Effects of Whey and Soy Protein Supplementation with a 9 Month Progressive Resistance Training Program on Intracellular Signaling Pathways of Muscle Protein Synthesis

By

Russell Scott Emmons

Submitted to the graduate degree program in Health, Sports and Exercise Science and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Science in Education.

Chairperson Phil Gallagher, Ph. D

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Date Defended: August 8, 2013

The Thesis Committee for Russell Scott Emmons

certifies that this is the approved version of the following thesis:

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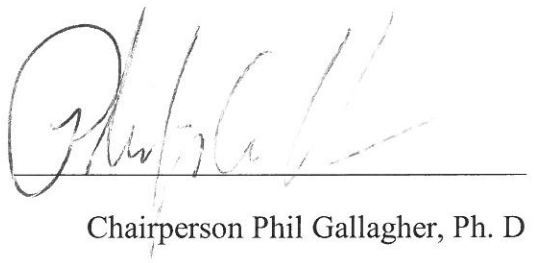

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List of Abbreviations

ANOVA	Analysis of Variance
BCAA	Branched Chain Amino Acids
EAA	Essential Amino Acids
4EBP1	Eukaryotic Initiation Factor 4E Binding Protein
eIF4E	Euakryotic Intitation Factor 4E
AA	Amino Acid
Akt	Protein Kinase B
BCA	Bicinchoninic Acid
ELISA	Enzyme Linked Immunosorbent Assay
EIA	Enzyme Immunoassy
FOXO	Forkhead Box O
INS	Insulin
IGF-1	Insulin Growth Factor
IRS	Insulin-Receptor Substrate
INS	Insulin
Leu	Leucine
MPS	Muscle Protein Synthesis
MPB	Muscle Protein Breakdown
mTOR	Mammalian Target of Rapamycin

NPB	Net Protein Breakdown
p70s6k	70 kDa ribosomal protein s6k1
PI3K	Phosphatidylinositol 3-Kinase
1 RM	Rep Max
Resistance Protocol	Acute Resistance Training Bout
Resistance Program	Full 9-Month Progressive Resistance Training Program
Tsc1/2	Tuberous Sclerosis Complex 1 and 2 proteins

Abstract

Introduction: Resistance training and protein supplementation have been shown to synergistically stimulate the Akt cascade in human skeletal muscle. The purpose of this study was to explore the effects of a combined protein supplementation protocol combined with a 9 month progressive resistance training protocol on the changes in intracellular signaling of protein synthesis. **Methods**: 19 healthy, young, non-obese sedentary individuals were recruited for participation and randomly assigned to either consume a whey, soy, or placebo group to complete a 9 month progressive resistance training protocol. Muscle biopsies were collected before and 10 minutes after an acute resistance exercise bout before resistance and upon completion of the resistance training program. **Results:** There was no significant changes in the stimulation of Akt, p70s6k, or 4ebp1 pre-resistance training protocol versus post-resistance training program and regardless of supplementation group. No significant differences were seen in the basal activity levels of Akt, p70s6k, or 4EBP1. No significant strength gains in bilateral leg extension regardless of treatment group. **Conclusion:** Protein supplementation combined with a 9 month resistance training protocol does not change skeletal muscle's response to an acute resistance exercise bout.

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I would like to thank my advisor, Dr. Phil Gallagher for his continued guidance, patience, and support throughout this project. He has helped me, for better or worse, develop into the person I am today, and fostered my ambition to become successful in the field of academia.

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Chapter I

Introduction

Proper nutrition plays a key role in maintaining skeletal muscle mass; regulating the balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB). This balance is called the net protein balance (NPB). The main contributor to a positive or neutral NPB is muscle protein synthesis. MPS is highly responsive to the availability of extracellular essential amino acids (EAA) (Howarth, Moreau, Phillips, & Gibala, 2009) . Whey protein is a naturally occurring byproduct of cheese and curd manufacturing; valued for possessing a high spectrum of amino acids. Considered a "fast" protein due to its ability to quickly move through the acidic environment of the stomach, whey enters the small intestine more rapidly in comparison to other milk based products such as casein. Upon entering the duodenum, whey protein is more slowly hydrolyzed in comparison to casein, providing a longer time period for increased amino acid absorption throughout the length of the small intestine (Boirie et al., 1997). Whey protein has been widely researched due to possessing a high ratio of branched chain amino acids, and has been implicated with health benefits for cardiovascular disease, osteoporosis, skeletal muscle repair, and tissue growth (Marshall, 2004).

Whey protein is a major source of the branched chain amino acids (BCAA) leucine, iso-leucine, and valine. BCAA's compose approximately 33% of myofibullar protein within skeletal muscle (Layman, 2002). Leucine is of particular interest, as it has been associated with directly stimulating the anabolic pathway protein kinase B (Akt)/mammalian target of rapamycin (mTORC1)/ 70-kDa ribosomal protein kinase (p70s6k) (Wang & Proud, 2006). Within murine models, leucine has been shown to directly stimulate the mTORC1 and the subsequent phosphorylation of proteins downstream including eukaryotic-initiation factor 4E binding-

protein (4EBP1) and 70 kDa ribosomal protein S6K1 (p70s6k1) (Kimball & Jefferson, 2004; Nagasawa, Kido, Yoshizawa, Ito, & Nishizawa, 2002; Proud, 2004). Stimulation of mTORC1 results in increased MPS, and offsets MPB pathways associated with the catabolism of skeletal muscle. Research into human subjects with leucine and EAA supplementation has produced conflicting results on the effects of NPB. Although leucine and EAA supplementation has been shown to be taken up by skeletal muscle and lead to MPS. It also appears that without an adequate amount of indispensable extracellular amino acids, MPS will revert back to basal levels (Bohe, Low, Wolfe, & Rennie, 2003; Boirie et al., 1997). MPS in response to amino acids or protein supplementation is dose dependent (Cuthbertson et al., 2005; Moore et al., 2009).

Soy protein is another popular vegetable derived source of EAAs. Although soy protein sources contain lower amounts of BCAAs in comparison to both whey and casein, it has been show to stimulate MPS within both mouse and human models (Anthony et al., 2007; Butteiger et al., 2013; Luiking, Engelen, Soeters, Boirie, & Deutz, 2011). Soy protein has been shown to stimulate the mTORC1 pathway and its downstream effectors p70s6k and 4EBP1 (Anthony et al., 2007), although to a lesser degree in comparison to whey. Caution surrounds the use of soy protein as a supplement due to controversy of the effects of soy isoflavones. Still, soy protein serves an excellent source of EAAs and a potent stimulator of MPS within skeletal muscle.

Resistance training is a far more potent stimulator of MPS than feeding within skeletal muscle. The anabolic response from skeletal muscle secondary to resistance training alone has been well established (Chesley, MacDougall, Tarnopolsky, Atkinson, & Smith, 1992; Phillips, Tipton, Aarsland, Wolf, & Wolfe, 1997; Welle, Thornton, Jozefowicz, & Statt, 1993). Acute bouts of resistance training robustly activate the insulin-like growth factor-1 (IGF-1)/Akt/mTORC1 pathway. Activity within MPS pathways elevates 10 minutes after resistance

exercise and will continue to be up regulated for 24 to 48 hours (Creer et al., 2005; Phillips et al., 1997). However, a positive shift in NPB and protein accretion will only occur if there is an adequate source of amino acids are available (Biolo, Tipton, Klein, & Wolfe, 1997; Rasmussen, Tipton, Miller, Wolf, & Wolfe, 2000; Tipton, Ferrando, Phillips, Doyle, & Wolfe, 1999).

The combination of resistance exercise and protein supplementation produces a synergistic effect that elevates MPS more robustly than either individually. Supplemental whey protein administered in conjunction with an acute resistance training bout has been shown to increase MPS via the mTORC1 protein cascade for 2 hours post exercise (Farnfield, Carey, Gran, Trenerry, & Cameron-Smith, 2009). Whey protein supplementation significantly increased the phosphorylation of mTORC1, 4EBP1, and p70s6k when compared to placebo. These results are agree with previous studies by Rasmussen et al. (2000) and Wilkinson (2007), who observed increased MPS signaling in response to resistance training and protein supplementation.

The Akt/mTORC1 pathway has been implicated in promoting muscle hypertrophy and inhibits the catabolic pathways leading to MPB. Activation of the Akt/mTORC1 pathway can occur through a myriad of different stimuli including exercise, ingestion of amino acids, and elevated insulin level. Subsequent downstream effectors phosphorylated include p70s6k and 4EBP1. Inhibition of 4EBP1 causes disassociation with eukaryotic initiation factor 4E (eIF4E), allowing protein synthesis to occur. Both supplemental whey protein and exercise have been shown to stimulate the Akt/mTORC1 pathway. Previous studies have investigated the short term effects of supplemental whey protein combined with exercise on intracellular protein signaling, but none have looked at the effects over a 9 month periodized resistance training protocol.

I. Statement of the Problem

Whey protein in combination with resistance training has been shown to have a positive effects of stimulating MPS. However, there has been no research investigating the long term effects on stimulating the Akt/mTORC1 pathway within skeletal muscle. It is possible that a combination of protein supplementation along with a periodized resistance training protocol will elicit a greater effect in activating the Akt/mTORC1 pathway.

Thus, the following hypotheses will be tested on 25 (n = 25) normal, young (18-34 yrs) sedentary individuals:

1. Activation of the Akt, p70s6k, and 4EBP1 pathway will be increased following completion of the 9 month periodized resistance training protocol compared to pre-workout status with protein supplementation in the whey soy and whey groups.

   Protein supplementation and resistance training will result in increased change of activation of Akt, 4EBP1, p70s6k. This will result in the subsequent increased activation of P70S6K and phosphorylation of 4EBP1 (inhibition).

2. The P:T ratio of Akt, p70s6k and 4EBP1 at basal levels will be higher in the whey and soy groups compared to the placebo group following the resistance training program.

   Whey supplementation will result in equal activation of the Akt/mTORC1 pathways. This should cause an equal phosphorylation rates between placebo soy and whey groups post resistance training program.

Specific Aim #1

To determine if there is a difference a change in the phosphorylation ratio of Akt, p70s6k, and 4EBP1 between whey, soy, and placebo groups.

Specific Aim #2

Determine if there is a significant difference in P:T ratio of Akt, p70s6k, and 4EBP1 between basal levels before and after the resistance training program.

II. RATIONALE FOR THE INVESTIGATION

While whey protein has been shown to increase activity of the Akt/mTORC1 pathway within skeletal muscle, most research has focused on the short-term effects of supplementation and resistance exercise on MPS. It is important to determine the long term effects of whey protein supplementation on protein synthesis. A better understanding of the relationship between the long term effects of protein supplementation and resistance exercise would allow for maximizing muscle hypertrophy as a therapeutic intervention. This information would be of particular interest in cases of sarcopenia, diabetes, and sedentary populations.

Chapter II

Review of Literature

Akt/mTOR Pathway

Skeletal muscle mass is maintained through an intricate balance of muscle protein synthesis (MPS) and muscle protein breakdown (MPB). In normal healthy(Bamman et al., 2001) skeletal muscle, the net protein balance (NPB) is preserved through ingestion of high-protein meals resulting in hyperaminoacidema, elevating muscle protein synthesis (Burd et al., 2011). Heightened levels of MPS appear to be transient, returning back to basal levels after 24 hours (Creer et al., 2005). Resistance exercise is also a potent stimulator of acute myofibular proteins synthesis (Adams & Haddad, 1996; Adams, Haddad, & Baldwin, 1999; Bamman et al., 2001; D'Aniello, D'Onofrio, & Pischetola, 1990; Smith, 2000; Yan, Biggs, & Booth, 1993; Yang, Alnaqeeb, Simpson, & Goldspink, 1996). Consequences from exercise and amino acid consumption lead to up-regulation of a myriad of different intracellular signaling pathways associated with MPS. The purpose of this review is to outline the protein kinase B (Akt)/ mammilan target of rapamycin (mTOR) pathway; including the resultant activation of downstream effectors 70-kDa ribsomal protein S6k1 (p70S6k) and phosphorylation of eukaryotic-initiation factor 4E binding-protein (4EBP1).

Mechanisms of action

The Akt/mTOR pathway lays downstream of the insulin growth factor-1 (IGF-1)/ phosphatidylinotiol 3 kinase (PI3K) pathway. Released in response to different stimuli, IGF-1 binds to an insulin receptor and activates insulin receptor substrate (IRS-1) resulting in phosphorylation of PI3K (Phillips, 2009). Activation of the IGF-1/PI3K pathway can be induced by electrically induced muscle contraction, loads induced stretching, and elevated insulin levels,

and resistance training (Yang et al., 1996). Once activated, PI3K moves to phosphorylate Akt molecules. Phosphorylation of Akt, in either a PI3K dependent or independent fashion, is necessary for promoting cell survival and inhibition of apoptosis. Activation of Akt leads to inhibition of several targets including Bad (Cardone et al., 1998), Forkhead transcription factors (FOX O) (Latres et al., 2005), and tuberous sclerosis complex 1 and 2 (TSC 1/2). TSC 1/2 is also an inhibitor of the mTORC1 complex (Glass, 2003; Rommel et al., 2001). Another function of an activated Akt protein is the phosphorylation of mTORC1. Once activated, mTOR phosphorylates several proteins downstream including p70s6k and 4EBP. A positive signal to p70s6k1 causes phosphorylation to the S6 protein of the 40S ribosomal subunit and increased mRNA translation (Pullen, N. and Thomas, G.) Another target of mTORC1 is 4EBP, an inhibitor of protein synthesis bound to eIF4E (eukaryotic initiation factor-4E) (Pause et al., 1994).

Phosphorylation, or inhibition, of 4EBP1 disrupts the 4EBP1*eIF4E complex and allowing increased mRNA translation and protein synthesis. Both eIF4E and p70s6k are crucial in stimulating downstream effectors that lead to up regulation of ribosomal subunit activity and translation of 5'terminal polyprimidine (TOP-sequences) mRNA's leading to increased protein synthesis [34]. The Akt/mTORC1 pathway is not only crucial for the role it plays in elevating protein synthesis, but also in deterring apoptosis. The NPB is a result of the interconnection between MPS and MPB; so it is imperative to note the effects of Akt/mTORC1 pathway on MPB and the protection from apoptosis leading to skeletal muscle atrophy.

Akt/mTOR Activation

The Akt/mTOR pathway has been implicated in both murine and human models to stimulate skeletal muscle hypertrophy and protection from skeletal muscle atrophy (Bolster, Kimball, & Jefferson, 2003; Bolster, Kubica, et al., 2003; Laurent, Sparrow, & Millward, 1978;

Pain, 1996; Rommel et al., 2001). Stimulation via adaptive hypertrophy, Akt is phosphorylated and activated. Several murine models have demonstrated the importance of the Akt/mTORC1 pathway in maintaining skeletal muscle mass. Activation of the IGF-1/PI3K pathway has been implicated in stimulating the Akt/mTOR pathway and causing skeletal muscle hypertrophy (DeVol, Rotwein, Sadow, Novakofski, & Bechtel, 1990; Goldspink, Garlick, & McNurlan, 1983). Increased skeletal muscle mass has also been observed in transgenic mice over expressing IGF-1 through skeletal muscle-specific promoters (Coleman et al., 1995; Musaro et al., 2001). IGF-1 lies upstream of the Akt/mTOR pathway; and over expression leads to activation of MPS and increased skeletal muscle hypertrophy. Other studies utilizing constitutively active forms of PI3K or Akt via electroporation have induced muscle fiber hypertrophy both *in vivo* (Bodine et al., 2001; Pallafacchina, Calabria, Serrano, Kalhovde, & Schiaffino, 2002) and *in vitro* (Rommel et al., 2001; Rommel et al., 1999). Lai et al. (2004) also demonstrated that conditionally inducing activation of Akt in transgenic mice was enough to elicit rapid skeletal muscle hypertrophy accompanied by activation of the downstream elements p70s6k and a decrease in the amount adipose tissue.

Further support for the importance of the Akt/mTOR pathway was also demonstrated in transgenic mice deficient in Akt. Knockout Akt -/- transgenic mice were significantly smaller, but also had shorter life spans when exposed genotoxic stress and spontaneous apoptosis in a wide variety of somatic cells in comparison to wild type mice (Chen et al., 2001). Cho et al. (2001) examined rats deficient in Akt; observing growth defects, decreased body weight and increased mortality rate. These studies taken together demonstrate the importance of the Akt/mTORC1 pathway in promoting skeletal muscle hypertrophy; but also in regulating existing skeletal muscle mass and promoting skeletal muscle cell myotube differentiation.

Activation of the Akt/mTOR pathway can happen independently of the IGF-1/PI3K pathway. Several studies have examined the effects of skeletal muscle hypertrophy in the absence of the IGF-1/PI3K. Utilizing an over-expression form of a dominant negative form of IGF-1 receptor, Spangenburg et al. observed similar skeletal muscle hypertrophy in response to mechanical overload between transgenic and wildtype mice. They also observed equal activation levels of the mTORC1 pathway and its downstream elements (Spangenburg, Le Roith, Ward, & Bodine, 2008). Evidence from this study also concurred with mTOR activation independent of PI3K/Akt regulation (Hornberger et al., 2004; O'Neil, Duffy, Frey, & Hornberger, 2009). Miyazaki et al. demonstrated mTORC1 activation independent of PI3K/Akt and was likely due to activation of the mitogen activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) with inhibition of PI3K by wortmannin. mTOR was significantly activated 1 day after mechanical loading, with Akt not becoming significantly active for 2-3 days after, indicating that there are multiple and redundant methods to activate the mTOR pathway and stimulate MPS (Miyazaki, McCarthy, Fedele, & Esser, 2011). Similarly, the branched chain amino acids (BCAA) have been shown to up regulate mTORC1 activity (Deldicque, Theisen, & Francaux, 2005).

Administration of rapamycin, an inhibitor of mTOR, has been shown to block the phosphorylation of p70s6k1 and 4EBP1; lending evidence to support the crucial point of mTORC1 activation and its downstream targets for protein synthesis (Dickinson et al., 2011). Activation of mTOR leads to phosphorylation of p70s6k and 4EBP1; with both pathways leading indirectly to increase translation of mRNA.

Insulin and Amino Acids

Insulin (INS) has been shown to enhance protein synthesis (Garlick & Grant, 1988; Pain, Albertse, & Garlick, 1983; Pain & Garlick, 1974) and increase translation of mRNA within skeletal muscle (Gautsch et al., 1998; Kimball, Jefferson, Fadden, Haystead, & Lawrence, 1996). Fujita et al. (2006) demonstrated that insulin increased protein synthesis and mRNA translation as long as there was an adequate supply of blood flow and amino acid supply. It has been postulated and shown by Gautsch et al. that there is a co-regulatory relationship between INS and AA uptake (Gautsch et al., 1998). With adequate amino acid supply, increasing insulin concentrations leads enhanced protein synthesis that is not necessarily dependent on Akt (Kwon, Marshall, Pappan, Remedi, & McDaniel, 2004). Resulting activation of the Akt/mTOR pathway leads to high phosphorylation rates of p70s6k and 4EBP1 resulting in skeletal muscle hypertrophy (Farrell et al., 1999; Gautsch et al., 1998).

Amino acids are also a positive signal for protein synthesis and activation of the Akt/mTOR pathway. The branched chain amino acid, leucine (Leu), activates protein synthesis at mTOR and the downstream protein factors p70s6k1 and 4EBP1 (Baum et al., 2005; Kimball & Jefferson, 2005). It should also be noted that although mixed AA are sufficient to stimulate MPS, they do not inhibit MPB (Biolo et al., 1997; Greenhaff et al., 2008). Glynn et al. (2006) demonstrated that ingesting an excess amount of Leu was sufficient to stimulate mTOR signaling, but not enough for overall protein anabolism within skeletal muscle (Glynn et al., 2010) MPS in response to amino acids or protein supplementation is dose dependent (Cuthbertson et al., 2005; Moore et al., 2009). It should be noted that although Leu supplementation is enough to stimulate MPS; without the availability of indispensable AA MPS will return to basal levels.

Resistance Training

Exercise has been shown to be a potent stimulator of the Akt/mTOR pathway (Bolster, Kimball, et al., 2003; Burd et al., 2011; Laurent et al., 1978; Moore et al., 2009; Phillips et al., 1997; Tipton et al., 1999). MPS increases by two-fold to five-fold after a single bout of heavy resistance exercise in young adults (Phillips et al., 1997). Stimulation of Akt appears within 10 minutes of recovery (Bolster, Kubica, et al., 2003) and remains elevated for 24 to 48 hours (Creer et al., 2005; Phillips et al., 1997). However, net protein accretion only occurs in the presence of feeding and without proper nutrition, MPS will return to basal levels (Rasmussen et al., 2000; Tipton, Borsheim, Wolf, Sanford, & Wolfe, 2003; Tipton et al., 1999). Differences in MPS responses to exercise have been noted athletes trained in either dynamic endurance or high-intensity training when subjected to opposite training stimuli (Coffey et al., 2006). MPS was blunted when exposed to their regular training stimulus, and was enhanced when exposed to different exercises. This contraction mode-specific response was further elucidated by Wilkinson et al. (2008) in a longitudinal study, who observed a blunted response of MPS to resistance exercise when subjected to the same protocol after 10 weeks(Wilkinson et al., 2008). Taken together, resistance training is a potent stimulator of MPS and the Akt/mTOR pathway and with proper nutrition will result in skeletal muscle hypertrophy. However, without the introduction of increasing stimuli, a training damping-induced response will result in a lowered MPS.

Soy

Introduction

The availability of AA serves as an important factor in regulating skeletal muscle mass, as previously stated. Soy protein is a rich source of nitrogen and EAAs necessary for long-term maintenance of skeletal muscle mass (Istfan, Murray, Janghorbani, Evans, & Young, 1983). Soy protein is considered a "medium" speed protein in terms of digestion. When compared to whey, nitrogen kinetics has demonstrated a more delayed appearance of amino acid appearance in plasma serum (Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009). However, in the same study Tang et al. (2009) also showed soy is still more rapidly digested when compared to casein. Some controversy surrounds the use of soy protein supplementation due to the caution with the effects of soy isoflavones to the body.

Akt/mTORC1 interactions

Research into the effects of soy protein and the Akt/mTOR pathway is limited. Most research into protein supplementation and the Akt/mTOR pathway focuses on the effects of rapidly digested proteins (whey) versus slow (casein). Anthony et al. (2006) investigated the effect of acute treadmill exercise and various protein supplementations on protein synthesis with the skeletal muscle of rats. They observed an elevated activation of mTOR, p70s6k, and 4EBP1 in both whey and soy supplementation when compared to a carbohydrate mix (Anthony et al., 2007). Although both were significantly higher than the carbohydrate mixture, the whey protein group activation of mTOR, p70s6k, and 4EBP1 was still higher in comparison to the soy group. It appears that whey possessing a higher content of the Leu lead to a higher activation of the mTORC1 pathway. Although research into the effects of soy on the Akt/mTOR pathway is limited, soy protein is still a promoter of MPS. Several studies utilizing fractional synthesis rates

(FSR) have seen an increase in MPS in response to exercise and soy supplementation in both mouse and human models (Butteiger et al., 2013; Luiking et al., 2011; Norton et al., 2009). In mouse models Butteiger et al. (2013) showed that MPS peaked at 90 minutes for a whey protein blend in comparison to the soy protein blend, which peaked at 135 minutes. These observations further demonstrate the slower digestion and availability of amino acids for MPS of soy in comparison to whey.

Whey

Introduction

Whey is a natural byproduct of cheese and milk possessing; possessing a high content of essential amino acids (EAA) in comparison to other protein sources including casien, soy, and wheat gluten (Walzem, Dillard, & German, 2002). Milk processing results in casein and whey byproducts; casein making up the curds while whey remains in the aqueous solution (Marshall, 2004). Whey protein also is also made up of various components including alpha-lactalbumin, beta-lactoglobulin, bovine serum albumin, lactoferrin, immunoglobulins, lactoperoxidase enzymes, glycomacropeptides, and lactose (Walzem et al., 2002). Recently, whey protein supplements have become commercially successful and implied to have various benefits ranging from immune modulation to improved muscular strength and body composition (Marshall, 2004). Whey protein is considered a “fast” digesting protein in comparison to other supplementation products including soy and casein In particular; whey protein contains a high amount of Leu. Upon ingestion, amino acid availability in plasma serum peaks approximately 1 hour after consumption (Farnfield, Trenerry, Carey, & Cameron-Smith, 2009). Several studies have demonstrated that ingestion whey protein supplements, either before or immediately

following exercise resistance training, enhances muscularity and strength gains in young males (Cribb, Williams, Stathis, Carey, & Hayes, 2007; Willoughby, Stout, & Wilborn, 2007). The various claims of the therapeutic effects have launched numerous studies into whey supplementation.

Akt/mTOR Interactions

Whey protein has been widely investigated for the possible effects on MPS. Investigations examining the effects of resistance exercise/whey protein ingestion on fractional synthesis rates have produced conflicting results. Akt/mTOR and the subsequent downstream factors p70s6k and 4EBP1 are further enhanced in response to nutritional supplementation with resistance training when compared with placebo (Blomstrand, Eliasson, Karlsson, & Kohnke, 2006; Dreyer et al., 2006). Farnfield et al. demonstrated an acute resistance training bout with whey protein isolate enhanced phosphorylation of mTORC1, p70s6k, and 4EBP1 at 2 hours post exercise versus a placebo in young males. They did not see any difference between the two groups of Akt activation at any time point or differences in phosphorylation levels at 4 or 24 hours after exercise (Farnfield, Carey, et al., 2009). Another study conducted by Farnfield et al. examined the effects of whey protein isolate supplementation versus placebo with a 12 week resistance training protocol in young and old men on the Akt/mTOR pathway. They observed a significant activation of mTOR in the young men group compared to placebo in both untrained state and following the protocol. In the older men, they observed a significant activation of mTOR, 4EBP1, and p70s6k in an untrained state, and no significant differences between placebo and whey protein isolate in the trained state (Farnfield, Breen, Carey, Garnham, & Cameron-Smith, 2012). Speculation points to AA or whey protein supplementation enhancing activation of mTOR, p70s6k, and 4EBP1; and when combined with resistance training leads to increases in

overall activation of the Akt/mTOR pathway resulting in further MPS. However, the long term results of whey protein supplementation in combination with resistance training merit further research in to the effects of the Akt pathway.

CHAPTER III

METHODS and PROCEDURES

Protein supplementation and resistance training have been shown to have beneficial results on the Akt/mTOR pathway and muscle hypertrophy. However, much of the previous research has shown conflicting results as the overall effect of protein supplementation, combined with resistance training, on the intracellular protein signaling over time. Ergo, elucidation of the relationship between a combination of protein supplementation and a progressive resistance training program over a 9 month period would allow for clarification on interventions meant to increase muscle hypertrophy.

Experimental Design

The primary objective of this experiment was to measure changes in the Akt/mTOR pathway following 9-month resistance training program with whey supplementation. Subjects were divided into three treatment groups: resistance training + whey supplementation, resistance training + soy supplementation, resistance training + placebo supplementation. Following group assignment, subjects were put through a 9 month progressive resistance training program (3 sessions / week) and given supplements (40 g/day whey protein, 40 g/day soy protein, 40 g/day) twice a day. Muscle biopsies were obtained at two points from the vastus lateralis at baseline (resting and following exercise session) and upon conclusion (resting and following exercise session) of the 9 month resistance training program. Muscle samples were analyzed for differences in proteins associated with the Akt/mTOR/pS70S6K pathway.

Subjects

The participant population was recruited from the University of Kansas and the surrounding communities. 19 subjects successfully completed the full workout program and all

required addendums. Participants were compensated for successful completion of the 9 month program. Subjects had to meet the following inclusion criteria: young male and female individuals with no resistance-training in the past 12 months, low amounts of endurance training (Less than 3 hours per week), overweight (BMI) and stable weight (±2.27 kg) for 3 months. Female participants had to present a negative pregnancy test at baseline and were reevaluated at 3, 6 and post-study. Exclusion criteria for subjects: cardiovascular or metabolic disorders, medications including cardiac drugs, thyroid, steroids, insulin, beta blockers, SSRI's, binge drinking/more than 3 alcoholic drinks per day or 18 per week, use of smoking/tobacco products, or depression.

Resistance Training Protocol

All participants completed a 40-week progressive resistance training program. Participants performed 3 training sessions per week with 48 hours rest in-between each training session. Individual exercise sessions were monitored by an exercise supervisor whose responsibility was to verify participants completed exercise prescription and documentation of exercise, sets, repetitions, and load. The first 6 training sessions were completed with minimal self-selected weight for familiarization with the exercise protocol. Following familiarization, 1 repetition max (RM) were determined for all exercises used to program for initial training load. 1-RM was reevaluated at the beginning over every 4 week training cycle to adjust exercise prescription. Each training session consisted of a 5-10 minute warm up session on a bike ergometer or treadmill. The resistance training exercises consisted of chest press, shoulder press, triceps push down, back extension, leg press, lat pulldown, leg curl, calf raises and abdominal crunches on Paramount Advanced Performance equipment. Specific programing and sets can be viewed in the Appendix B.

Supplement Protocol

Subjects were randomly assigned to a supplement group: whey supplement, soy supplement, or placebo. Supplements were given in 20 gram doses to be consumed twice daily. On training days, subjects will consume 1 protein drink immediately after training and 1 protein drink with breakfast (3/week). On non-training days, subjects will be given a pre-measured amount of supplement to be consumed at the approximate times they are consumed during training days. Following consumption, subjects were instructed to bring in empty containers of protein supplements. To ensure compliance with supplementation protocols, subjects were required to randomly submit urine samples. Urine samples were measured for P-aminobenzoic acid, which was added to supplement. Failure of compliance with supplementation protocol below 90% within any 3 month time) period (1-3 months, 3-6 months, 6-9 months of the study results in subject expulsion from the study. Protein supplementation doses and placebo were prepared to be iso-caloric, iso-nitrogenous, and of similar texture and color.

Time Line

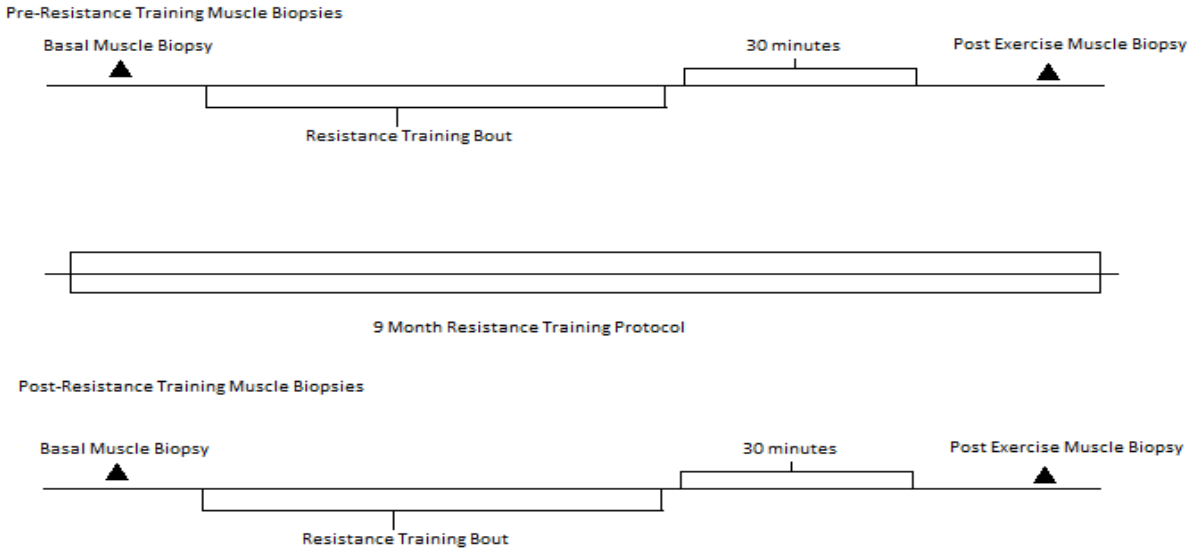

Figure 3-1 Timeline of events

Acute Resistance Training Bout

Subjects were run through an acute resistance training bout during the muscle biopsy to ensure maximal activation of proteins in the quadriceps. After the baseline muscle biopsy was obtained, subjects were taken to the weight room to determine their 1 rep max (RM) on the bilateral leg extension. Following determination of their 1 RM, subjects were given a 5 minute rest period. Subjects completed 3 sets at 80% 1 RM (2 x 10, 1 set to failure) with a minute rest period in between each set. Subjects were then led back to the Applied Physiology Lab for the post-resistance training protocol muscle biopsy.

Muscle Biopsy

Muscle samples were obtained from the right vastus lateralis from each subject to determine the quantitative values of skeletal muscle Akt, mTOR, p70s6k, and 4EBP-1. Percutaneous muscle biopsies were obtained from the lateral side of the right vastus lateralis at two time points: baseline and upon conclusion of the resistance exercise bout. Subjects came into the Applied Physiology Laboratory after fasting for 8 hours. The skin of the right vastus lateralis (mid-belly) was thoroughly cleaned and sterilized with beta-iodine at the site of the muscle biopsy. After ensuring the entry site was sterile, a local anesthetic (1.0-1.5 ml of 1% lidocain HCl 10 mg/ml) was applied. A 10 minute waiting period followed to ensure the anesthesia adequately desensitized the area. A number 11 sterile scalpel blade was used to create a 1 cm incision through the skin and fascia tissues. Muscle samples were then obtained using a 5.0 mm biopsy needle (check type). Muscle samples were immediately placed into storage for future analysis. Following initial muscle biopsy, subjects were run through a lower body resistance training bout on a leg extension to stimulate protein synthesis within the quad muscles. Upon completion,

subjects were instructed to lie supine for 10 minutes to ensure adequate activation of protein signaling. A second muscle biopsy was performed 3 cm from the original incision.

Intracellular Protein Signaling

Total protein concentrations of each sample were determined via a micro bicinchonic photometry assay (Thermo Scientific, pierce, Rockford, IL). Blanks, standards (bovine serum albumin) and samples (diluted 1:500) were pipetted, in triplicate, into a 96 well flat bottom plate. A copper sulfate solution was added to individual wells. The plates were set to incubate for 2 hours; and absorbance measured at 562 nm (Synergy Microplate Spectrophotometer, Bio-Tek R® Instruments, Inc, Winooski, Vermont).

Western Immunoblotting

To determine the total and phosphorylated levels of Akt, p70sk6, and 4EBP-1, western immunoblotting techniques were utilized. Protein samples (80 µg/ml) were loaded into each lane, and separated via SDS-Page (mini Protean 3, Bio-Rad, Herculeas, CA). Tailor made gels were used dependent on protein size. Proteins were transferred to a polyvinylindine diflurodide (PVDF) membrane. A blocking buffer (5.0 % non-fat dry milk, in tris-buffered saline/Tween 20 (TBS-T: 20 mM Tris-Base (pH 7.6), 137 mM NaCl) was used to prevent non-specific binding. Specific primary antibodies (rabbit IgG) were utilized for proteins of interest (Cell Signaling Technologies, Beverly, MA); diluted in an antibody buffer (5.0% BSA in TBS/T and incubated overnight at 4°C. PVDF membranes were then washed three times in TBST-T. Following the wash, a secondary antibody (rabbit IgG in 5.0% nonfat dry milk) labeled with horerasdish peroxidase was applied and incubated at room temperature for 1 hour. A second bout of washing three times in TBST-T was applied, and the membranes incubated within a chemilumiscent substrate (GE Healthcare-Amersham, Piscataway, NJ) for 5 minutes. Light emissions were

ascertained using Alphaview Flurochem HD2 and FC2 (Protein Simple, Santa Clara, CA). The signal intensities were quantified using Alphaview densitometry analysis software (Protein Simple, Santa Clara, CA).

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) program version 20 (Chicago, IL) was used to conduct statistical analysis of protein activation. Means and standard deviations were calculated for intramuscular content of total, phosphorylated, and phosphorylated to total levels (P:T ratio) of Akt, p70s6k, and 4EBP1 utilizing 3 x 2 analysis of variance (ANOVA) with a Bonferonni post hoc test. A probability level of 5% ($\alpha<0.05$) was set to determine statistical significance unless otherwise stated.

Chapter IV

Results

Akt. A 3 x 2 way ANOVA was utilized to compare the phosphorylation, total content, P:T ratio levels of Akt between pre-resistance training and post-resistance training groups across treatment groups (whey, soy, and placebo). . There was no significant difference in groups vs time points ($p = .689$) between treatment groups ($p = .687$) or time points ($p = .275$) for changes in P:T ratio across time points. The ratio of phosphorylation to total Akt expression at pre-resistance training time point 1 and post-training training time point 1 was also analyzed; no significant differences were detected comparing groups vs time points ($p = .525$) or between treatment groups ($p = .368$) or between time points ($p = .927$). There was no significant difference detected between group vs time points ($p = .779$) or treatment groups ($p = .074$) or between time points ($p = .190$) for phosphorylated levels of Akt. No significant difference was detected between groups vs time points ($p = .268$) or treatment groups ($p = .565$) or between time points ($p = .605$) for total Akt content. Overall, there was no significant differences observed in Akt activation as a result of the resistance training protocol or protein supplementation.

p70s6k. A 3 x 2 way ANOVA was utilized to compare the P:T difference of p70s6k between pre-resistance training and post-resistance training groups across treatment groups. There was no significant difference groups vs time points ($p = .358$) or between treatment groups ($p = .371$) or between time points ($p = .626$). The level of activation at pre-resistance training time program point 1 and post-resistance training program time point and no significant difference comparing groups vs time points ($p = .774$) or between treatment groups ($p = .372$) or between time points ($p = .689$). There was no significant differences detected between group vs

time points (p = .867) or treatment groups (p = .118) or time points (p = .931) for changes in phosphorylated levels. No significant difference was detected between groups vs time points (p = .611) or between time points (p = .263) for total p70s6k content. A significant difference was detected between groups; with placebo content being significantly higher (p = .006) than whey and no significant difference between placebo and soy (p = .409). There was no significant difference between whey vs soy (.069)

4EBP1. A 3 x 2 way ANOVA was utilized to compare the phosphorylation ratio of 4EBP1 compared to total expression (P:T) between pre-resistance training and post-resistance training groups across treatment groups (whey, soy, and placebo). There was no significant difference for groups vs time points (p = .272) or between treatment groups (p = .489) or time points (p = .964) for P:T ratio. The results also showed no significant differences between pre-resistance training program time point 1 and post-resistance training program time point 1 P:T ratio between groups vs time points (p = .616) or between treatment groups (p = .309) or between time points (p = .665). There was no significant difference detected between group vs time points (p = .419) or treatment groups (p = .516) or time points (p = .871) for phosphorylated levels. Total 4EBP1 content was not significantly different in groups vs time points (p = .471) or between groups (p = .483) or between time points (p = .841). Overall, there was no significant inhibition of 4EBP1 as a result of the resistance training protocol or protein supplementation.

Strength data. A 3 x 2 way ANOVA was used to measure changes in 1 RM on a bilateral leg extension pre-resistance training program versus post resistance training program. No

significant differences were detected between groups vs time points ($p = .889$) or between time points ($p = .084$). The amount lifted was significantly higher in the placebo group vs whey ($p = .025$) that was independent of time points. There was no significant difference between the whey vs soy ($p = 1.00$) or soy vs placebo ($p = .160$)

Figure 4-1. Changes in P:T ratio pre-resistance training and post-resistance training program. There was no significant difference in groups vs time points (p = .689) between treatment groups (p = .687) or time points (p = .275) for Akt. There was no significant difference groups vs time points (p = .358) or between treatment groups (p = .371) or between time points (p = .626) for p70s6k. There was no significant difference for groups vs time points (p = .272) or between treatment groups (p = .489) or time points (p = .964) for 4EBP1.

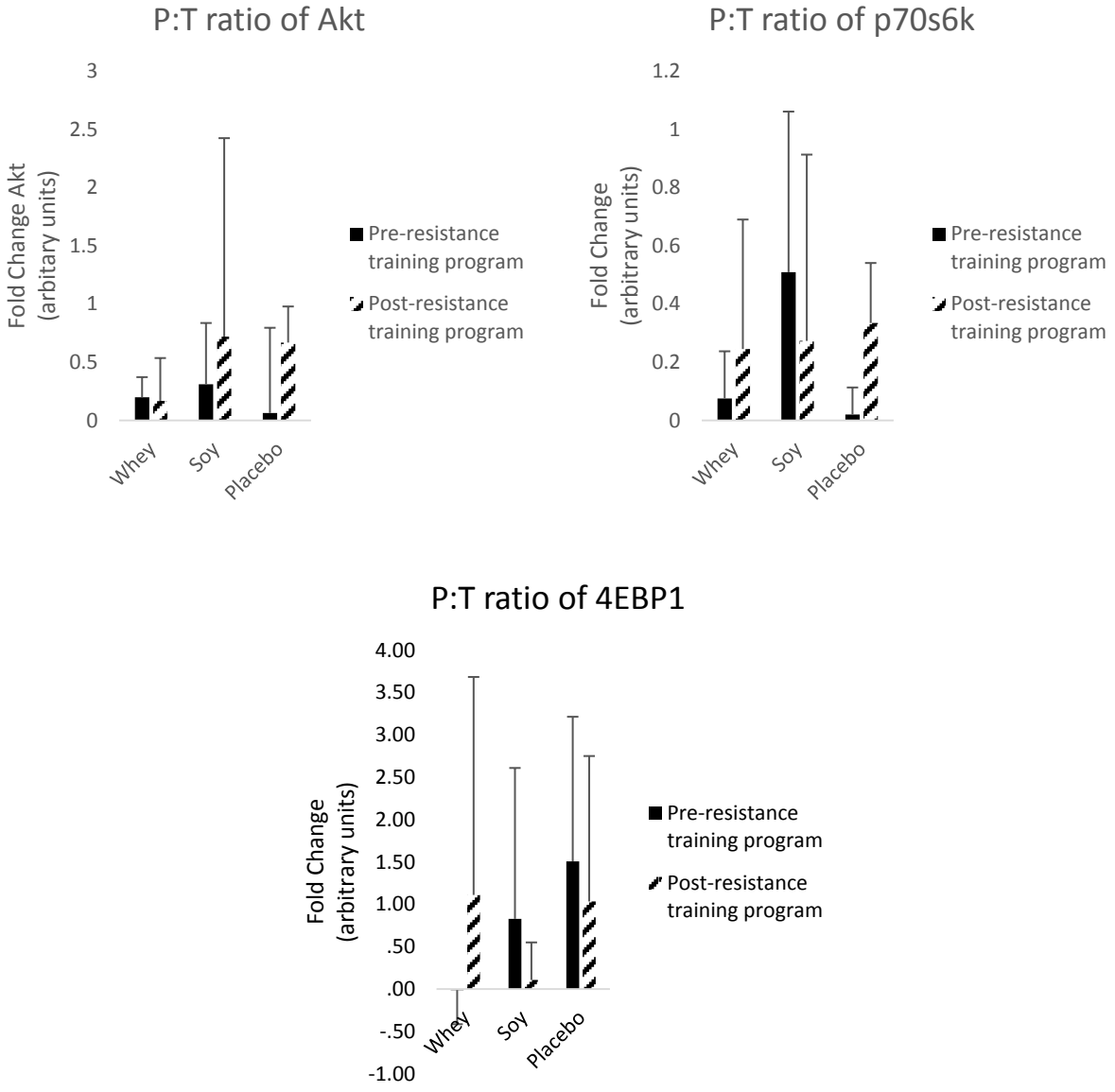

Figure 4-2. Changes in basal levels of P:T ratio. There was no significant differences were detected comparing groups vs time points (p = .525) or between treatment groups (p = .368) or between time points (p = .927) for Akt. There was no significant difference comparing groups vs time points (p = .774) or between treatment groups (p =.372) or between time points (p = .689) for p70s6k. There was no significant difference detected comparing groups vs time points (p = .616) or between treatment groups (p = .309) or between time points (p = .665).

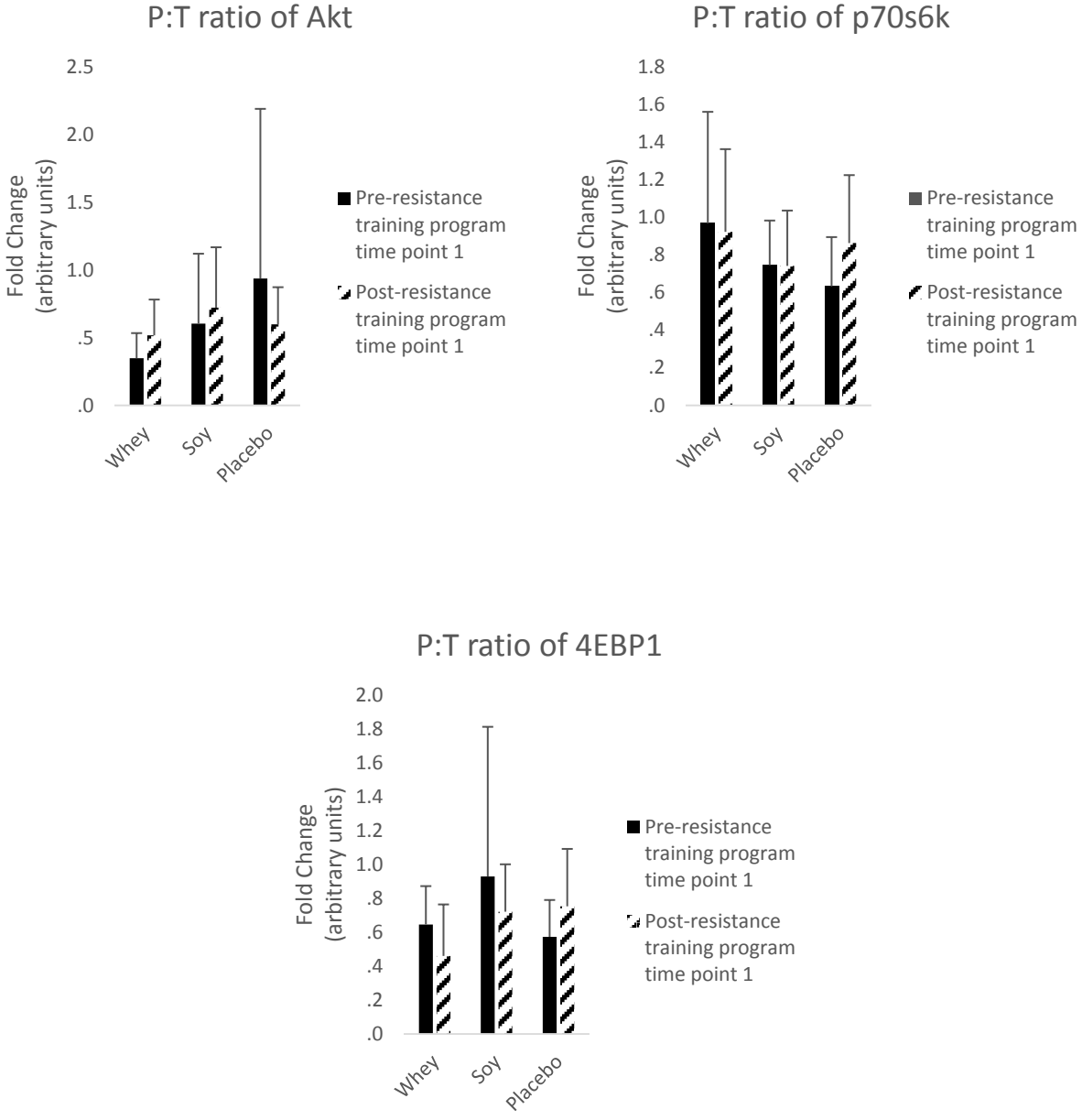

Figure 4-3. Changes in phosphorylated proteins. There was no significant difference detected between group vs time points (p = .779) or treatment groups (p = .074) or between time points (p = .190) for Akt. There was no significant differences detected between group vs time points (p = .867) or treatment groups (p = .118) or time points (p = .931) for p70s6k. There was no significant difference detected between group vs time points (p = .419) or treatment groups (p = .516) or time points (p = .871)

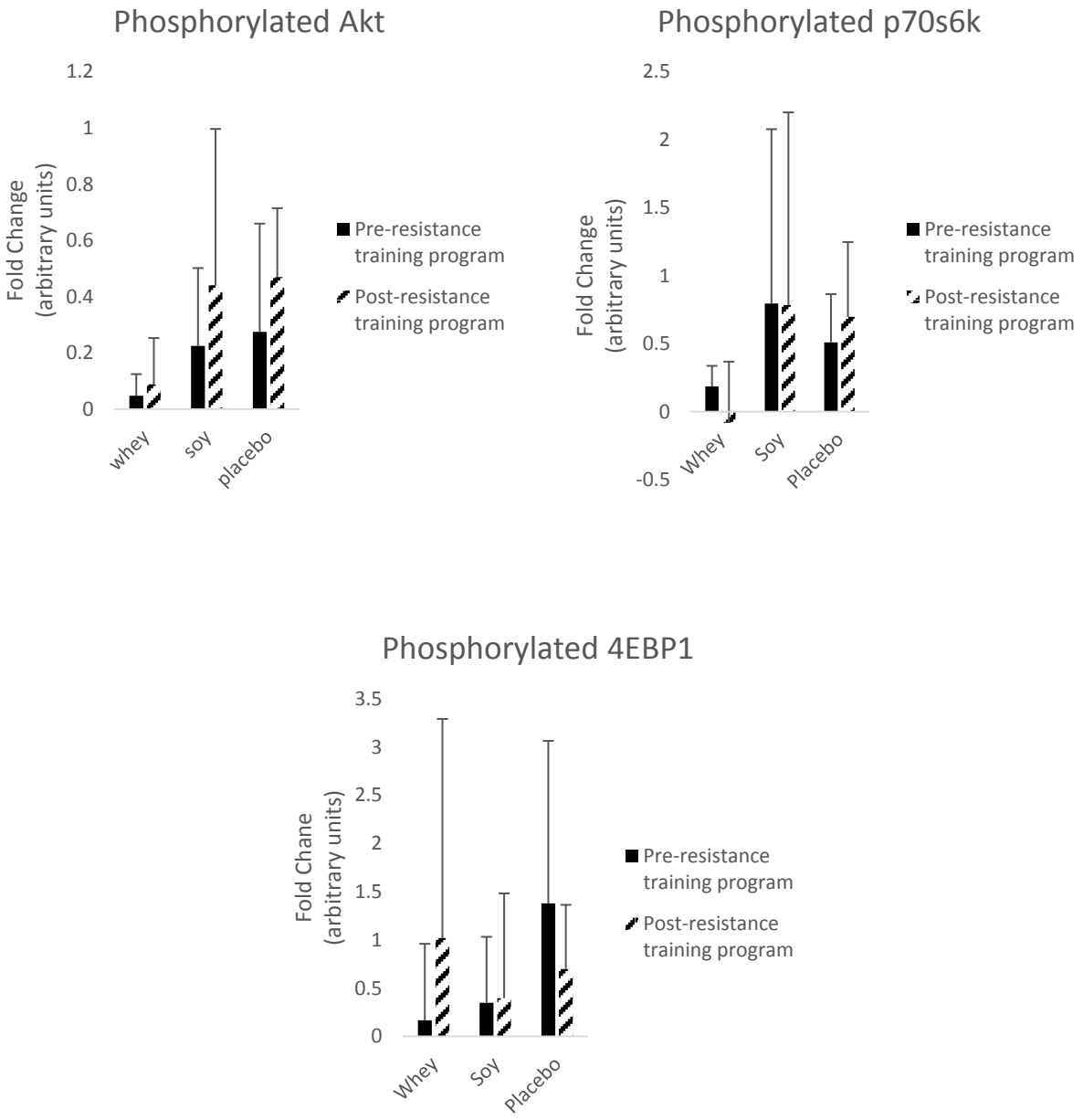

Figure 4-4. Changes in total proteins. No significant difference was detected between groups vs time points (p = .268) or treatment groups (p = .565) or between time points (p = .605) for Akt. No significant difference was detected between groups vs time points (p = .611) or between time points (p = .263) for p70s6k. * - indicates a significant difference between whey vs placebo treatments (p = .006) No significant differences were detected comparing groups vs timepoints (p= .471) or between treatment groups (p = .483) or between time points (p = .841) for 4EBP1

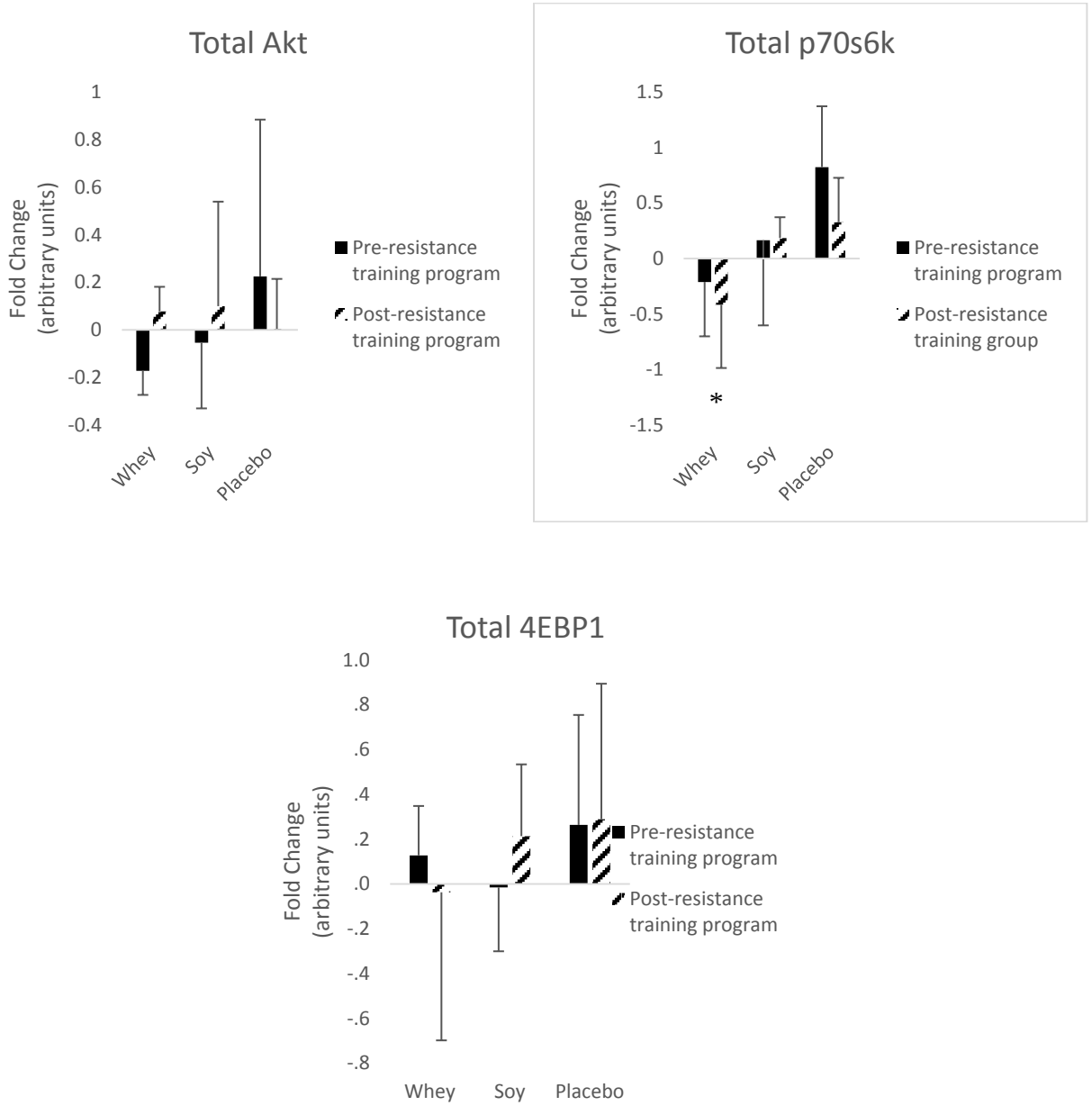

Table 4-1. Strength Changes of 1 RM pre-resistance training vs post-resistance training program. There was no significant difference between group vs time point (p = .889) or between time points (p = .089). A significant difference was detected between the whey and placebo groups (p = .025).

Group	Time point	Leg extension 1 RM (lbs)	SD
Whey*	Pre-resistance training program	137.1 ±	51.5
	Post-resistance training program	161.5 ±	47.5
Soy	Pre-resistance training program	130.0 ±	45.4
	Post-resistance training program	143.8 ±	28.3
Placebo	Pre-resistance training program	93.8 ±	39.6
	Post-resistance training program	121.3 ±	36.4

*Significant difference (p = .025) between whey and placebo groups

Chapter V

Discussion

There has been a multitude of studies examining the effects of protein supplementation combination with resistance training on intracellular signaling. The positive effects of both resistance training and protein supplementation on MPS has been well documented (Biolo et al., 1997; Rasmussen et al., 2000; Tipton et al., 1999); (Kimball & Jefferson, 2004; Nagasawa et al., 2002; Proud, 2004). Most of the research out to date has focused on the acute effects of synergistic effects of resistance training combined with protein supplementation. The aim of the current study was to observe any changes in signaling to Akt, p70s6k, and 4EBP1 as a result of either whey or soy protein supplementation combined with a 9-month progressive resistance training protocol. Our data demonstrated there were no significant changes in Akt, p70s6k, or 4EBP1 phosphorylation ratios pre-resistance training protocol versus post-resistance training protocol regardless of supplementation with whey, soy, or placebo. The data also led to observations noting no significant changes in basal level of active Akt, p70s6k, and inhibited 4EBP1 pre-resistance training protocol versus post-resistance training protocol.

Although there are many studies investigating the effects of whey protein supplementation and resistance exercise on intracellular signaling; none have investigated the effects over a 9 month training period. However, there are several studies that can be related and used to help further elucidate our results. Farnfield et al. (2009) conducted an experiment observing the acute effects of whey protein supplementation with resistance training protocol. Utilizing a Cybex NORM dynamometer and performing 3 sets of 12 at 60°/sec, they observed an increase signaling of Akt, mTOR, p70s6k, and 4EBP1 with whey protein supplementation at 2 hours post exercise in comparison to placebo. Several different factors might have contributed

to differing results. Muscle biopsies were obtained at 2, 24, and 48 hours post exercise. It could be that significant stimulation takes a certain amount of time to reach peak levels, as our biopsies were obtained after 10 minutes of recovery. Also, subjects also consumed there supplement immediately after exercise. It appears that the amino acids availability affects intracellular signaling; as they did not detect any significant differences between the two groups was seen at 4 or 24 hours post exercise. The method of exercise has to be taken into consideration as well. Utilizing maximal contractions at a fixed speed on the Cybex dynamometer might affect the response of intracellular signaling in comparison to a single leg extension machine. Also taken into consideration is that all subjects were males in Farnfeild et al. (2009); whereas the present study had a mixed population.

Farnfield and colleagues took the results from this study and moved to further elucidate the effects of resistance training with whey protein supplementation on intracellular signaling in young and old men (Farnfield et al., 2012). They constructed a 12 week progressive resistance training protocol utilizing 7 exercises: leg press, bench press, seated rows, leg extension, dumbbell shoulder press, and sit ups. Subjects performed 3 training sessions per week and regularly consumed whey protein supplements or a placebo. Farnfield et al. (2012) observed increased mTOR activation before beginning the resistance training program; and increased phosphorylation of Akt, p70s6k, and 4EBP1 between whey protein supplementation and placebo that did not reach significance in the young men group. Following the resistance training program, the young men's group still had significantly enhanced activation of mTOR and an increased activation of Akt, p70s6k, and 4ebp1 that did not reach significance. This study falls more in line with our own and shows that training status does not affect mRNA translation in young men. These findings concur with our own. However, the same considerations must be

taken into effect as with Farnfield et al. (2009), with the use of the Cybex dynamometer on acute resistance training days and the consumption of supplementation on testing days.

Resistance exercise boosts muscle hypertrophy through a myriad of signal transduction pathways, including PI3K/Akt pathway. Akt is a serine/threonine kinase whose site of activation is at Ser473 (Allessi, et al., 1996). Human studies have demonstrated that Akt activation might be a relatively short period; with studies showing maximal activation at 1 hour post exercise but not immediately after (Rommel et al., 2001). Supplementary studies have observed a small, but still significant, increase in Akt phosphorylation with the consumption of 100 g of BCAA at 1 hour post exercise (Bloomstead et al., 2010). Data from our study correlates with the previous research (Farnfield et al., 2009; Farnfield et al., 2012). Although we saw an increase in the phosphorylation of Akt after an acute resistance training bout, it did not reach significance nor was there a significant difference before and after the resistance training program.

P70s6k is a serine/threonine kinase laying downstream of mTOR that is closely associated with skeletal muscle hypertrophy (Long et al., 2000). Phosphorylation occurs at several serine and threonine residues, however full activation appears to happen with phosphorylation at Thr189 (Pearson et al., 1995). Previous research has suggested that optimal activation of p70s6k is dependent on the availability of AA to see further phosphorylation of rps6 (Karlson et al., 2004; Koopman et al., 2006). Also, a dose-dependent relationship has been observed in the phosphorylation of p70s6k and increasing leucine consumption in rats (Crozier et al., 2005). In our study, we did not see any significant changes in the activity of p70s6k when comparing P:T ratios pre-resistance training versus post-resistance training protocol or in the phosphorylation levels of p70s6k. These results are agree with results seen by Farnfield and colleagues while studying the effects of whey protein ingestion in combination with a 12-week

progressive resistance training program; who did not see a significant change in p70s6k phosphorylation levels as a result of their protocol.

4EBP1 is a downstream target of mTOR. Phosphorylation of 4EBP1 happens a Thr 37/46 residues for maximal activation (Gingras et al., 1999). Our data showed that there was no significant change in the reaction of 4EBP1 before and after the resistance training program. These results agree with a previous study conducted by Farnfield et al. (2009); who did not see any change in 4EBP1 phosphorylation as a result of exercise alone. Long et al. (2000) saw a significant decrease in 4EBP1 phosphorylation as a result of exercise.

The results from the present study did not see a significant increase in muscular strength of bilateral leg extension as a result of a 9-month resistance training program regardless of treatment group. These results contradict previous studies. Farnfield et al. (2012) saw a significant increase in muscular strength associated with the concentric and eccentric movements utilizing Cybex isokinetic leg extension following a 12 week resistance training protocol regardless of treatment group. Farnfield and colleagues also saw significant increases in the predicted maxes of the machine bench press, bilateral leg extension, and leg press. Crib et al. (2006) saw significant strength improvements in barbell bench press, squat, and cable pull down with consumption of whey protein isolate in combination with resistance training. The whey ingestion group gains were also significantly higher when compared to a casein supplementation group.

Limitations

A lack of analyzing the activity of mTOR is a major limitation of this study. mTOR is considered a crucial point in the PI3K/Akt pathway and moderator of hypertrophy in relation to the presence of AA.

The time points at which we retrieved muscle biopsies could have compromised results. Biopsies were obtained 10 minutes after exercise. Previous research has shown that activation of muscle protein synthesis does occur 10 minutes post exercise, but optimal activation may take up to an hour to happen.

The lack of leg extensions being integrated into the resistance training protocol is a limiting factor. Having subjects perform an unfamiliar exercise might induce higher intracellular signaling that might cloud or cause a misrepresentation of intracellular signaling.

The sample size of our groups is a limiting factor. Densitometry analysis produces large variability between subjects; and a small sample size can obscure results causing a misrepresentation or miss possible significant interactions.

Future Research

There is still plenty to be elucidated about the role of resistance training plus protein supplementation on skeletal muscle hypertrophy. Obviously, including the effects of a 9 month resistance training protocol on mTOR phosphorylation needs to be further explored. Also exploring for any significant changes to the downstream proteins ribosomal protein s6, eukaryotic initiation factor 4e and eukaryotic initiation factor 2 kinase.

Conclusion

There were no significant changes in the response of intracellular signaling before and after a 9 month progressive resistance training program. We did not see any significant differences in the phosphorylation of Akt, p70s6k, and 4EBP1. Our study also presented data showing no significant changes in the basal levels of Akt, p70s6k, and 4EBP1. The reaction of mRNA translation appears to be independent of training status in our population.

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Appendix A

Whey Raw

Blot		Akt-P			P-Average	Akt Total			T-Average
117	p1	196356	218214	208187	207586	8305527	8162129	7733597	8067084
	p2	732692	741450	698603	724248	10862820	9668389	6771792	9101000
	t1	928813	931455	842107	900792	11338222	11602209	8669584	10536672
	t2	2063580	2261668	2125318	2150189	692783	8309645	6945463	5315964
Control Blot	ct	850405	892495	830904	857935	12883559	12973194	12406706	12754486
104	p1	4684609	5035860	4918391	4879620	3164762	3217087	3293208	3225019
	p2	5055525	5011052	5210490	5092356	1999122	1788216	1791013	1859450
	t1	6823104	6137005	5993707	6317939	4442479	4132554	4628781	4401271
	t2	1.2E+07	11803225	1.1E+07	11634867	5511692	6352326	6512545	6125521
130	p1	3477272	3506223	3037737	3340411	6213356	6519550	5863765	6198890
	p2	1.2E+07	12020803	1.2E+07	12056356	6193861	7305234	6888994	6796030
	t1	3437896	3630365	4084471	3717577	6173803	5873301	6771064	6272723
	t2	1.2E+07	13456929	1.2E+07	12676207	7291753	7938064	8702976	7977598
409	p1	5343590	5498485	5346437	5396171	7068544	6577402	7542650	7062865
	p2	7157882	6761393	6684953	6868076	5658493	6362719	5831711	5950974
	t1t	8687398	7901019	8293399	8293939	4012173	4625727	3915942	4184614
	t2	5602264	5099007	5919522	5540264	6163322	4488253	6121577	5591051
Control Blot	ct	1.4E+07	13603655	1.3E+07	13672737	4811383	5540747	5399921	5250684
148	p1	939759	1001153	1013148	984687	11905668	10117296	11247170	11090045
	p2	813130	897872	898256	869753	11334472	9141337	9284293	9920034
	t1	3095345	3255963	3240618	3197309	9409389	13249426	12957598	11872138
	t2	4597463	4950977	5030463	4859634	8683833	10037657	9697598	9473029
156	p1	2872395	3002217	3282640	3052417	14503082	16584318	15647884	15578428
	p2	4944867	5414382	5056764	5138671	14654642	14107915	14367974	14376844
	t1	2400404	2659972	2441831	2500736	8109914	8885236	11638357	9544502
	t2	4990850	5018798	4960868	4990172	9594216	8958869	11143003	9898696
240	p1	2830659	3009284	2873522	2904488	2746838	2447614	3273569	2822674
	p2	5612322	5715940	5351216	5559826	11262868	12733271	13287208	12427782
	t1	2321230	2443652	2198916	2321266	3341530	3376931	3084003	3267488
	t2	4976870	5065566	4975117	5005851	3088616	3194106	3390675	3224466
Control Blot	ct	1.5E+07	13876914	1.4E+07	14093857	7265719	7209480	7205442	7226880
149	p1	1513221	1847981	1816626	1725943	4447242	4919801	5882104	5083049
	p2	1E+07	10924831	1E+07	10482162	5919596	5758529	5919596	5865907
	t1	6571337	7707735	6877640	7052237	8370012	8725347	9102013	8732457
	t2	1.3E+07	13442713	1.2E+07	12833826	10135029	8868483	12198796	10400769
342	p1	4102533	4149254	4121721	4124503	7245205	7372048	7762930	7460061
	p2	5080882	5074159	4675480	4943507	8040017	7663218	7183975	7629070
	t1	4095117	4500168	4254452	4283246	8802721	7709584	8229106	8247137
	t2	6725921	6290329	6620000	6545417	7445045	7663218	7426777	7511680
450	p1	5456993	5232884	4877844	5189240	8845400	7897001	9001955	8581452
	p2	2638871	2995869	2414948	2683229	6797111	7556084	6504546	6952580
	t1	2741349	2554158	2740350	2678619	3744179	3579450	3336301	3553310
	t2	6259569	5967315	5889531	6038805	3419715	3856819	3469345	3581960

Control	ct	1.1E+07	10926867	1.1E+07	10792693	8064310	8979996	8062718	8369008
Blot									
155	p1	1000092	1049890	1099483	1049822	2769543	2947471	2437502	2718172
	p2	794502	795861	799000	796454	1489747	1879609	1872340	1747232
	t1	2006266	2046250	2111926	2054814	5458163	4816552	4311735	4862150
	t2	2237421	2294504	2112186	2214704	5963565	4849409	6160245	5657740
227	p1	966655	100757	952809	673407	1330806	1207227	1846150	1461394
	p2	3189522	3559499	3370201	3373074	4389588	4419816	4129748	4313051
	t1	3416165	3512915	3572462	3500514	3984126	3474620	4127466	3862071
	t2	9830145	9984160	9590218	9801508	4971683	4925730	4597810	4831741
340	p1	1354325	1781121	1627463	1587636	6084017	7151887	6084017	6439974
	p2	2640589	2697021	2709560	2682390	4224533	3914575	3964382	4034497
	t1	2619566	3342184	2778616	2913455	8779952	7532357	7962189	8091499
	t2	5912726	6591322	5833662	6112570	5713801	5810583	5713801	5746062
Control	ct	9857017	952731	9253309	6687686	6367407	7189864	7247331	6934867
Blot									
305	p1	2515668	2441188	2773670	2576842	3351112	4281832	4022357	3885100
	p2	2867901	2840763	2566835	2758500	2785718	3517330	2741164	3014737
	t1	1897545	1897098	1930640	1908428	3222495	3334125	2521236	3025952
	t2	2036818	2006889	2087082	2043596	5063914	3819788	4224559	4369420
134	p1	1834037	1739910	2047485	1873811	4570615	3274409	3900412	3915145
	p2	1612675	1603282	1596652	1604203	1573086	1475330	1475330	1507915
	t1	1888322	1903390	2188413	1993375	4079890	3855056	4537714	4157553
	t2	3879601	382366	4255488	2839152	4418105	4163437	3640924	4074155
335	p1	938406	843253	1021756	934472	2815176	3559367	3318188	3230910
	p2	1131037	1011141	1145053	1095744	3279007	3571148	2049076	2966410
	t1	1318316	1318316	1436416	1357683	2026285	2029857	2816670	2290937
	t2	3706106	3554115	3754819	3671680	2422116	2681767	2746927	2616937
Control	ct	8677250	8307080	8110552	8364961	6933649	7084023	7412857	7008836
Blot									
413	p1	3594703	3078627	3988282	3553871	1660062	1478005	1352434	1496834
	p2	5529042	5597506	7720985	6282511	3695725	3560680	3237182	3497862
	t1	2979683	3156378	2328259	2821440	3581796	3334559	3936681	3617679
	t2	7896500	8030914	7933855	7953756	6172092	5375471	3159785	4902449
414	p1	6151247	7961902	7090971	7068040	8473231	8574579	8582600	8543470
	p2	6062442	8037922	8040323	7380229	3898924	4805972	4340445	4348447
	t1	3070747	3417304	2901691	3129914	3182070	2986166	2628862	2932366
	t2	1456103	1550444	1542338	1516295	2716310	2514295	2950415	2727007
415	p1	2415069	2686141	2768281	2623164	5941975	5111016	5102326	5385106
	p2	2891944	3136239	3364536	3130906	3674259	4311974	4864589	4283607
	t1	1889555	1845951	1972899	1902802	2639802	2898118	3224688	2920869
	t2	2276511	2962185	3276650	2838449	2997815	2762683	2961546	2907348
Control	ct	5796369	6509295	6174899	6160188	8247252	8155203	8259920	8220792

P70S6k Raw

Blot	p70s6k-p	P-	p70s6k	T-Average

					Average				
117	p1	181842	163860	147175	164292	205797	199507	188511	197938
	p2	4652943	4211844	4681394	4515394	1845730	1781537	1753116	1793461
	t1	696419	758669	715009	723366	1524656	1532477	1522661	1526598
	t2	5536526	5376694	5586269	5499830	2160615	2010497	2148966	2106693
Control Blot	ct	1175913	1188756	1245079	1203249	949396	951665	995742	965601
104	p1	1618549	1269946	1376353	1421616	2964089	2161354	2201349	2442264
	p2	1464065	1324473	1558340	1448959	1215959	1163130	1252265	1210451
	t1	1650235	1650255	1509067	1603186	2791477	2730142	2642701	2721440
	t2	2684402	2387085	2392570	2488019	2496796	3062411	3345582	2968263
130	p1	2100745	1996494	2262524	2119921	3660016	2337606	2663511	2887044
	p2	2122914	2108098	2309021	2180011	3039408	3559003	2933659	3177357
	t1	2111917	2445327	2270911	2276052	3206382	2403763	3160527	2923557
	t2	2675425	2754834	2779980	2736746	3132855	2995141	3347474	3158490
409	p1	2518506	2540335	2408656	2489166	3346845	2780509	3271191	3132848
	p2	2667687	2674359	2617678	2653241	2643692	2902857	3521883	3022811
	t1t	2039926	1907061	1893052	1946680	2083408	2381402	2123553	2196121
	t2	3418442	3156726	3192725	3255964	2430991	2463806	2417238	2437345
ct Blot	ct	2769710	2877122	2889031	2845288	2596557	2485873	2361958	2481463
105	p1	445498	605948	545641	532362	473695	494786	497621	488701
	p2	658688	878109	888808	808535	606248	592888	599944	599693
	t1	534914	647629	545641	576061	307281	288070	352655	316002
	t2	346891	347636	364385	352971	424540	488316	483157	465338
227	p1	823122	791473	791473	802023	337004	415246	408023	386758
	p2	737802	597255	634759	656605	500735	508128	493498	500787
	t1	474342	495058	515342	494914	310693	371211	374816	352240
	t2	652166	586755	616979	618633	194985	275472	246395	238951
203	p1	407321	381462	412308	400364	234326	284612	290176	269705
	p2	887651	760386	747540	798526	496024	572083	521245	529784
	t1	700896	593851	602310	632352	321761	406012	448906	392226
	t2	1033217	858945	879121	923761	344799	477900	476044	432914
ct Blot	ct	1194353	857663	945607	999208	657578	599402	630779	629253
148	p1	1035932	992209	1108488	1045543	1409955	1535316	1493552	1479608
	p2	1508550	1508550	1383931	1467010	973435	979801	810281	921172
	t1	599327	5.50488	611066	403466	686831	856467	787153	776817
	t2	728810	973574	718469	806951	864433	856467	905492	875464
156	p1	673216	558043	634854	622038	684170	603989	591488	626549
	p2	594866	882383	589158	688802	615135	649230	696272	653546
	t1	667878	645109	691995	668327	977260	1031925	1065774	1024986
	t2	455622	430704	441025	442450	419728	493539	476807	463358
234	p1	246905	299556	310074	285512	518028	565817	578179	554008
	p2	851727	829234	868487	849816	1464614	1536563	1490590	1497256
	t1	455573	399556	464920	440016	499951	582429	629872	570751
	t2	1197498	1234125	1254312	1228645	111552	1147373	1151829	803585
ct Blot	ct	811825	1078765	1020821	970470	757211	737177	697759	730716
155	p1	1490836	1384241	1515639	1463572	426172	421336	437460	428323

	p2	1663330	1678974	1610051	1650785	190266	185948	185464	187226
	t1	2688894	2989818	3103321	2927344	605864	593851	597893	599203
	t2	3210445	3369211	3513849	3364502	596454	543645	584306	574802
227	p1	1399626	1379942	1242757	1340775	356233	313259	325054	331515
	p2	4768257	4737519	4521802	4675859	448571	392283	425573	422142
	t1	4920645	4688771	4215802	4608406	358715	372316	400147	377059
	t2	5091405	4783941	4852881	4909409	424194	376361	441136	413897
340	p1	3821308	4196565	3857335	3958403	476884	463993	459259	466712
	p2	4396033	4268125	5067747	4577302	596500	582952	567849	582434
	t1	4937104	5530781	5369702	5279196	414149	447710	452849	438236
	t2	5889957	5614444	5814340	5772914	411832	381320	415698	402950
	ct	5013261	4949193	5022324	4994926	537405	521241	521407	526684
Blot									
413	p1	1800458	1771572	1976506	1849512	4406613	1288244	1468752	2387870
	p2	3582545	3758905	3445385	3595612	3934735	3722275	4250981	3969330
	t1	3430649	4785664	4055048	4090454	6097101	3184970	3606876	4296316
	t2	6837932	6749107	6200819	6595953	4910578	5596215	5995229	5500674
414	p1	5987416	5880096	4162096	5343203	6508322	6084728	6586671	6393240
	p2	5809721	6979195	5459439	6082785	4205814	4743137	4948842	4632598
	t1	5941673	5905201	4243009	5363294	3517539	3877305	3771982	3722275
	t2	3661171	3557478	3017073	3411907	1378487	1391664	1482909	1417687
415	p1	4683149	4963970	4326917	4658012	3846346	3636371	3846346	3776354
	p2	4845837	5036410	4531313	4804520	4459466	4120388	4193315	4257723
	t1	4641089	4037959	4170729	4283259	4143151	3575590	4176868	3965203
	t2	5038143	5535600	5694327	5422690	4106216	4399613	4350177	4285335
ct	ct	2175323	2071895	2117079	2121432	1624897	1638306	1673163	1645455
Blot									
105	p1	14235455	14789103	14047681	14357413	2494577	2375356	2448044	2439326
	p2	15964602	15342562	13258213	14855126	1732027	1782211	1840826	1785021
	t1	11724785	11004973	11122195	11283984	2062849	2036272	2009187	2036103
	t2	6896348	7360410	7254409	7170389	909137	989993	952021	950384
134	p1	9231187	9010284	9022931	9088134	952900	973514	1014948	980454
	p2	9889199	9913546	9436607	9746451	1173081	1173081	1043220	1129794
	t1	6809937	6905585	6423647	6713056	1008477	1002191	1087495	1032721
	t2	6133640	6763309	6727955	6541635	876490	909850	912440	899593
314	p1	2540302	2685989	2686913	2637735	623701	707417	666424	665847
	p2	7351908	6400856	7105070	6952611	907026	883136	963815	917992
	t1	2237537	2566662	2609855	2471351	720701	706314	715422	714146
	t2	2375826	2741207	2939423	2685485	795395	893182	849161	845913
ct	ct	5785714	5656882	5694350	5712315	1377384	1491202	1375971	1414852

4EBP1 Raw

Blot		4ebp1-p			P-Average	4ebp1			T-Average
117	p1	2024425	2100781	2075843	2067016	16745434	15797238	15885594	16142755
	p2	3943204	4121677	4005278	4023386	16638172	17178936	15379040	16398716
	t1	2279574	2195495	2261916	2245662	17392653	16838436	17054758	17095282
	t2	4880040	5196059	5057292	5044464	26859984	27925542	28684965	27823497

ct	ct	3970558	4055994	3978517	4001690	28180304	27367771	26704202	27417426
Blot									
104	p1	6604019	6393559	6388767	6462115	9586111	8385470	8702094	8891225
	p2	8340785	8618697	8277981	8412488	8396646	7509189	7614759	7840198
	t1	2075389	1907910	2022079	2001793	6496701	5385186	5414176	5765354
	t2	2833500	3085144	3045285	2987976	8328978	6986790	7957278	7757682
130	p1	3714719	3596000	3509906	3606875	5593522	5566487	5559625	5573211
	p2	3881306	3790614	4205557	3959159	6773992	6017443	6484742	6425392
	t1	6519936	6180035	6883750	6527907	7976852	7182330	7671402	7610195
	t2	6750016	6292606	6724313	6588978	11122285	10997492	10997492	11039090
409	p1	9898259	9361354	10058936	9772850	12646093	11877207	12231221	12251507
	p2	23386253	22715729	23317826	23139936	14582313	14821405	14957049	14786922
	t1	4409445	4259080	4286031	4318185	4719841	4635825	4635825	4663830
	t2	3908158	3148428	3090179	3382255	3620194	4097133	4385320	4034216
	ct	7335704	7020472	7307386	7221187	8134902	7407036	8321997	7954645
Blot									
105	p1	2059123	2227283	2239763	2175390	11010728	10111805	11183108	10768547
	p2	1074602	1153348	1118874	1115608	12050849	10023580	11055288	11043239
	t1	1129977	1234623	1189620	1184740	11009284	8895749	10403088	10102707
	t2	9898015	100030542	10053624	39994060	19452487	17437493	18759189	18549723
227	p1	1585870	1656077	1626722	1622890	27401889	22025358	25797963	25075070
	p2	1372953	1328328	1374856	1358712	21783659	25398332	30572897	25918296
	t1	1473709	1354097	1420232	1416013	32480363	21636117	22991578	25702686
	t2	1042146	1035209	110069	729141	25292470	22375524	28962025	25543340
203	p1	1485708	1418902	1418560	1441057	11249049	9653293	11967990	10956777
	p2	1454280	1415650	1486308	1452079	15842423	13220706	16503043	15188724
	t1	1388972	1291577	1238772	1306440	13199314	12854158	15008579	13687350
	t2	2350663	2401198	2439865	2397242	15836787	14753908	15939591	15510095
	ct	6579996	6532869	6458278	6523714	23224975	21218700	22049322	22164332
Blot									
148	p1	2900511	3198982	3437850	3179114	32604520	32028893	31080366	31904593
	p2	4466768	4440099	4603007	4503291	24604520	24400637	24973746	24659634
	t1	4162625	4240164	4346767	4249852	36761915	38291587	36745492	37266331
	t2	3414092	3333436	3329072	3358867	33596333	33138189	33446867	33393796
156	p1	4125541	3913362	4180128	4073010	31714545	31801510	32426132	31980729
	p2	2553717	2550393	2634420	2579510	26933354	25807769	26295802	26345642
	t1	3776223	3483144	3836847	3698738	33142181	35088556	32671683	33634140
	t2	1948601	2009838	2081799	2013413	20434816	21629745	21711285	21258615
234	p1	1883282	1877603	2083703	1948196	21175828	22277754	21087493	21513692
	p2	17202997	17583744	17733723	17506821	32283194	31901668	31611515	31932126
	t1	2670844	2522051	2908074	2700323	15651598	14969402	14943373	15188124
	t2	3736227	3646724	3985522	3789491	15713359	15371010	15019792	15368054
	ct	4096474	4354997	4223732	4225068	28814640	28715739	28113215	28547865
Blot									
149	p1	2871395	3044710	2909944	2942016	44117912	46305944	43592292	44672049
	p2	2603373	2883130	3017765	2834756	36833919	38456535	36541234	37277229
	t1	3727608	3933666	3855811	3839028	48270892	48093743	46793093	47719243
	t2	3848840	4085715	3991074	3975210	62221972	57663755	56864190	58916639

342	p1	55021887	5744717	5758770	22175125	54810224	56289324	56941643	56013730
	p2	18576296	18409957	18822856	18603036	47544929	50070314	48897402	48837548
	t1	6400052	6146807	5919504	6155454	54515063	55363609	51526324	53801665
	t2	3576151	3660481	3515251	3583961	44926859	47847688	48943572	47239373
450	p1	5521267	5139021	5252689	5304326	45431240	49327864	49141790	47966965
	p2	19666283	19245519	18976959	19296254	38829653	41168451	39317593	39771899
	t1	4359956	4354180	4337924	4350687	42522246	41133949	42133015	41929737
	t2	16510372	16873175	16786017	16723188	34991589	34740279	18145944	29292604
	ct	8677183	8937346	9044325	8886285	68043926	68185829	68497092	68242282
Blot									
413	p1	2613878	2521787	2698370	2611345	8124969	8034261	8790755	8316662
	p2	4187680	4168957	4175047	4177228	13850296	13597129	14388587	13945337
	t1	5838276	5390439	5736256	5654990	14285800	14942613	15062884	14763766
	t2	12664190	12459200	11689271	12270887	22165376	21912638	21765313	21947776
414	p1	6948061	6274477	6796863	6673134	10636409	10091150	10472512	10400024
	p2	3252400	3506759	3597848	3452336	9703756	10058481	9706534	9822924
	t1	2830780	2799724	2823811	2818105	11035353	11033327	10387349	10818676
	t2	2899887	3122113	2994521	3005507	4621977	4120016	5159696	4633896
415	p1	2480781	2848557	2789574	2706304	7760592	8237974	8410391	8136319
	p2	3805616	4007337	3942438	3918464	11274095	11640682	12111896	11675558
	t1	6156838	6702478	6631844	6497053	7054294	7814518	7835535	7568116
	t2	24812238	23545648	23517132	23958339	13038713	11793312	12703007	12511677
	ct	5992362	5827533	5781669	5867188	6137966	6182511	6094651	6138376
Blot									
305	p1	4517560	4665544	4394236	4525780	7847603	7623662	7165262	7545509
	p2	21202221	20727686	20542751	20824219	4034810	4306829	3728693	4023444
	t1	4483572	3825574	3912784	4073977	6247842	5869520	4787161	5634841
	t2	3918064	3399213	3627157	3648145	6449866	5971266	6402699	6274610
134	p1	6080026	5252299	5684046	5672124	8940304	8218653	8963798	8707585
	p2	9174775	7491460	7863124	8176453	1637283	17422535	16550240	11870019
	t1	4683155	3699594	3729715	4037488	18480016	17155747	15030729	16888831
	t2	3572184	2897242	3021654	3163693	16201556	13214996	12762305	14059619
335	p1	3394497	2972655	2689385	3018846	14303954	13451293	13502446	13752564
	p2	3622354	3727790	3767488	3705877	24189361	24390429	2394192	16991327
	t1	3802559	3201560	3039919	3348013	20139118	18771826	18279221	19063388
	t2	18001722	16773638	16830697	17202019	29318531	29960678	29465765	29581658
	ct	8581339	8593768	8525331	8566813	9723844	9721575	9558511	9667977

Whey Normalized

P/T	P1	P2	T1	T2	PreChange	PostChange
104	1.628109374446	1.051706431499	0.551261479389	0.729421838123	-0.576402942947	0.178160358734
117	0.382551158994	1.183060978928	1.270953521000	6.013167762147	0.800509819934	4.742214241147
130	0.206940880450	0.681273005833	0.514609000957	0.610207549698	0.474332125383	0.095598548741
134	0.401014257509	0.891383029658	0.401728990104	0.583892645090	0.490368772148	0.182163654987
148	0.045528755052	0.044957652007	0.138094586504	0.263048435191	-0.000571103045	0.124953848687
149	0.263297192347	1.385670080193	0.626231063404	0.956830102764	1.122372887846	0.330599039360
155	0.400498507695	0.472685858355	0.438234402809	0.405914877766	0.072187350660	-0.032319525043
156	0.100471131359	0.183277130201	0.134349332442	0.258498805912	0.082805998842	0.124149473470
227	0.477829009301	0.810967806450	0.939883245414	2.103543718800	0.333138797149	1.163660473386

234	0.527630603875	0.229397444218	0.364277143200	0.796051517594	-0.298233159657	0.431774374394
305	0.555734740134	0.766664672307	0.528439975999	0.391880166841	0.210929932173	-0.136559809158
335	0.242338953185	0.309499348153	0.496554656301	1.175583837225	0.067160394968	0.679029180924
340	0.255640255377	0.689437441951	0.373371931581	1.103102498053	0.433797186574	0.729730566471
342	0.428719630192	0.502467195210	0.402729955845	0.675685157048	0.073747565018	0.272955201203
409	0.293403395113	0.443207804155	0.761142197238	0.380537483580	0.149804409042	-0.380604713658
413	3.168456693268	2.396901815312	1.040783814280	2.165104524400	-0.771554877956	1.124320710119
414	1.104038960000	2.264932981767	1.424406440877	0.742022719185	1.160894021767	-0.682383721692
415	0.650056130410	0.975394092999	0.869362881615	1.302877923732	0.325337962590	0.433515042117
450	0.468907486543	0.299265006273	0.584550459557	1.307297630410	-0.169642480270	0.722747170854

P70S6K Normalized

Group	P1	P2	T1	T2	PreChange	PostChange
104	1.016050135	1.043975791	0.513767493	0.539360219	0.027925656	0.025592727
105	0.686015496	0.849061485	1.148017296	0.477683175	0.163045989	-0.670334121
117	0.666084712	2.020439077	0.380255303	2.095029453	1.354354365	1.71477415
130	0.640394788	0.598376158	0.678972314	0.755677694	-0.042018629	0.07670538
134	2.295865495	2.136712874	1.610038366	1.801104867	-0.159152621	0.191066501
148	0.532061067	1.199108391	0.63494307	0.69402486	0.667047325	0.05908179
155	0.77252006	0.792988411	0.304250284	0.801066602	0.020468351	0.496816318
156	0.747528573	0.793569239	0.490950044	0.718975375	0.046040666	0.228025331
203	0.934836658	0.949204043	1.015294222	1.343776974	0.014367385	0.328482752
227	0.426455407	1.167948983	1.288731309	1.250714767	0.741493576	-0.038016542
234	0.38803775	0.427361203	0.58048169	1.151226906	0.039323453	0.570745216
314	0.98119513	1.875892036	0.857129863	0.786314345	0.894696906	-0.070815519
340	0.909380714	0.828675323	1.270226151	1.510654832	-0.080705391	0.240428681
409	0.692940581	0.765503838	0.77307173	1.165049013	0.072563257	0.391977283
413	0.600763375	0.702606967	0.738468897	0.930076213	0.101843591	0.191607316
414	0.648242544	1.018438471	1.117583679	1.866697244	0.370195927	0.749113565
415	0.956719839	0.875244753	0.837849132	0.981492262	-0.081475086	0.14364313

4EBP1 Normalized

P/T	P1	P2	T1	T2	PreChange	PostChange
104	0.89466243	1.18197857	0.38247696	0.42428470	0.28731613	0.04180774
105	0.68634057	0.34322180	0.39842356	7.32517132	-0.34311877	6.92674776
117	0.87730279	1.68099012	0.90001825	1.24218578	0.80368732	0.34216753
130	0.71291503	0.67875882	0.94490987	0.94490987	-0.03415621	0.00000000
134	0.735130246	0.7773737	0.269791328	0.253943529	0.04224345	-0.01584780
148	0.673275636	1.23390968	0.770543437	0.679621221	0.56063405	-0.09092222
149	0.505757725	0.58398996	0.617818767	0.518149875	0.07823223	-0.09966889
156	0.860532175	0.66155872	0.743041725	0.639937829	-0.19897346	-0.10310390
203	0.446846068	0.32480954	0.32428728	0.52511827	-0.12203653	0.20083099
227	0.219890547	0.17810692	0.187175217	0.096982585	-0.04178363	-0.09019263
234	0.611867897	3.70441326	1.201300153	1.666103482	3.09254536	0.46480333

305	0.676894867	5.84099846	0.815930551	0.656147706	5.16410359	-0.15978284
335	0.247727117	0.24613879	0.19819985	0.656255921	-0.00158833	0.45805607
342	3.040219187	2.92525211	0.878613186	0.582629503	-0.11496707	-0.29598368
409	0.878706617	1.72383841	1.019930846	0.923547904	0.84513179	-0.09638294
413	0.328502517	0.3133882	0.400735859	0.584936776	-0.01511432	0.18420092
414	0.671303605	0.36770178	0.272525111	0.678570402	-0.30360183	0.40604529
415	0.347994271	0.35112494	0.898156782	2.003386112	0.00313067	1.10522933
450	0.849222055	3.7258897	0.796836086	4.384240941	2.87666764	3.58740485

Appendix B

	Sets	Reps	Intensity
Cycle 1. Weeks 1-4	2	15	50% 1RM
Cycle 2. Weeks 5-8	3	12	60% 1RM
Cycle 3. Weeks 9-12	3	10	60% 1RM
Cycle 4. Weeks 13-16	4	8	75% 1RM
Cycle 5. Weeks 17-20	4	6	90% 1RM
Cycle 6. Weeks 21-24	3	10	60% 1RM
Cycle 7. Weeks 25-28	4	8	75% 1RM
Cycle 8. Weeks 29-32	4	6	75% 1RM
Cycle 9. Weeks 33-36	4	6	90% 1RM
Cycle 10. Weeks 37-40	5	5	90% 1RM
Training volume and intensity will be decreased by 15-20% during the final week of each cycle.			

Appendix C – Acute resistance training protocol raw data

Subject	Group	Pre-1RM	80%	1 reps	2 reps	3 reps	Post 1RM	80%	1 reps	2 reps	3 reps
117	2	170	140	10	10	10	180	150	10	10	18
130	2	210	175	10	10	10	170	140	10	10	10
132	3	110	90	10	10	19	150	120	10	10	12
134	1	190	150	10	10	11	170	140	10	10	11
148	2	120	100	10	10	10	140	110	10	10	12
149	3	140	110	10	10	9	160	130	10	10	14
155	1	130	100	10	10	13	190	150	10	10	10
156	1	140	100	10	10	12	150	120	10	10	14
214	3	50	40	10	10	13	90	70	10	10	8
227	2	70	60	10	10	14	90	70	10	10	11
234	3	40	30	10	10	20	70	60	10	10	15
305	2	130	100	10	10	10	160	130	10	10	22
314	2	120	100	10	10	15	150	120	10	10	14
319	3	100	80	10	10	19	130	104	10	10	10
335	1	220	180	10	10	12	250.25	200	10	10	16
340	3	150	120	9	7	6	170	140	9	8	10
342	2	140	110	10	10	6	130	100	10	10	12
409	1	110	90	10	10	20	130	100	10	10	13
413	3	70	60	10	10	16	100	80	10	10	13
414	1	80	60	10	10	16	130	100	10	10	8
415	2	80	60	10	10	16	130	100	10	10	10

450	3	90	70	10	10	10	100	80	10	10	10