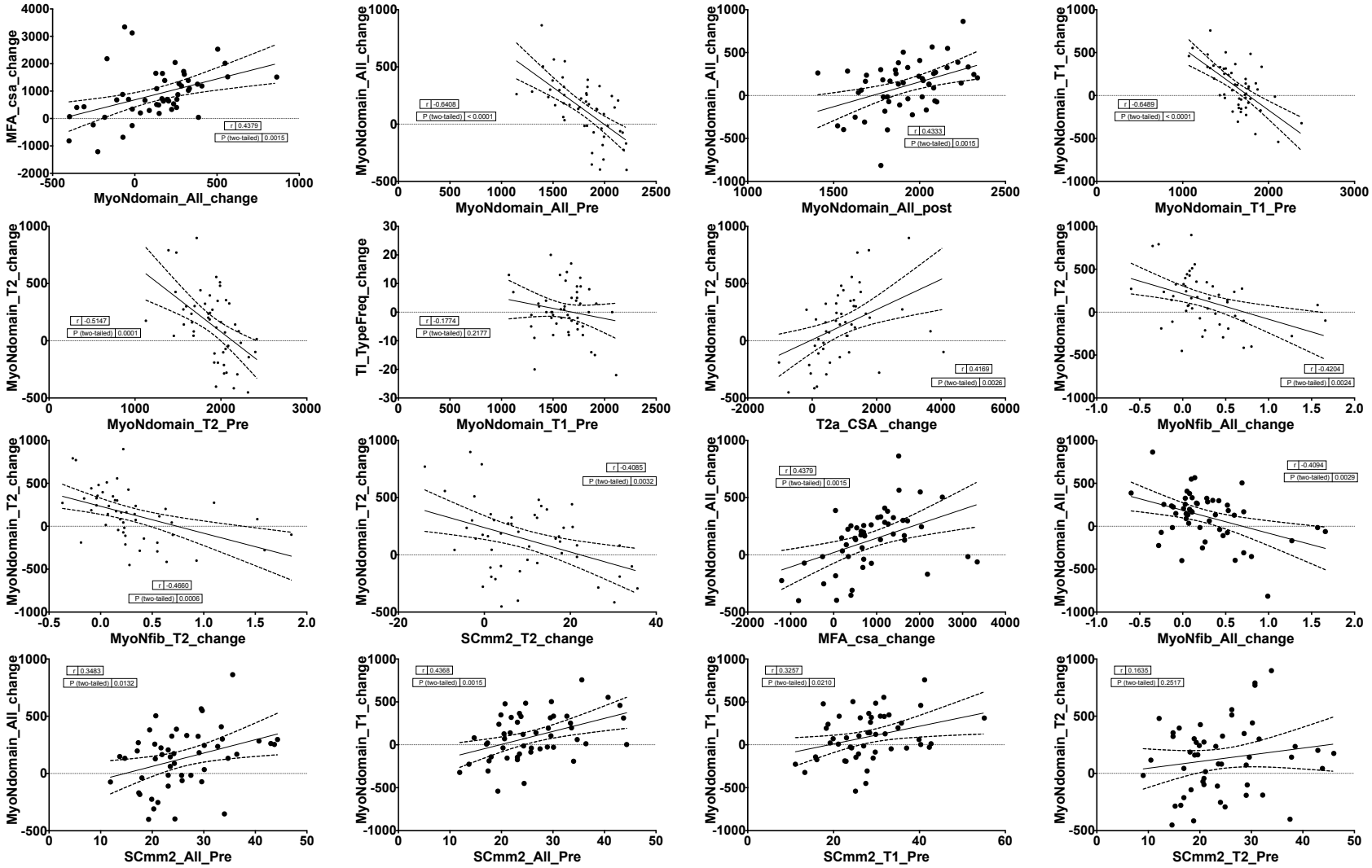


Figure B.5.3. Correlation between lean mass, strength and myofiber cross-sectional area

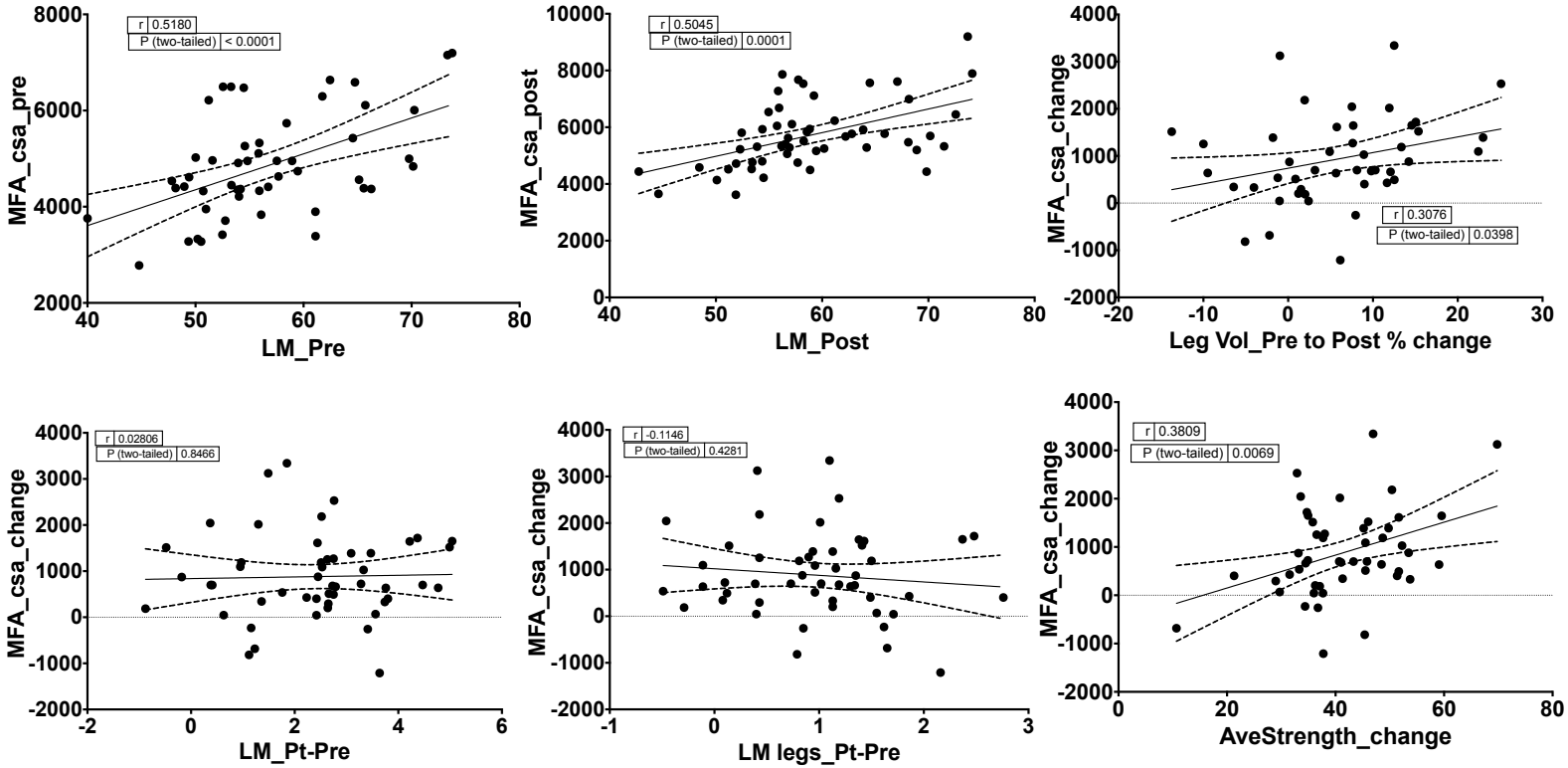
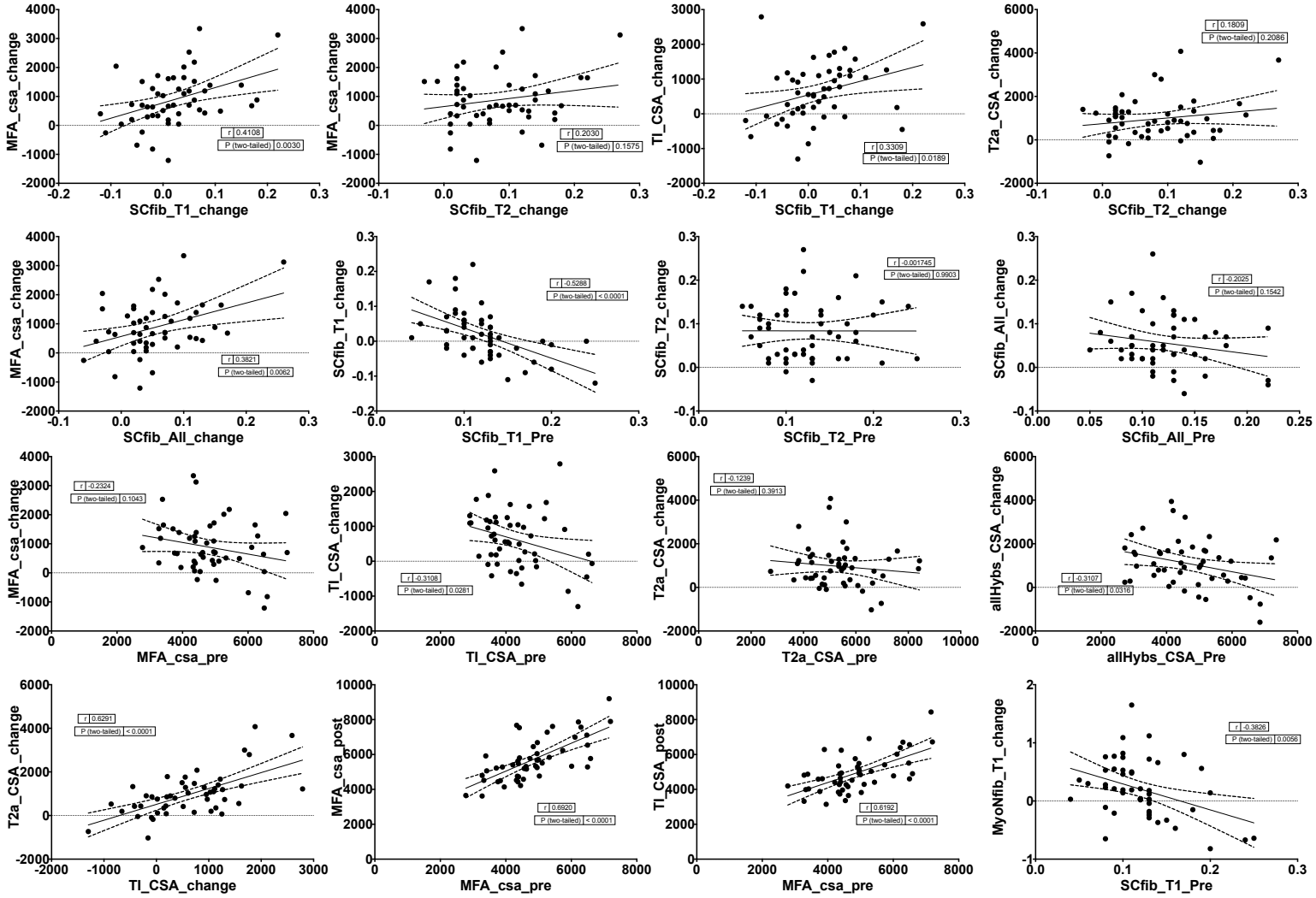


Figure B.5.4. Correlation between lean mass, strength and myofiber cross-sectional area



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Vita

Paul Timothy Reidy was born on December 23rd, 1985 to Timothy and Judith Reidy in Milwaukee, Wisconsin. He was educated at home until his junior year of high school. He attended Charles B. Whitnall high school in Greenfield, WI for two years and graduated the spring of 2004. He moved to Anderson, Indiana to attend a private liberal arts university, Anderson University. He studied graduated in 2008 with a Bachelor of Arts, majoring in Exercise Science and a minor in History. Thereafter, he went to the legendary Ball State University Human Performance Laboratory, located in Muncie, Indiana, where he worked as a graduate research assistant and received his Masters of Science in Exercise Physiology. After graduation, he worked as a laboratory technician for a few months and was married to Mollie Faye Ringer before moving to Galveston, Texas to start his doctoral studies in biomedical science. At UTMB, he worked in the laboratory of Dr. Blake Rasmussen as a graduate research assistant while taking classes in biology, public health and rehabilitation science. At BSU and UTMB Paul was involved in muscle physiology/biology research and publication. He has taught laboratory classes at AU, BSU and mentored students at BSU and UTMB. At UTMB he has also took a teaching skills class. He was the recipient of many graduate school awards and scholarships at UTMB.

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PUBLISHED:

A. ARTICLES IN PEER-REVIEWED JOURNALS:

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B. OTHER:

INFLUENCE OF AEROBIC TRAINING ON SKELETAL MUSCLE PROTEIN COMPOSITION.
A THESIS FOR THE DEGREE MASTERS OF SCIENCE
ADVISOR: MATTHEW P. HARBER, PHD
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C. ABSTRACTS:

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