Abstract

Skeletal Muscle Eukaryotic Elongation Factor 2 (eEF2) Response to Acute Resistance Exercise in Young and Old Men and Women: Relationship to Muscle Glycogen Content and 5'-AMP-Activated Protein Kinase (AMPK) Activity

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Sarcopenia is associated with an age-related decrease in skeletal muscle mass, which can result in decreases in strength and physical functioning in the older population. Resistance training interventions are not completely effective in stimulating muscle protein synthesis in aged muscle and thus do not completely combat age-related atrophy. Phosphorylation (and thus theoretically activation) of the energy-sensing molecule 5'-AMP-activated protein kinase (AMPK, known to inhibit the muscle protein synthesis pathway) has been shown to be elevated for up to three hours in response to a resistance training bout in the muscles of older, but not younger individuals. Data in rats indicate that, in response to muscle contractions, elevated AMPK activity can accentuate the inhibitory phosphorylation (and thus deactivation) of its downstream intermediate, eukaryotic elongation factor 2 (eEF2, which normally stimulates protein translation and synthesis). AMPK activity is inhibited by high muscle glycogen levels. Interestingly, older individuals exhibit a lower muscle glycogen content compared to younger individuals, which may account for the greater AMPK phosphorylation response to resistance exercise in older individuals. The relationship between muscle glycogen content, AMPK activity, and eEF2 phosphorylation in response to an acute bout of resistance exercise has not yet

been examined in young or old individuals. We hypothesized that inhibitory eEF2 phosphorylation would be higher in response to an acute resistance exercise bout in the skeletal muscles of older versus younger individuals. We further hypothesized that this higher eEF2 phosphorylation response would be related to a higher AMPK activation, and that higher AMPK activation would be related to lower glycogen content, in the skeletal muscles of older versus younger individuals. Seven young (21.7 ± 0.8 yrs) and 10 old (67.0 ± 2.6 yrs) untrained but physically active men and women performed 3 sets of leg extensions at a 10-repetition maximum resistance until failure after an overnight fast. Muscle biopsies were obtained from the vastus lateralis pre-exercise (PRE), immediately post exercise (0P), 1-hour post exercise (1P), and 2-hours post exercise (2P). Glycogen content was measured in muscle samples, as were the phosphorylations, by western blot, of AMPK (Thr172), acetyl-CoA carboxylase (ACC, a marker of in vivo AMPK activity) (Ser79), and eEF2 (Thr56). Muscle glycogen content was significantly lower in the old vs. young subjects at the PRE time point and decreased in response to exercise in both age groups; however, glycogen content decreased to a greater degree in young subjects such that it was equal between young and old at all post-exercise timepoints. As expected, AMPK phosphorylation was significantly increased in the old subjects immediately post exercise, but no such response was noted in the young. However, no age-related differences were observed in AMPK activity as measured by ACC phosphorylation, which was significantly elevated at 0P and 1P in both age groups. Similarly, the eEF2 phosphorylation response (elevated vs. PRE at 0P and decreased vs. PRE at 1P and 2P in both age groups) was also not affected by age. Regardless of age, higher muscle glycogen content was associated with lower AMPK activity (as assessed by phospho-ACC content) at 0P and 1P, and this lower AMPK activity was associated with lower inhibitory phosphorylation of eEF2 at those same timepoints.

These findings indicate the possibility that higher muscle glycogen content may result in lower AMPK activation and consequently lower inhibitory eEF2 phosphorylation in response to a resistance training session in the muscles of both younger and older individuals, thereby potentially enabling greater translation elongation, protein synthesis, and muscle growth regardless of age.

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CHAPTER 1: INTRODUCTION

Sarcopenia

In the future of our country, the proportion of Americans to fall into the "aged" or "older" category is expected to increase significantly. To compound this "baby boom" generation reaching older adulthood, the lifespan has also increased drastically (Mokdad et al., 2004). There is a strong connection between declines in physical function with advancing age, which is accelerated as age continues to increase (Beckett et al., 1996). A decrease in physical function is of concern due to the resulting inability to perform usual activities of daily living (Beckett et al., 1996). A portion of this physical disability can be attributed to a reduction in skeletal muscle as associated with aging (Janssen, Heymsfield, Wang, & Ross, 2000) . At about 45 years of age the decrease begins, this age-associated decline in muscle mass is known as sarcopenia (Janssen, Baumgartner, Ross, Rosenberg, & Roubenoff, 2004). There is roughly a 30% decrease in muscular strength when comparing the second and seventh decades of life (Rogers & Evans, 1993). The decrease in muscle mass is mostly associated with atrophy of the type II fibers (Lee, Cheung, Qin, Tang, & Leung, 2006).

It is estimated that approximately 45% of the elderly United States population can be categorized as having sarcopenia, which encompasses both men and women (Janssen, Baumgartner et al., 2004). Sarcopenia is associated with an increase in disability which leads to increases in healthcare expenditures. Approximately $18.5 billion, or 1.5% of the healthcare costs in the US in 2000, is due to sarcopenia. Every individual suffering from sarcopenia does not necessarily experience a decrease in their functionality, but depending on the severity, the risk can range from 1.5 to 4.6 times higher than an individual without sarcopenia. If sarcopenia could be eliminated approximately 85.6% and 26.0% of men and women, respectively, disability

cases could be prevented, helping to alleviate a portion of the economic burden from healthcare (Janssen, Shepard, Katzmarzyk, & Roubenoff, 2004).

Resistance Training

Resistance training has been shown to improve muscular strength and potentially reverse or hinder the negative effects of sarcopenia (Marini et al., 2008; Rogers & Evans, 1993). Resistive exercises have proven to increase the muscular strength and volume in old individuals (Kryger & Andersen, 2007). Older individuals who were exposed to persistent exercise training, possibly lifelong, have been reported to exhibit larger muscle sizes and mechanical performance (Aagaard, Magnusson, Larsson, Kjaer, & Krustrup, 2007). However, others have found that older subjects exhibit a blunted hypertrophic response to progressive resistance training when compared to younger subjects who have underwent the same exercise regimen (Kosek, Kim, Petrella, Cross, & Bamman, 2006; Raue, Slivka, Minchev, & Trappe, 2009). Several studies have also compared old and young rat muscles and have revealed that when introduced to an overload the old muscles hypertrophy to a lesser extent than that of the young (Thomson & Gordon, 2005). There is a disparity in the ability of the muscle to hypertrophy based on fiber type. It appears that slow twitch fibers (type I) can significantly enlarge, while fast twitch (type II) fibers sometimes show little to no improvement (Kosek et al., 2006; Thomson & Gordon, 2005)

Protein Synthesis

Protein synthesis is a process in which proteins are synthesized within the muscle by various intracellular processes. Exercise training can lead to an increase in the size of the muscle due to an increase in the contractile proteins in the muscle. During the post exercise period,

protein synthesis will ideally outweigh the degradation of protein within the muscle and therefore result in an increase in the available proteins (Drummond, Miyazaki et al., 2008). Age-related losses are present between young and old subjects in expression of genes for the regulation of muscular protein synthesis at baseline levels (Drummond, Miyazaki et al., 2008). Similar differences can also be seen between age groups in response of protein synthesis itself to a single bout of leg extension exercise (Kumar et al., 2009; Sheffield-Moore et al., 2005). It is possible that these critical differences in protein synthesis account for the lower skeletal muscle hypertrophy seen in older individuals with resistance training

Signaling Pathways

The processes of protein synthesis are abundantly regulated by upstream signaling pathways. Akt/mammalian target of rapamyacin (mTOR) is critical for protein translation, synthesis of new proteins and therefore muscle hypertrophy (Bodine et al., 2001). An increase in effectiveness and ability for the process to proceed is promoted by mTOR and its control of activity of certain downstream intermediates, such as 70-kDa ribosomal protein S6kinase (p70S6k) and eukaryotic translation elongation factor 2 kinase (eEF2) (Kimball, Horetsky, & Jefferson, 1998). The p70S6k protein stimulates the translation process of a variety of ribosomal proteins and elongation factors including the downstream activation of eEF2 (Terada et al., 1994). That is, p70s6K stimulates global translation by first specifically recruiting and translating 5' tract of pyrimidine (5'TOP) mRNA, which encodes for specific proteins of the ribosomal machinery itself as well as for eEF2 (Ryazanov, Shestakova, & Natapov, 1988). Translation elongation is also augmented by p70s6K's inhibition of eukaryotic elongation factor 2 kinase (eEF2k), which, when active, would otherwise phosphorylate and deactivate eEF2 (Ryazanov et al., 1988). Signaling through these crucial components (mTOR, p70s6k, and

eEF2) displays an increase in activity when stimulated with an acute bout of contractile activity in young muscle (Bodine et al., 2001; Parkington, Siebert, LeBrasseur, & Fielding, 2003), but has been shown to be suppressed in aged fast-twitch muscle in response to overloading and/or resistance exercise (Kumar et al., 2009; Parkington, LeBrasseur, Siebert, & Fielding, 2004; Thomson & Gordon, 2006). The inhibitory phosphorylation of eEF2 at Thr^{56} is higher in old rat skeletal muscle and tends to further increase under overload conditions (Thomson & Gordon, 2006). Correspondingly, the p70s6k and eEF2 signaling pathways are both inhibited after electrical stimulation in old rat skeletal muscle (Fick & Gordon, 2007). Although one finding in humans indicated that the phosphorylation of eEF2 (Thr^{56}) was not different between the young and old age groups after resistive exercise, that exercise was performed after the ingestion of an essential amino acid (EAA) and carbohydrate rich drink (Drummond, Dreyer et al., 2008). Theoretically, that EAA stimulus would bypass AMPK and enhance the responses of mTOR and its downstream effects such as p70s6k and eEF2 signaling (as well as protein synthesis). Thus, the effect of resistance exercise in young and old subjects under normal (without an EAA stimulus) remains to be determined.

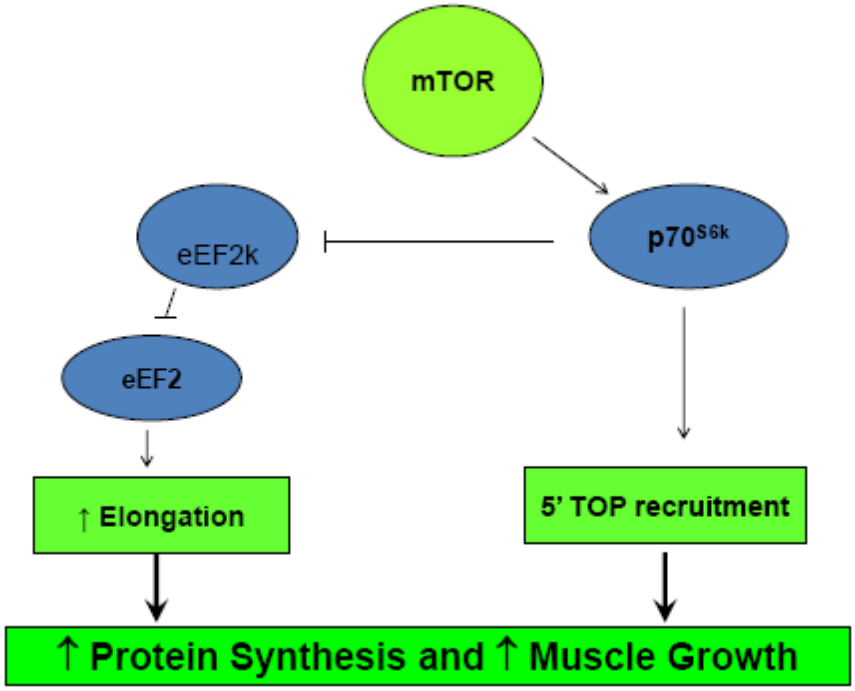

Figure 1.1. The mTOR signaling pathway. Mammalian target of rapamyacin (mTOR) activates 70 kDa ribosomal protein s6 kinase (p70s6k) which inhibits eukaryotic elongation factor 2 kinase (eEF2k) from deactivating eukaryotic translation elongation factor 2 (eEF2). Thereby increasing elongation and recruitment and translation of 5' tract of pyrimidine (5' TOP) resulting in increased protein synthesis and muscle growth.

AMPK

5'-Adenosine monophosphate-activated protein kinase (AMPK), in essence, is an energy sensing protein that responds based on the ratio of AMP/ATP (Winder & Hardie, 1999). It is activated during instances of metabolic demand, such as resistive exercise, and stimulates the generation of ATP while impeding processes that are metabolically expensive, such as protein synthesis (Thomson & Gordon, 2005). AMPK "turns off" protein synthesis by inhibiting the signaling to activate mTOR and thereby shutting down the downstream proteins from allowing translation elongation and the recruitment of 5'TOP, as reviewed (Gordon, Lake, Westerkamp, & Thomson, 2008). In some tissues, AMPK may also bypass mTOR-p70s6k signaling to more

directly inhibit protein synthesis by stimulating eEF2k, which subsequently inhibits eEF2 (Browne, Finn, & Proud, 2004).

During a bout of exercise muscular ATP stores stay relatively constant, however; AMP levels can become elevated and therefore increase the ATP/AMP ratio (Hardie & Sakamoto, 2006). AMPK senses this shift in energy stores it reflexively initiates the synthesis of ATP, through energy saving processes such as glucose uptake and lipid catabolism (Hardie & Sakamoto, 2006). In turn, AMPK phosphorylates (an activation mechanism) and activates intermediates that block mTOR activity in attempt to replenish ATP and conserve energy (Hardie & Sakamoto, 2006). AMPK activation has a strongly negative correlation with overload-induced muscular hypertrophy (Thomson & Gordon, 2005), and a significantly higher activation in old (O) versus young (Y) participants after resistance exercise (Drummond, Dreyer et al., 2008). This same effect of higher AMPK activation has also been seen in O rats as compared to Y when in overloaded fast twitch muscle (Thomson & Gordon, 2005). Although, this likely occurs in an attempt to restore the energy imbalance of the AMP/ATP ratio typically observed in old skeletal muscle post exercise (Bastien & Sanchez, 1984), the result may also be an inhibition of the response of the mTOR pathway typically observed in young muscle. In fact, the elevated AMPK phosphorylation observed in aged rat fast-twitch muscle during overload is associated with a diminished response of markers of mTOR activity (p70S6k and eukaryotic elongation factor binding protein-1) as well as eEF2 (Thomson et al. 2006). An acute resistance training bout did elicit an increase in the phosphorylation of AMPK, (which is normally an inhibitor of mTOR, p70s6k, eEF2, and protein synthesis) in the muscles of elderly, but not young (Drummond, Dreyer et al., 2008). Thus, AMPK is one potential mechanism inhibiting the protein synthesis and hypertrophy response to overload in old muscle.

Glycogen and Diet in the Elderly

When comparing diet between young and old subjects, it has been shown that the elderly not only consume fewer calories, but also fewer carbohydrates (Lieberman, Wurtman, & Teicher, 1989). Between the two groups, most of the snacks consumed are carbohydrate rich; however, the elderly are eating less snacks as compared to the young (Lieberman et al., 1989), which could account for some of the decrease in carbohydrate consumption. In accordance with a reduction of consumed carbohydrates, the elderly have been shown to have lower muscle glycogen content, trained and untrained, when compared with young subjects (Cartee, 1994). Young subjects that are introduced to an exercise bout exhibit a decrease in available glycogen stores post exercise session (Harber et al., 2008).

It is suggested that the degree of AMPK activation during exercise is in part regulated by the availability of fuel in the skeletal muscle (Wojtaszewski et al., 2003). When comparing a rested muscle during low carbohydrate state, or glycogen depleted, with a carbohydrate-rich state, or high glycogen content, AMPK activity levels were seen to be elevated in times of low glycogen content (Wojtaszewski et al., 2003). This suggests there is some connection between the AMPK phosphorylation and the amount of available muscle glycogen. During exercise the glycogen depleted state exhibits an enhanced uptake of circulating fuels that could potentially be associated with the elevated AMPK activation (Wojtaszewski et al., 2003).

Specific Aim

Resistance training has been proven to elicit hypertrophy in older subjects, but to a lesser extent than young counterparts (Kosek et al., 2006; Raue et al., 2009). In response to overload or resistance training, elderly humans and rats have shown to have a decreased skeletal muscle activation of mTOR and p70s6k (Kumar et al., 2009; Parkington et al., 2004; Parkington et al.,

2003; Thomson & Gordon, 2006) as well as a lower protein synthesis response compared to young muscle (Kumar et al., 2009). The inhibitory phosphorylation of eEF2 is higher in older vs. younger rat skeletal muscle, and also tends to increase with overload only in old (Thomson & Gordon, 2006). Another key finding was that p70s6k and eEF2 signaling are both inhibited in old versus young rat muscles after electrical stimulation (Fick & Gordon, 2007). Another study in humans observed that the eEF2 phosphorylation response to resistance exercise did not differ between age groups when all subjects had ingested an essential amino acid (EAA) and carbohydrate drink prior to exercise (which would theoretically bypass AMPK and enhance the responses of mTOR and its downstream effects such as p70s6k and eEF2 signaling as well as protein synthesis) (Drummond, Dreyer et al., 2008). The eEF2 response to acute resistance exercise has not been examined in younger vs. older humans under normal circumstances (i.e., in the absence of an EAA/carbohydrate drink). Interestingly, the acute resistance training bout did elicit an increase in the phosphorylation of AMPK (normally an inhibitor of mTOR, p70s6k, eEF2, and protein synthesis in the absence of an EAA/carbohydrate drink) in the muscles of elderly, but not young, individuals in that study (Drummond, Dreyer et al., 2008). Low levels of muscle glycogen have been shown to stimulate AMPK activity (Wojtaszewski, Jorgensen, Hellsten, Hardie, & Richter, 2002). Thus, the lower muscle glycogen content (Cartee, 1994) with age could explain the increased muscle AMPK phosphorylation and activity with age observed at rest and in response to overloading/resistance exercise (Thomson 2005, Drummond 2008) that may subsequently lead to the decrease in muscle mass and/or diminished anabolic response to resistance exercise commonly seen in aged muscle. Therefore, **the aim of this study was** to examine the eEF2 phosphorylation response to resistance exercise and its relation to muscle glycogen content and AMPK activation in old versus young subjects. **We hypothesized**

that inhibitory eEF2 Thr56 phosphorylation would be higher in response to acute resistance exercise in the skeletal muscles of older versus younger individuals. We further hypothesized that this higher eEF2 phosphorylation response would be related to higher AMPK activation, and that higher AMPK activation would be related to lower muscle glycogen content, in the skeletal muscles of older versus younger individuals.

CHAPTER II: REVIEW OF LITERATURE

Sarcopenia

As individuals age they tend to experience a decrease in their physical ability which tends to accelerate with advancing age (Beckett et al., 1996). The population of Americans categorized as older adults is continuously increasing, so this burden of disability is becoming a public health concern (Janssen, Shepard et al., 2004). The cause of the physical impairments can be attributed to several different rationales; most specific to this study is sarcopenia. Sarcopenia is the wasting away of skeletal muscle associated with advancing age (Gordon et al., 2008). After the age of 45 muscle begins to atrophy and progressively worsens with age (Hughes, Frontera, Roubenoff, Evans, & Singh, 2002). Concurrently with decreasing muscle mass, as individuals age they also tend to lose muscular strength (Rooyackers, Balagopal, & Nair, 1997). The trend has shown that the prevalence of sarcopenia increases from the third to the sixth decade of life, indicating increasing cases of sarcopenia with increasing age (Janssen, Heymsfield, & Ross, 2002). The skeletal muscle tends to decrease not only in size but also in the number of fibers (Welle, 2002) and is mostly associated with the atrophy of the type II, fast twitch fibers (Lee et al., 2006).

Sarcopenia is directly related to an decrease in functional ability, (Janssen et al., 2002), and those suffering from the disease report a decreased quality of life when compared to non-sarcopenic counterparts (Janssen, Shepard et al., 2004). With 45% of the elderly population being to some degree sarcopenic, and therefore potentially losing physical ability, it puts a strain on the healthcare industry, financially speaking (Janssen, Baumgartner et al., 2004). Approximately $18.5 billion dollars alone can be attributed to sarcopenia related costs (Janssen, Shepard et al., 2004). That is a significant amount of money going towards a very preventable

disease. By exercise alone, which will be discussed farther in later sections, a person could spend roughly $600 per month on gym-based exercise and exercise counseling in an exercise facility in attempt to combat this age related process. On the other hand, the same study revealed a person can expect to spend approximately $900 a month in costs due to sarcopenia (Sevick et al., 2000). This financial standing alone indicates how serious of a problem sarcopenia is, and it will only worsen as the amount of Americans over 65 increases. To date, sarcopenia accounts for a similar portion of healthcare funding as osteoporosis, however it has yet to see a public health campaign aimed at reducing the prevalence of it (Janssen, Shepard et al., 2004).

Several lifestyle choices can lead an individual in this down spiraling course, leading to muscle mass decline and disability. First and foremost is the lack of physical activity. It has been shown that older persons engaged in higher levels of physical activity have a slower rate of decline in motor performance (Buchman, Boyle, Wilson, Bienias, & Bennett, 2007). Another common trend among elderly is a decrease in the amount of calories consumed (Janssen, Shepard et al., 2004). Not only do they consume fewer calories per meal, but they also are consuming less protein, (Janssen, Shepard et al., 2004) as well as less carbohydrates(Lieberman et al., 1989). Sarcopenia can also have an effect on various diseases, including diabetes, osteoporosis and obesity (Janssen, Shepard et al., 2004). With this growing problem it is of the utmost concern to prevent this epidemic from advancing any farther than it already has. Not only does it put a strain on our financial system, but it also decreases quality of life in the older population (Janssen, Shepard et al., 2004). One of the best known interventions is resistance training, which causes the muscle to hypertrophy, a quick response to training (Janssen, Shepard et al., 2004). While resistance training can help combat the decrease in muscle, it appears that

hypertrophy and strength gains are substantially reduced in old versus young subjects (Kosek et al., 2006; Trappe et al., 2003).

Resistance Training

One approach commonly utilized to delay the progression of sarcopenia and encourage muscular growth is resistance training (Marini et al., 2008). Frail individuals can see an advantageous increase in fiber size as well as strength when taken through a chronic resistance training routine (Kryger & Andersen, 2007). Individuals who have continuously exercised throughout their lifespan tend to have larger muscle sizes and muscular performance (Aagaard et al., 2007). For older individuals experiencing the decline in muscle mass, resistance training can assuage this process (Thompson, 1994). The age related protein decreases are only partially reversed with resistance exercise, it does not entirely reverse the process of degeneration (Balagopal, Schimke, Ades, Adey, & Nair, 2001). While older individuals can experience gains in their muscular strength, it tends to be a blunted response when compared to the gains a younger individual is able to achieve (Kosek et al., 2006). This same trend can be seen in the hypertrophic response in aged versus young rats when looking at fast twitch fibers (Thomson & Gordon, 2005). When comparing the genders and their abilities to increase muscular strength and hypertropic response women tended to have smaller increases than men (Bamman et al., 2003). There is also a discrepancy in the enlargement of the type of muscle fiber and its response to exercise. The type II, or fast twitch fibers are the most susceptible to atrophy with age (Hortobagyi et al., 1995; Thompson, 1994), and are also the fiber type that shows no significant size improvements with resistance training (Frontera, Meredith, O'Reilly, Knuttgen, & Evans, 1988; Trappe et al., 2001), this decreased amount of hypertrophy is also seen in fast-twitch muscles of aged rats (Haddad & Adams, 2006; Thomson & Gordon, 2005).

Protein Synthesis

Protein synthesis is the process involving the translation of mRNA to create proteins from various amino acids within the muscle. This process consists of three main steps, initiation, elongation, and termination. The rate in which these processes occur is in part liable for the either hypertrophy of atrophy of the skeletal muscle. In order for the muscle to grow the rate at which protein synthesis must exceed the rate at which protein degradation occurs (Phillips, Tipton, Aarsland, Wolf, & Wolfe, 1997). Post resistance exercise, the rate of protein synthesis is shown to surpass that of breakdown and therefore result in an increase in overall protein mass availability (Phillips et al., 1997). The normal response to a bout of resistance exercise, as seen in young individuals, is an increase in the rate of protein synthesis (Phillips et al., 1997). This muscle protein synthesis response to a bout of resistance exercise is comparatively lower than that of young human subjects (Drummond, Dreyer et al., 2008; Kumar et al., 2009). These same findings of lower rates of muscular protein synthesis are also found in the muscles of aged versus young rats when introduced to weight lifting exercise (Tamaki et al., 2000). This could potentially explain why when an old muscle is introduced to an overload they are not capable of producing a hypertrophic response similar to that of young muscles (Thomson & Gordon, 2005). It has been shown that even at rest elderly individuals exhibit lower levels of skeletal protein synthesis (Yarasheski, Zachwieja, & Bier, 1993). The atrophy, and lack of hypertrophy, as seen after a bout of resistive exercise is more inclined to strike type II fibers (Lee et al., 2006). This noteworthy difference in the degree of protein synthesis in the muscle of varying cohorts can potentially explain the age-related atrophy of type II fibers.

mRNA Translation into Protein

Protein synthesis is initiated by either an increase in the available amino acids or by resistance exercise (Kimball, Farrell, & Jefferson, 2002). The process begins in part because of the stimulation of mRNA translation (Kimball et al., 2002). Protein synthesis is partially regulated by the initiation and elongation factors that are responsible for the rate of mRNA translation (Bodine et al., 2001). The first stage of translational initiation the met-tRNAi binds to a 40S ribosomal subunit to form a tertiary complex, which includes eukaryotic initiation factor 2 eIF2, GTP and met-tRNA (Kimball et al., 2002). This allows the initiation factors to form a 43S preinitiation subcomplex (Kubica, Kimball, Jefferson, & Farrell, 2004). This complex is responsible for scanning the mRNA strand until it arrives at the start codon, and once it reaches this location an 80S complex is formed, prepared for translation elongation (Kimball et al., 2002).

Translation is based on the pairing of a codon on the mRNA and the anticodon on the tRNA caring the amino acid to form a polypeptide chain (Frank, Gao, Sengupta, Gao, & Taylor, 2007). The elongation cycle is a three step process that is consistently repeated which is catalyzed by a ribosome and two GTPases (Frank et al., 2007). Eukaryotic elongation factor 2 (eEF2) is responsible for directing the tRNAs into the exit site for removal (Browne & Proud, 2002). Translation termination is responsible for the release of the newly created polypeptide chain and its removal from the ribosome (Bolster, Crozier, Kimball, & Jefferson, 2002).

Protein Translation via mTOR Signaling

Protein synthesis is vastly dependent upon upstream cellular signaling pathways that are responsible for controlling the process. One of the most critical pathways involves Akt/mammalian target of rapamyacin (mTOR) and its activation directly influences an increase

in hypertrophy as well as a decrease in atrophy (Bodine et al., 2001). Following a bout of resistance exercise a signal is transmitted through this mTOR pathway and activation is increased (Drummond, Dreyer et al., 2008). In response to the increase of the signaling through this pathway, the rate of protein synthesis is consequently augmented accordingly. Moreover, if this pathway is pharmacologically inhibited protein synthesis is suppressed post exercise training session (Kubica, Bolster, Farrell, Kimball, & Jefferson, 2005), as is muscular hypertrophy during overload (Bodine et al., 2001). Akt is responsible for activating mTOR by its phosphorylation at Ser2448 and deactivation of mTOR occurs by the activation of the tuberous sclerosis complex (TSC) at the same location (Inoki, Li, Zhu, Wu, & Guan, 2002). mTOR plays an integral role in protein translation, through its stimulation of initiation and elongation. Downstream from mTOR, 4E-BP1 (a binding protein) is phosphorylated which allows the formation of the translational initiation complex eIF4F (eukaryotic initiation factor 4E; the beginning stages of initiation). This deters the binding and deactivation of eIF4F and its binding to the 40S ribosomal subunit (Inoki et al., 2002; Kimball et al., 2002; Kimball et al., 1998).

Another crucial role of mTOR is its role in the activation of 70-kDa ribosomal protein s6kinase (p70s6k) which affects the protein synthesis process in two ways. Initially, it stimulates the translation portion of protein synthesis by recruiting 5'tract of pyrimidine (5'TOP) mRNAs to the ribosome (Ryazanov et al., 1988). This is crucial for the improvement of translation because 5'TOP mRNAs specifically encode for proteins of the ribosomal machinery itself, as well as for proteins such as eEF2 (Levy, Avni, Hariharan, Perry, & Meyuhas, 1991). The other important function of p70s6k is to prevent the deactivation of eEF2 via eEF2k which thereby increases translation elongation (Ryazanov et al., 1988).

Several findings indicate that the mTOR-p70S6k-eEF2 pathway may be less responsive to resistance exercise and/or overload in old versus young skeletal muscle. mTOR signaling was decreased in old rats when compared to young when stimulated with a single bout of contractile activity (Funai, Parkington, Carambula, & Fielding, 2006; Parkington et al., 2004; Parkington et al., 2003). Ablation surgeries of young and old rats have revealed similar findings, with mTOR phosphorylation being significantly elevated after an overload-induced situation in young, but not in older, skeletal muscles (Thomson & Gordon, 2006). p70s6k signaling is significantly lower in old versus young rat skeletal muscle in response to electrical stimulation (Fick & Gordon, 2007). Another key finding was that eEF2 signaling was inhibited in the old rats in response to electrical stimulation in that study (Fick & Gordon, 2007). Likewise, young animals, after overload-induced exercise, exhibit an increase of total eEF2 protein content, while old muscles showed no such response (Thomson & Gordon, 2006). A study in humans indicated that a resistance exercise session elicited no differences in eEF2 phosphorylation response between age groups after the ingestion of an essential amino acid (EAA) and carbohydrate-rich drink immediately prior to the exercise bout (Drummond, Dreyer et al., 2008). However, EAA (especially leucine), would theoretically bypass or override AMPK signaling to directly enhance mTOR signaling and consequently its downstream effects, such as p70s6k phosphorylation, eEF2 phosphorylation, and protein synthesis (Drummond, Dreyer et al., 2008). Interestingly, that study observed that the acute resistance training bout resulted in an increased of 5'-Adenosine monophosphate-activated protein kinase (AMPK) phosphorylation (which would normally act as an inhibitor of mTOR signaling in the absence of the EAA drink) in the muscles of the older individuals, but not the young (Drummond, Dreyer et al., 2008). This indicates the potential for a lower responsiveness in the protein synthesis signaling pathway due to AMPK

signaling under normal conditions (i.e., in the absence of EAA ingestion) and ultimately a potentially lower efficiency of older muscle to have a hypertrophic response to resistance exercise and or overload.

AMPK

5'-Adenosine monophosphate-activated protein kinase (AMPK), plays an integral role in the regulation protein synthesis. AMPK is located upstream from mTOR and has a negative effect on the initiation of protein synthesis (Bolster et al., 2002). AMPK is a protein known for its function in sensing the energy balance within in the cell and responding accordingly (Winder & Hardie, 1999). AMPK is activated when there is a shift in the AMP/ATP ratio and available ATP decreases (Winder & Hardie, 1999). This occurs during times where ATP production is reduced or when ATP usage is higher, such as a bout of resistive exercise or hypoxic state (Hardie & Sakamoto, 2006). AMPK is activated to fulfill two purposes. First, it stimulates processes responsible for generating ATP and restores the energy balance within the cells (Winder & Hardie, 1999). ATP production is increased through glycolysis, increased glucose uptake, fat oxidation and mitochondrial biogenesis (Hardie & Sakamoto, 2006). Secondly, it simultaneously inhibits processes that are metabolically expensive, such as protein synthesis (Bolster et al., 2002; Winder & Hardie, 1999). So when AMPK senses this energy imbalance it inhibits mTOR signaling, and other downstream proteins that allow translation elongation and the recruitment of 5'TOP (Bolster et al., 2002). AMPK is also capable of directly inhibiting protein synthesis by bypassing the mTOR-p70s6k pathway and stimulating eEF2k, which in turn inhibits eEF2 (Browne et al., 2004). Essentially, AMPK regulates the availability of ATP for use and growth of the cell or promotes the production of the energy source. Depending on the

AMPK activation, either diminished hypertrophy or atrophy can be expected (Gordon et al., 2008).

AMPK appears to negatively regulate skeletal muscle hypertrophy and may be a reason underlying the diminished hypertrophy of fast-twitch muscle in older animals (Gordon et al., 2008; Thomson & Gordon, 2005, , 2006) . Although muscle AMPK activity has been seen to remain elevated 1-2 hours after an acute bout of resistive exercise in young men (Dreyer et al., 2006), this response is more accentuated with age. In sedentary old men at 1 hour and 3 hrs post resistance exercise, AMPK levels are significantly higher than seen in young men under the same conditions (Drummond, Dreyer et al., 2008). These findings are consistent with old versus young overload induced muscle growth in Fisher 344 x Brown Norway F1 hybrid male rats (Thomson & Gordon, 2005). The researchers observed a significantly higher AMPK phosphorylation in plantaris muscles (fast twitch) from old versus young rats, but not in the soleus (slow twitch) (Thomson & Gordon, 2005). The findings also indicated a strong negative correlation between AMPK phosphorylation and the degree of hypertrophy in these fast twitch plantaris muscles (Thomson & Gordon, 2005). These findings are all consistent with the notion that AMPK phosphorylation has a negative influence on mTOR and its downstream intermediaries and the percent of muscle hypertrophy. The phosphorylation of mTOR is significantly higher in young versus old muscles after overload (Thomson & Gordon, 2006), and downstream markers of mTOR activity (p70s6k phosphorylation and 4E-BP1 phosphorylation) were negatively correlated with the increased AMPK activity with age in that study.

To further prove whether hyperactivation of AMPK negatively regulates fast-twitch muscle growth in aged animals, a local perfusion of 5-aminoimidazole-4-carboxamide-1beta-D-ribofuranoside (AICAR; an AMPK activator) was continuously administered to a fast-twitch

plantaris muscle of young animals (Gordon et al., 2008). This revealed a decreased response in the overload-induced growth of the young fast-twitch muscle fibers. On the contrary, old rats received a continuous local perfusion of an AMPK inhibitor, Compound C, on the fast-twitch plantaris muscle (Gordon et al., 2008). This showed an improved growth in aged muscles, to levels seen in young saline treated muscle, which further confirms AMPK's inhibitory role in hypertrophy (Gordon et al., 2008).

In addition to the possibility that the effects of AMPK inhibits skeletal muscle hypertrophy by inhibiting protein synthesis, AMPK may be partly responsible for the enhanced degradation of proteins observed in aged muscle (Nakashima & Yakabe, 2007). Specifically, the initiation of forkhead box (FOXO), which is an atrophy related protein, has been linked to increased AMPK activation (Nakashima & Yakabe, 2007). When these responses are compared between old and younger individuals there is a considerably higher activation after a bout of exercise in the older subjects (Raue, Slivka, Jemiolo, Hollon, & Trappe, 2007). Although the protein degradation processes are not the focus of the current project, the increased atrophy-related gene expression coupled with the decreased protein synthesis response, (all in part potentially due to enhanced AMPK activity) is a potential source for the atrophy and diminished hypertrophy in type II muscle fibers with age.

Glycogen and Diet

With advancing age there tends to be a deterioration of diet and caloric intake which contributes to a calorically lower state associated with aging (Vanitallie, 2003). Dietary intake of the elderly has been studied in attempt to identify the specific inadequacies. Specifically, total calories as well as carbohydrate consumption were significantly lower in older individuals when compared to equally healthy young subjects (Lieberman et al., 1989). Long term low

carbohydrate consumption has been associated with a reduction in glycogen stores (Astrup, Meinert Larsen, & Harper, 2004). This information indicates that the elderly may be unintentionally consuming a low carbohydrate diet and thus lowering their total glycogen stores.

When individuals were placed on low carbohydrate or high carbohydrate diets, and performed exercise bouts, the high carbohydrate subjects were found to have a higher muscle glycogen content (Green, Ball-Burnett, Jones, & Farrance, 2007). AMPK senses a shift in the available energy stores in the form of AMP/ATP. AMPK sensing of energy stores can also extend to an intermediate reserves of energy, glycogen for example (Hardie & Sakamoto, 2006). Little research has examined the connection between manipulation of glycogen content and AMPK activity, but it is known that high levels of muscle glycogen can suppress the activation of AMPK by AICAR in perfused rat muscle (Wojtaszewski et al., 2002). AMPK activity is also somewhat sensitive to the fuel status of the muscle in human subjects (Wojtaszewski et al., 2003). When young subjects were introduced to a low versus high carbohydrate diets, AMPK activation was significantly lower in glycogen loaded states (Wojtaszewski et al., 2003).

Specific Aim

Resistance training has been shown to elicit hypertrophy in young subjects, but the response is diminished in older individuals (Kosek et al., 2006; Raue et al., 2009). In response to overload or resistance training, older humans and rats exhibit a decreased response in skeletal muscle activation of mTOR and p70s6k (Kumar et al., 2009; Parkington et al., 2004; Parkington et al., 2003; Thomson & Gordon, 2006). In addition, older muscle shows a lower protein synthesis response compared to young muscle (Kumar et al., 2009). Older rat skeletal muscle demonstrates a higher inhibitory phosphorylation of eEF2 when compared to younger skeletal muscle. This trend seems to increase with overload, but only in the old muscle (Thomson &

Gordon, 2006). Additionally, a study conducted on humans demonstrated that after consuming an essential amino acid and carbohydrate drink and completing a resistance exercise bout, the phosphorylation of eEF2 did not significantly differ between age groups (which would hypothetically bypass AMPK and augment the responses of mTOR and its downstream effects, such as p70s6k and eEF2 signaling as well as protein synthesis) (Drummond, Dreyer et al., 2008). The eEF2 response to acute resistance exercise has not been examined in younger versus older humans under normal circumstances. Remarkably, the resistance exercise session in that study was sufficient to elicit an increase in AMPK phosphorylation (which would normally inhibit mTOR, p70s6k, eEF2, and protein synthesis in the absence of an EAA/carbohydrate drink) in the muscles of elderly, but not young, individuals in that study (Drummond, Dreyer et al., 2008). AMPK activity has been shown to be stimulated during periods of low muscle glycogen content (Wojtaszewski et al., 2002). Therefore, the lower muscle glycogen content (Cartee, 1994) of aged muscle could explain the increased muscle AMPK phosphorylation and activity that is observed in older individuals and rats at rest and in response to overloading/resistance exercise (Thomson 2005, Drummond 2008). These variables could subsequently be contributing to the decrease in muscle mass and/or diminished anabolic response to resistance exercise commonly seen in aged muscle. **The aim of this study was** to examine the eEF2 phosphorylation response to resistance exercise and its relationship to muscle glycogen content and AMPK activation in old versus young subjects. **We hypothesized** that inhibitory eEF2 Thr56 phosphorylation would be higher in response to acute resistance exercise in the skeletal muscles of older versus younger individuals. We further hypothesized that this higher eEF2 phosphorylation response would be related to higher AMPK activation, and that higher

AMPK activation would be related to lower muscle glycogen content, in the skeletal muscles of older versus younger individuals.

CHAPTER III: METHODS

Subjects

Participants in this study consisted of 7 young subjects (4 male and 3 female) between the ages of 18 and 30 years old and 10 older subjects (5 male and 5 female) between the ages 55 and 85 years old. Inclusion criteria included healthy, lean men and women with no known, current or past, cardiovascular disease, diabetes or hypertension. Prior to participation subjects underwent a telephone or email prescreening to verify age, height, weight, BMI, medical history, current medications and exercise history. Subjects were required to have a BMI less than, or close to, 30 and be physically inactive. Inactivity was defined as engaging in less than three days per week of moderate intensity physical activity for more than six months prior to enrolling in the study. This study was approved by the East Carolina University Institutional Review Board for Use of Human Subjects (APPENDIX A). Flyers, email and word of mouth were all utilized around the East Carolina University (ECU) campus to recruit subjects.

Table 3.1. Subject Characteristics.

	Young Adults (n=7)	**Old Adults (n=10)**
Age (years)	21.7 ± 0.8	67.0 ± 2.6*
BMI (kg/m^2)	23.5 ± 1.1	29.0 ± .98*
Body Fat (%)	21.9 ± 1.9	32.6 ± 1.2*
Fat-Free Mass (kg)	58.1 ± 4.1	57.3 ± 2.4
Weight (kg)	74.5 ± 5.2	85.6 ± 3.5

*=Significantly different between groups. Data are presented as Mean ± SEM (standard error of the mean). Old adults were significantly older as a group and had a significantly higher BMI and body fat percentage. There were no significant differences between young and old adults for fat-free mass or weight. BMI: body mass index (calculated by the formula: $(kg)/m^2$). Body fat and fat free mass were obtained with the use of a four-site Durnin and Womersley method of skinfold measurement (Durnin & Womersley, 1974)

Experimental Design and Laboratory Visits

The laboratory visits and associated procedures are presented in Figure 3.1. The two laboratory visits were separated by one to two weeks.

Initial Visit (non-fasted)	Experimental Visit (overnight fast)
▪ Informed consent & medical history ▪ Height and weight measurements ▪ Skinfold measurements ▪ 10-repetition maximum determination ▪ Dietary recall analysis and counseling	▪ Exercise session -Bilateral leg extension -3 warm-up sets (50, 70, 90% of 10RM) -3 sets to failure (100% of 10RM) ▪ Biopsy- alternating legs -Pre-exercise, immediately post, 0 min, 60 min, 120 min post

Figure 3.1. 10 repetition max (10RM) was determined on the leg extension machine in the FITT building on the East Carolina University campus. Warm-up sets were conducted with 10 repetitions, 5-7 repetitions and 3-5 repetitions for each set (50%, 70%, 90% of the estimated 10RM), respectively, with the primary goal being muscular failure during the working sets. The 0 min time point marks the completion of the last set of the resistance exercise. 60 min and 120 min post exercise biopsies are taken 1 hour and 2 hours after the 0 min post biopsy, respectively.

Initial Visit

The initial session was held at the Fitness Instruction, Testing and Training (FITT) building on the campus of ECU. This initial visit consisted of a thorough screening as well as gathering baseline measurements, familiarizing the participants with the exercise equipment and determining their individual 10 repetition maximum on the Cybex leg extension machine.

During the initial visit participants filled out an informed consent document, health history questionnaire, and a 1-day dietary recall. Height and weight measurements were then taken in order to calculate BMI from the data. Next, a 4 site skinfold measurement was taken to determine body density. To remain consistent with previous research, the bicep, triceps, suprailiac, and subclavical skinfold sites were selected. Then, body fat was estimated using the Durnin and Womersley skinfold assessment and body density formula ([495/(1.1714 – 0.063 * LOG[sum of skinfolds] – 0.000406 * [age]) – 450]) (Durnin & Womersley, 1974; Siri, 1961).

The subjects then underwent a brief familiarization period with the resistance exercise equipment utilized during the exercise sessions to reduce the risk of injury or cardiovascular events due to inexperience with proper technique. Appropriate timing and breathing for the concentric and eccentric phases of the exercise was be explained and demonstrated when necessary. A 10 repetition maximum (10RM) was determined using the Cybex leg extension machine (Cybex International; Serial#: 485097W272216) during this initial visit as well. Subjects were asked to predict the maximum weight they could lift 10, this weight was divided in half for the warm-up. If the subject was unable to estimate a 10RM a conservative weight was selected and the subject rated the weight on a 1-10 RPE scale. Following the warm up, weight was increased between 5-20 pounds depending on the tolerance of the individual. Each set consisted of 10 repetitions with a 1-2 minute rest period between sets. Testing was terminated, and 10RM was determined when the participant could no longer successfully complete 10 repetitions at the set weight.

The subjects were provided with an information sheet containing detailed instructions for the 7-14 day period prior to the experimental session. Through the duration of the experiment the subjects were asked to abstain from exercise or donating blood. Two days prior to the

experimental visit the subjects were expected to avoid drinking alcohol, required to drink at least 64 ounces of water daily and drink caffeine only in moderation. Some older subjects were initially selected to participate in a high carbohydrate group. These individuals were briefly counseled on preferred food choices to increase carbohydrate intake, and maintain protein intake, prior to the experimental session (see "Attempt at Dietary Intervention" for more details). The subjects were instructed to drink plenty of fluids throughout the course of the study because it has been shown that dehydration can impair exercise performance (Shirreffs, 2005). Caffeine intake was monitored as well because it can have an ergogenic effect on performance (Burke, 2008). Subjects were to report for the experimental session after a 12 hour fast, and a 24 hour break from any exercise bouts. Prior to arriving in the morning the subjects were required to drink at least 16 ounces of water. The subjects arrived dressed for activity upon arriving at The East Carolina Heart Institute.

Attempt at Dietary Intervention

The young subjects and most of the older subjects were asked to maintain their normal diet throughout the course of the study. However, three older individuals were directed to consume a high carbohydrate diet three days prior to the experimental session in an attempt to increase muscle glycogen content through dietary intervention. During the initial visit, these subjects were provided guidelines and dietary counseling to assist them in making their food selections. When elderly individuals are introduced into a controlled setting and able to make free choices in meal selection they consume approximately 30% of calories from carbohydrates and 50% from fats (Wurtman, Lieberman, Tsay, Nader, & Chew, 1988). Initially, the ultimate goal of this investigation was to enhance muscle glycogen content via dietary intervention; therefore, the high CHO group was instructed to consume at least 60 to 70% of their calories

from carbohydrates, as well as minimize those coming from fat sources (leaving protein as unaltered as possible). This required the subjects to select low fat protein and carbohydrate food options. Essentially, this study design was aimed at elevating muscle glycogen content via a high carbohydrate diet. The three days before the experimental session all subjects were asked to fill out a diet log that was analyzed using Nutritionist Pro^{TM} software (version 4.04) to assess the caloric consumption and dietary composition.

Unfortunately, the attempt at accomplishing a "high CHO" group was unsuccessful, either due to lack of adherence or inadequate knowledge of appropriate food choices. After analyzing the dietary logs for all subjects, no difference was shown between the "ad lib" group and the "high CHO" groups. Table 3.2 shows a thorough breakdown of consumed calories for all three of the groups. The dietary composition between the "ad lib" group and the group instructed to ingest a high carbohydrate diet were practically indistinguishable, and therefore were combined for further analysis. Each dietary component was normalized for body weight, but still no significant differences were found between the age groups for any variable. The mean (± SE) calorie distribution within the old group was (kcal/kg BW): Carbohydrate, 12.3 ± 2.3; Protein, 4.3 ± .48; and Fat, 8.0 ± 2.2. The mean (± SE) calorie distribution within the young group was (kcal/kg BW): Carbohydrate, 12.0 ± 2.1; Protein, 3.3 ± .55; Fat, 6.8 ± 3.2. Total kcals within the old and young groups (mean ± SE, Kcal/kg BW) were 24.4 ± 2.5 and 22.6 ± 3.5, respectively.

Table 3.2. Subject Dietary Characteristics.

	Old Ad Lib (n=5*)	**Old High CHO (n=3)**	**Young (n=7)**	**All Old (n=8*)**
% Carb	47.0 ± 5.3	48.4 ± 5.1	53 ± 4.6	47.6 ± 3.5
% Protein	18.2 ± 2.7	17.2 ± 1.8	17.7 ± .89	17.6 ± .89
% Fat	34.8 ± 5.0	34.4 ± 4.2	29.7 ± 2.4	34.6 ± 3.1
Total Kcal	1994.4 ± 259.1	1993.9 ± 68.8	1608.4 ± 183.8	1994.4 ± 139.4

Data are presented as Mean ± SEM (standard error of the mean). The "Ad Lib" group refers to the group with no caloric restrictions. The "High CHO" group was the group instructed to consume a high carbohydrate diet. No significant differences were found between the Ad Lib group and the high carbohydrate group on any of the measures of dietary intake. Since there was no significant difference between the two groups they will be combined for further analysis. All dietary information is based on % Kcal of the total consumed calories. All nutrition information was assessed using the Nutritionist Pro™ software, version 4.04. The data from the Old "Ad Lib" group is a majority sub-sample of the entire group of 7. Likewise, the data from the "All Old' group is a majority sub-sample of the entire group of 10. Dietary logs were not obtained from all subjects in this group.

Experimental Session

A week or two following the initial visit, the subjects reported to The East Carolina Heart Institute where they were met in the lobby. Subjects arrived fasted and prepared for a maximum effort on the leg extension machine. A muscle biopsy was taken from the vastus lateralis prior to any work, for baseline measurements, immediately after exercise, an hour after exercise and two hours after exercise. Figure 3.1 outlines the procedure for the experimental session.

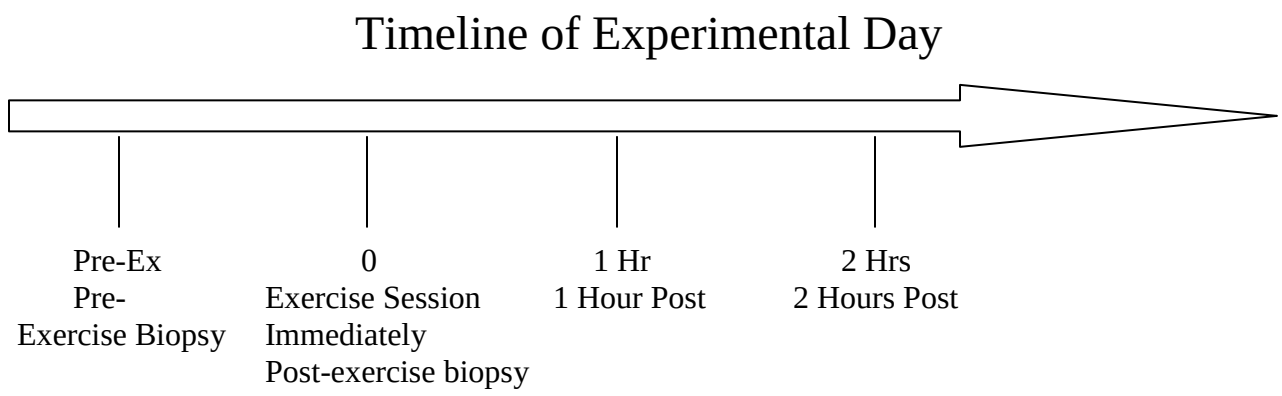

Figure 3.2. Timeline of events on experimental day. 0 is the exercise bout, a biopsy was taken immediately after exercise. 1 hr is one hour after the resistance exercise bout. 2 hr is two hours after the exercise bout.

Acute Resistance Exercise

The acute exercise bouts were performed on the Cybex leg extension machine (Cybex VR Leg Extension Model #4850, Cybex International, Medway, MA) after the first muscle biopsy was acquired. Proper technique for use on the leg extension machine was discussed again to prevent injury. An initial warm-up set was completed at 50% of the predetermined 10RM. Subjects were instructed to complete 10 repetitions for this warm-up set. Subjects were granted a 1.5 minute rest interval before the commencement of the second set. Warm-up set two was set at 70% of the predetermined 10RM with a completion goal of 5-7 repetitions. Another warm-up interval of 1.5 minutes was allotted and the subject was instructed to complete approximately 3-5 repetitions at 90% of their predetermined 10RM. The typical 1.5 minute rest session was allocated before the first work set began at the predetermined 10RM. The subject then completed three work sets until failure at the predetermined 10RM with 1.5 minutes between each work set. When the subject appeared to be nearing failure verbal encouragement was offered and 1-3 assisted repetitions were promoted. In the event that a work set allows 15 or more repetitions the weight was increased by five to ten pounds for each succeeding set.

Muscle Biopsies

A thorough explanation of the muscle biopsy procedure was explained to the subjects before the commencement of the procedure. The attending physician located the vastus lateralis by instructing the participant to flex the leg. The appropriate position was marked off on both legs prior to the procedure and, if necessary, the location was shaved. Three iodine swabs were used to sterilize the areas on the leg and a sterile field was placed over the area. Cold spray was used to numb the sight for the injection of lidocaine, approximately 5 mL of 1% lidocane was injected subcutaneously along the vastus lateralis. Approximately ¼ inch incision was made through the skin and a portion of the fascia using a No. 11 scalpel. Muscle biopsies were extracted using a 5-mm Bergstrom needle, with an application of suction to maximize the muscle removal.

Immediately after each biopsy, direct pressure and an ice pack were applied to the site. An appropriate amount of time was given for the bleeding to terminate. Once the bleeding stopped a steri strip bandage was placed on the site to hold the incision together. Several other wrappings were applied to further keep the site closed, including a band aid bandage, a tegaderm patch, and pressure wrap. This ensured the site healed properly, prevent water from reaching the incision, and result in as little scaring as possible.

A site was prepared on each leg prior to beginning the maximum exercise bout. The leg used for the biopsies was alternated for each subsequent biopsy. The first two biopsy sites were prepared at the beginning of the experimental visit. As soon as the subject completed the exercise session they were immediately moved back to the bed for the next biopsy, time point 0. Precisely one hour later the third biopsy was taken bilaterally, 5 cm proximally from the first, pre-exercise biopsy. The last biopsy was taken from the other leg proximal to biopsy two at

precisely 120 minutes after the exercise session. Each biopsy was allotted a new incision site that was sterilized in the same manner as the first two. Immediately after the biopsy was extracted from the leg, it was flash-frozen in liquid nitrogen and stored in a cryovial at -80° C until it was later used for western blotting analyses.

Sample Analyses

The muscle glycogen content was assessed by combining numerous methods from Passonneau and Lauderdale (1974), Passonneau and Lowry (1993), and Roy and Tarnopolsky (1998). Specifically, 5-15 mg of each sample of muscle were homogenized on ice in 53.3 volumes (3 mg/160 µL) of 0.1 M NaOH in a ground glass homogenizer. A 100 µL aliquot of every sample was neutralized with 100 µL of 0.2 M acetic acid and vortexing. Subsequently, two 50-µL aliquots of each of the samples were placed into duplicate tubes containing 200 µL of 0.1 M sodium acetate buffer (0.05 M acetic acid; 0.05 M sodium acetate, pH 4.6-4.7). Each remaining step was accomplished in a facsimile manner. In order to obtain free glucosyl units, glyogenolysis was then achieved by adding 10 µL of amyloglucosidase (10 µL/mL in 20 mM Tris buffer and 0.02% bovine serum albumin, pH 7.5; specific activity= 25.9 U/mg; Sigma-Aldrich # A7420, St. Louis, MO), followed by vortexing, and incubating at room temperature for 1 hour. The samples were then refined from any precipitate remaining in suspension by centrifugation at 16000 x g for 5 minutes. A commercially available glucose assay (based upon hexokinase and glucose-6-phosphate dehydrogenase reactions; Sigma-Aldrich # GAHK20) was then performed to establish the amount of free glucosyl units in each sample. The muscle glycogen content (µL/mg wet weight) of the original sample was then derived by comparing it to a standardized curve made with type III glycogen from rabbit liver (Sigma-Aldrich #G8876),

which were manipulated in the exact same way as the muscle samples in the present study, starting with the NaOH step.

AMPK phosphorylation, p70S6k phosphorylation, and eEF2 phosphorylation was assessed by western blotting technique to analyze the signaling proteins. The primary antibodies used to detect total and phospho-proteins are all commercially available: anti-AMPK [Cell Signaling Technology (CST); Danvers, MA; Cat. # 2532], rabbit monoclonal anti-phospho-AMPK (CST; Cat. # 4188), anti-acetyl CoA Carboxylase (streptavidin-HRP, GE Life Sciences, RPN1231), anti-phospho-Acetyl CoA carboxylase (Millipore Corporation; Temecula, CA; Cat. # 07-303), anti phospho-eEF2 (CST; Cat. # 2331), and anti total-eEF2 (CST; Cat. # 2332). A portion of each frozen muscle biopsy sample was homogenized using a buffer that consists of 50 mM HEPES (pH 7.4), 0.1% Triton X-100, 4 mM EGTA, 10 mM EDTA, 15 mM $Na_4P_2O_7 \bullet 10H_2O$, 100 mM ß-glycerophosphate, 25 mM NaF, 50 µg/ml leupeptin, 50 µg/ml pepstatin, and 33 µg/ml aprotinin. A ground glass homogenizer using a motor with variable speeds was used to perform all homogenizations. Homogenizations were performed on ice in order to prevent excessive heat build-up that may denature proteins. Total muscle protein homogenates were solubilized in sample loading buffer (50 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 2% ß-mercaptoethanol, 0.1% bromophenol blue) at a concentration of 1 mg/ml and boiled for 5 min. Proteins were then separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on multiple gels using various acrylamide percentages and gradients depending upon the molecular weights of the various proteins of interest, then western blotted for 2 hours at 4˚C onto a PVDF membrane at 100V in transfer buffer (25 mM Tris-base pH~8.3, 192 mM glycine, 20% methanol). Membranes were stained by Ponceau S to verify equal loading amongst lanes. For immunodetection, membrane strips were blocked for 1 hr in

blocking buffer [5.0% nonfat dry milk in TBS-T (20 mM Tris-base, 150 mM NaCl, 0.1% Tween-20), pH 7.6] then incubated overnight at 4ºC with primary antibody (1:1000 dilution in TBS-T with 5% BSA) overnight at 4º. The membranes were then serially washed in TBS-T, incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (Amersham Biosciences, Piscataway, NJ) in blocking buffer for 1 hr, and again serially washed in TBS-T. The HRP activity was detected using enhanced chemiluminescence reagent (Amersham Biosciences, Piscataway, NJ) and exposure to autoradiographic film. The IODs was then quantified by densitometry (Gel-Pro Analyzer, Media Cybernetics, Silver Spring, MD) and the concentration of the antigen present in each muscle was calculated as the IOD normalized to units of total muscle protein initially loaded on the gel. It should be noted that western blotting analysis was also attempted for phospho-p70s6k, but no bands were observed.

Statistics

An analysis of variance (ANOVA) with repeated measures was utilized to analyze the differences between and within the various groups using Statistica, version 6 (StatSoft, Inc., Tulsa, OK). The percent change data was evaluated using a one-way ANOVA. Post-hoc differences were determined by using Fisher's LSD Post-hoc test. Pearson Product-moment correlations were used for all correlational analyses. The significance level was set at an alpha level of $p \leq 0.05$.

CHAPTER IV: RESULTS

Subject Strength and Work Volumes

All subjects successfully completed the initial and experimental sessions. Performance for the exercise bouts were measured by total and relative volume for each group. The total volume was calculated by multiplying the total amount of weight lifted during the work sets by the number of completed repetitions during the experimental visit. Relative volume was standardized to fat free mass (kg) in order to represent the relative amount of weight lifted (data presented in Table 4.1).

Table 4.1. Assessment of Ten-repetition Maximum Strength and Work Volume Between Young and Old Adults

	Young Adults (n=7)	**Old Adults (n=10)**
Estimated 10 RM (kg)	48.53 ± 5.3	34.9 ± 3.4*
Total volume (kg resistance x repetitions)	1644 ± 223	1167 ± 183
Relative volume (kg resistance/kg FFM)	28 ± 1.9	19 ± 1.8*

*= Significantly different between groups. Data are represented as Means ± SEM (standard error of the mean). Older adults had significantly lower estimated 10 RM and relative volumes. Total volume (kg resistance) was calculated to evaluate the absolute amount of weight lifted during the working sets by each subject using the equation: Total volume = [(resistance $_{set\ 1}$(kg) x repetitions $_{set\ 1}$) +(resistance $_{set\ 2}$(kg) x repetitions $_{set\ 2}$) + (resistance $_{set\ 3}$(kg) x repetitions $_{set\ 3}$)]. Total volume was standardized by fat free mass to show the amount of weight lifted relative to lean body mass: relative volume = total volume (kg resistance) / fat free mass (kg).

Western Blotting Analysis

The present study aimed to determine the response of eEF2 phosphorylation at Thr56 to resistance exercise and its relation to muscle glycogen content and AMPK activation in the

skeletal muscle of young versus old individuals. It was hypothesized that inhibitory eEF2 Thr56 phosphorylation would be elevated in response to an acute resistance training session in the skeletal muscles of older versus younger individuals. It was further hypothesized that this higher eEF2 phosphorylation response would be related to a higher AMPK activation, and that higher AMPK activation would be related to lower glycogen content, in the skeletal muscles of older versus younger individuals. To assess AMPK activity, we measured acetyl oCoA carboxylase (ACC), an indicator of in vivo AMPK activity (Winder et al., 1997).

Glycogen

Muscle glycogen content was assessed at the PRE and all three post exercise time points for each of the groups. There was a significant difference in glycogen content between the young and old groups at PRE exercise. After the acute bout of resistance exercise both groups revealed a significant decrease in glycogen content at all time points within each individual age group, with no differences between groups after exercise (Figure 4.1).

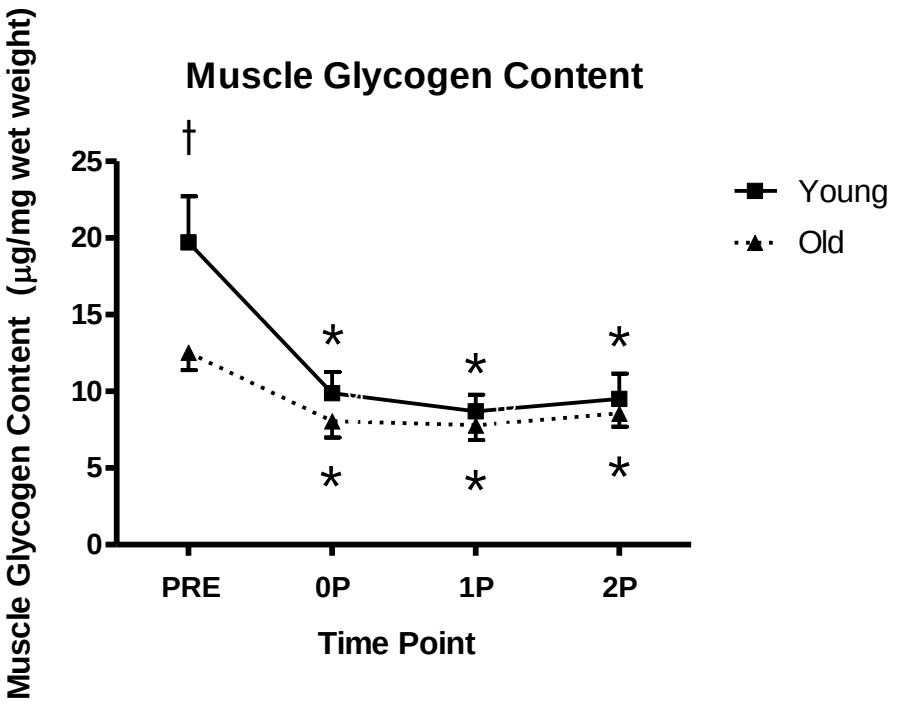

Figure 4.1. Mean ± SEM Muscle Glycogen Content in young and old subjects. Pre represents the pre exercise muscle biopsy, 0P is the immediately post exercise biopsy, 1P is the one hour post exercise muscle biopsy, and 2P is the two hour post exercise muscle biopsy. The young had significantly greater muscle glycogen content compared to the old at the PRE time point. The young and old subjects had significantly different muscle glycogen contents at all post exercise time points compared to the PRE time point within the individual age groups. Significance is set at $p \leq 0.05$.

AMPK

The old group had a significant increase in AMPK phosphorylation at Thr^{172} at the 0P time point compared to the PRE exercise value (Figure 4.2), but the young group had no such increase. There were no significant effects of age or time point on total AMPK (Figure 4.3). The phospho/total AMPK ratio revealed a significant main effect of time point at the 0P versus PRE exercise time point for both groups combined (Figure 4.4), but no effect of age.

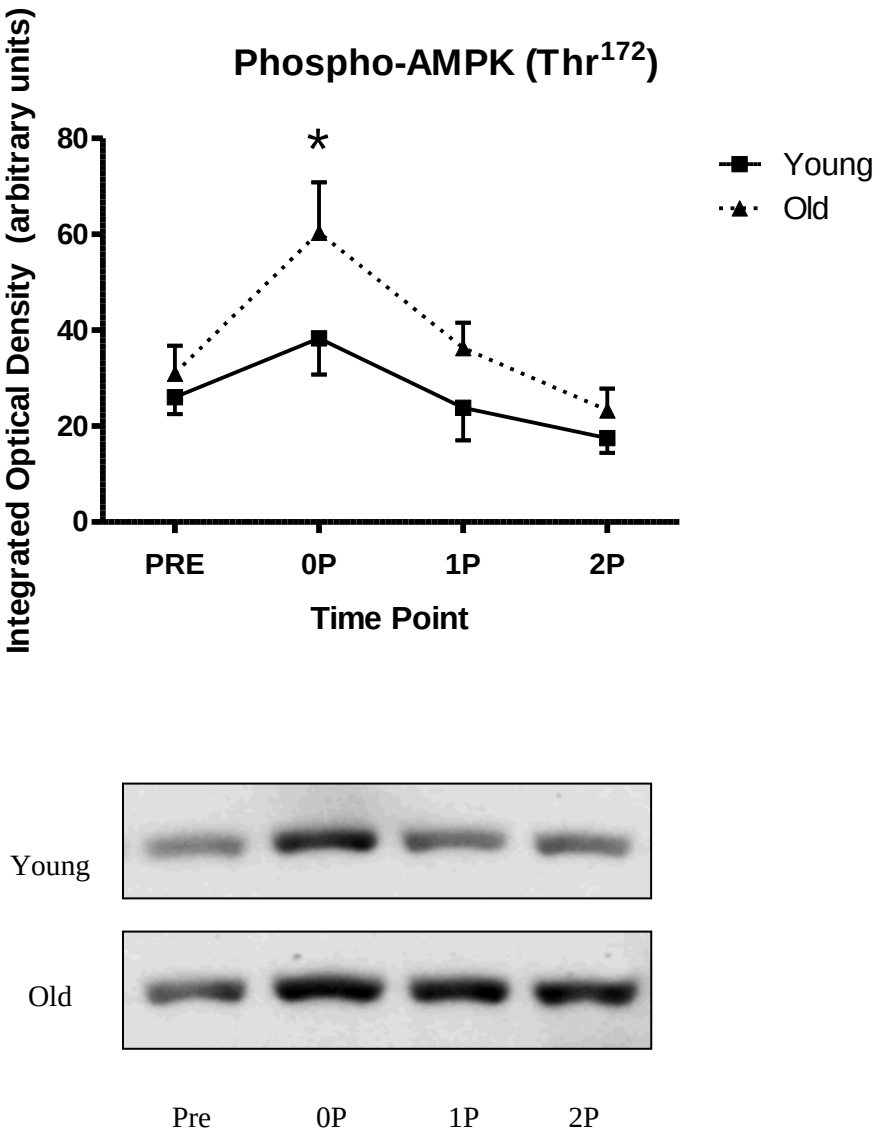

Figure 4.2. Mean ± SEM phospho-AMPK at Thr^{172} in young and old subjects and the representative western blots. PRE is the pre exercise muscle biopsy, 0P is the immediately post exercise muscle biopsy, 1P is the one hour post exercise muscle biopsy, and 2P is the two hour post exercise muscle biopsy. Phospho-AMPK was significantly increased from the PRE time point to the 0P time point within the old group. Significance is set at $p \leq 0.05$.

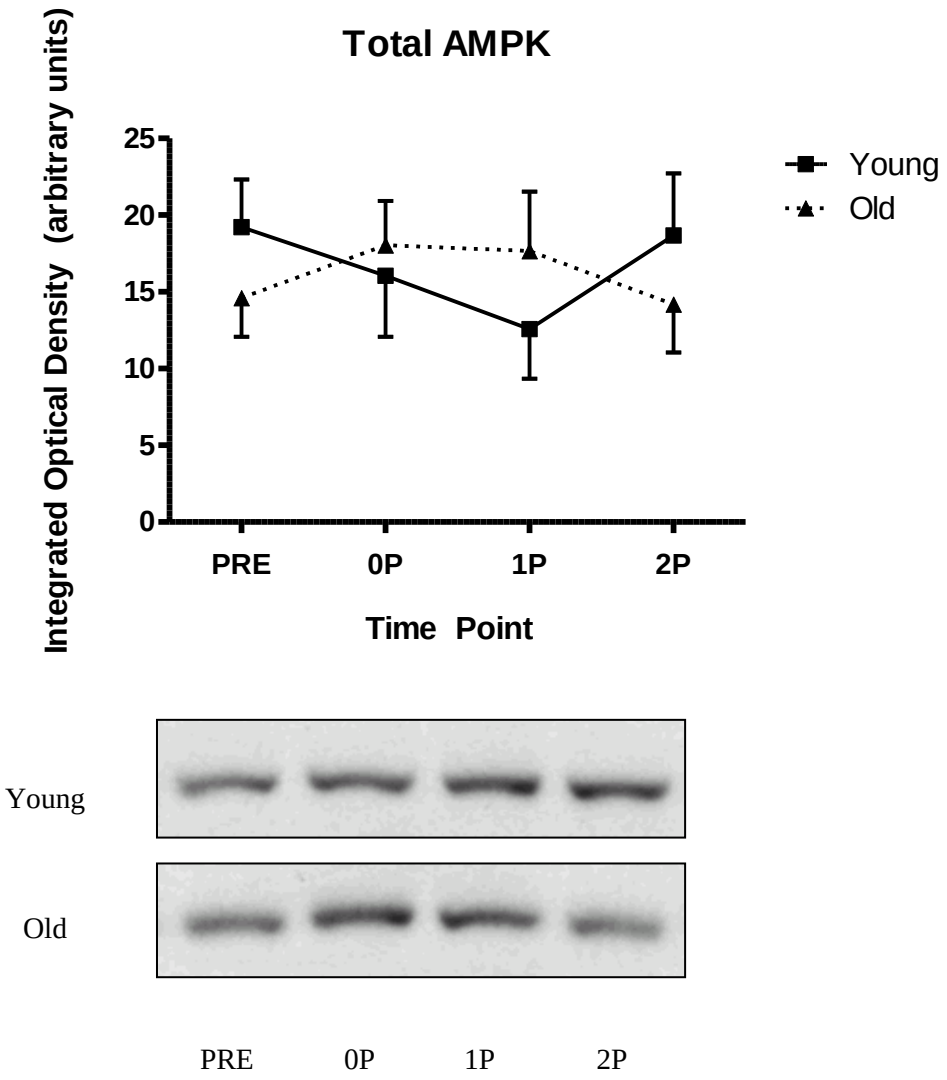

Figure 4.3. Mean ± SEM Total AMPK in young and old subjects and the representative western blots. PRE is the pre exercise muscle biopsy, 0P is the immediately post exercise muscle biopsy, 1P is the one hour post exercise muscle biopsy, and 2P is the two hour post exercise muscle biopsy. Total AMPK was not significantly different between age groups or from the PRE exercise time point. Significance is set at $p \leq 0.05$.

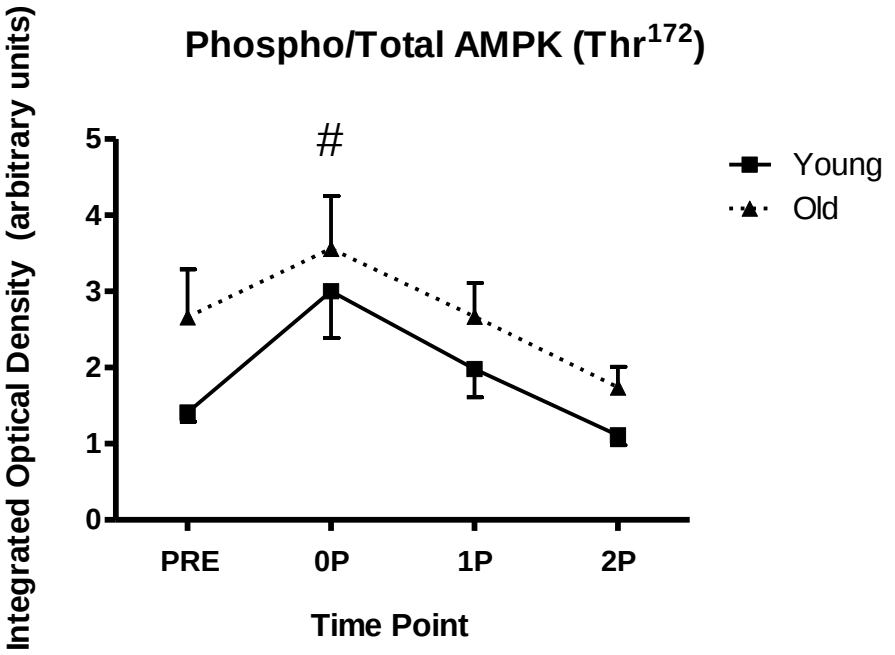

Figure 4.4. Mean ± SEM phospho/total AMPK ratio in young and old subjects and the representative western blots. PRE is the pre exercise muscle biopsy, 0P is the immediately post exercise muscle biopsy, 1P is the one hour post exercise muscle biopsy, and 2P is the two hour post exercise muscle biopsy. Phospho/total AMPK had a significant main effect of time point at 0P versus the PRE time point for both young and old groups combined. Significance is set at $p \leq 0.05$.

ACC

Acetyl CoA carboxylase (ACC), an in vivo indicator of AMPK activation (Winder et al., 1997), showed a significant main effect of time point in phosphorylation status at Ser^{79} immediately post exercise (0P) and at one hour post exercise (1P) (Figure 4.5) for both young and old groups combined. The same significant main effect of time point was seen in total ACC (decreased; Figure 4.6) and phospho/total ACC (increased; Figure 4.7) for both the 0P and 1P time points for both age groups combined. No differences existed between the age groups for phospho-ACC (Figure 4.5), total ACC (Figure 4.6) or phospho/total ACC (Figure 4.7).

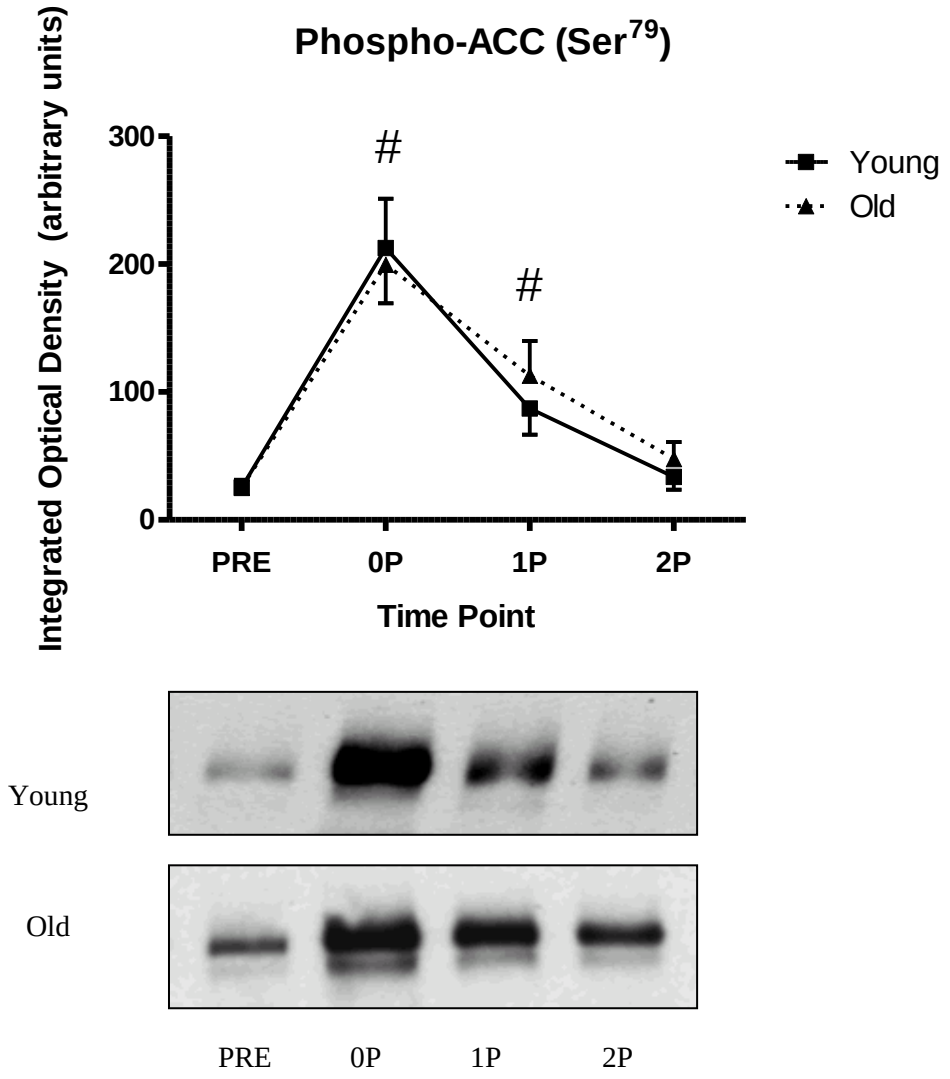

Figure 4.5. Mean ± SEM phospho-ACC at Ser^{79} in young and old subjects and the representative western blots. PRE is the pre exercise muscle biopsy, 0P is the immediately post exercise muscle biopsy, 1P is the one hour post exercise muscle biopsy, and 2P is the two hour post exercise muscle biopsy. Phospho-ACC had a significant main effect of time point at 0P and 1P versus the PRE time point for both young and old groups combined. Significance is set at $p \leq 0.05$.

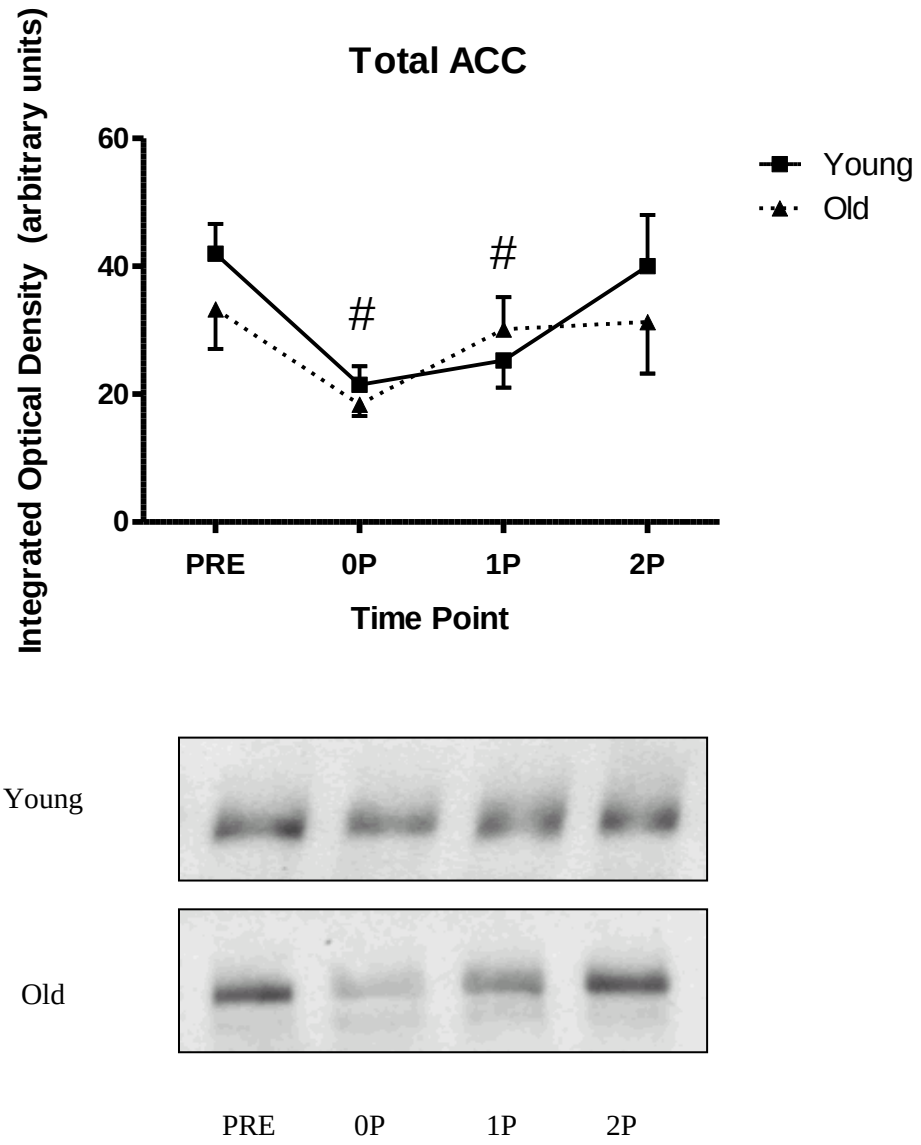

Figure 4.6. Mean ± SEM total ACC in young and old subjects and the representative western blots. PRE is the pre exercise muscle biopsy, 0P is the immediately post exercise muscle biopsy, 1P is the one hour post exercise muscle biopsy, and 2P is the two hour post exercise muscle biopsy. Total ACC had a significant main effect of time point at 0P and 1P versus the PRE time point for both young and old groups combined. Significance is set at $p \leq 0.05$.

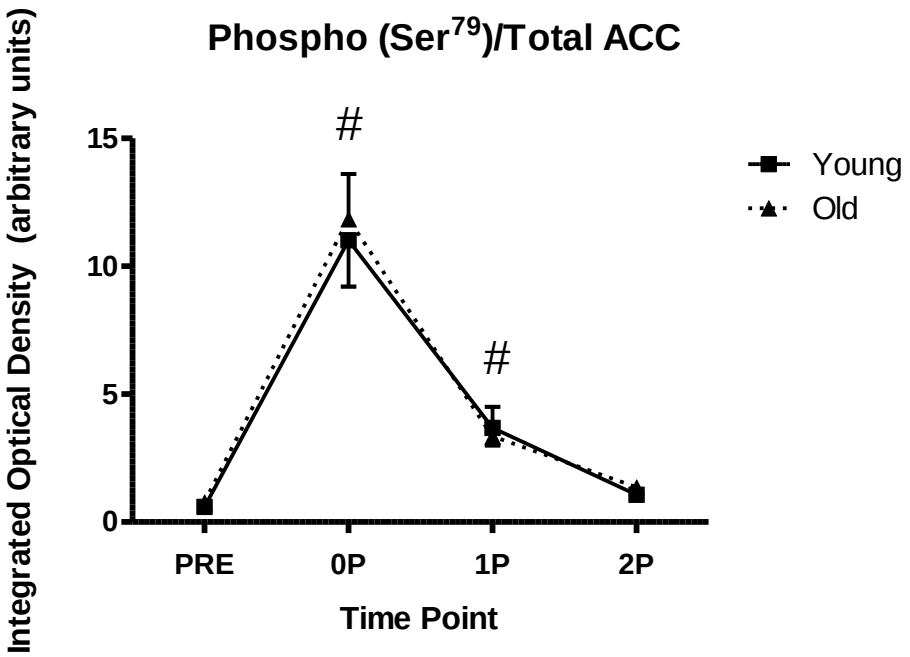

Figure 4.7. Mean ± SEM phospho/total ACC in young and old subjects. PRE is the pre exercise muscle biopsy, 0P is the immediately post exercise muscle biopsy, 1P is the one hour post exercise muscle biopsy, and 2P is the two hour post exercise muscle biopsy. Phospho/total ACC had a significant main effect of time point at 0P and 1P versus the PRE time point for both young and old groups combined. Significance is set at $p \leq 0.05$.

Phospho-ACC (Ser79) Correlations with Muscle Glycogen Content

The relationship between phospho-ACC and muscle glycogen content was examined at the PRE, 0P, 1P, and 2P time points. Under baseline conditions, or at the PRE exercise time point, no significant relationship exists between muscle glycogen content and the phosphorylation status of ACC at Ser^{79} (Figure 4.8). At the 0P and 1P time points, significant or near-significant correlations of -0.44 ($p=0.07$) and -0.64 ($p=0.006$) indicated a negative relationship between muscle glycogen content and phospho-ACC at those time points (Figures 4.9 and 4.10). This indicates that when muscle glycogen content is higher, the phosphorylation of ACC at Ser^{79} is lower one to two hours after resistance exercise. The relationship between phospho-ACC (Ser79) and muscle glycogen content is negative at all time points except the 2P time point, where it is non-significantly positive. The relationship between muscle glycogen content and phospho (Ser79)/ total ACC ratio demonstrated a similar pattern: $r = -0.43$, $p = 0.076$ at PRE; $r = -0.45$, $p = 0.06$ at 0P; $r = -0.69$, $p = 0.002$ at 1P; and $r = 0.02$ (N.S.) at 2P.

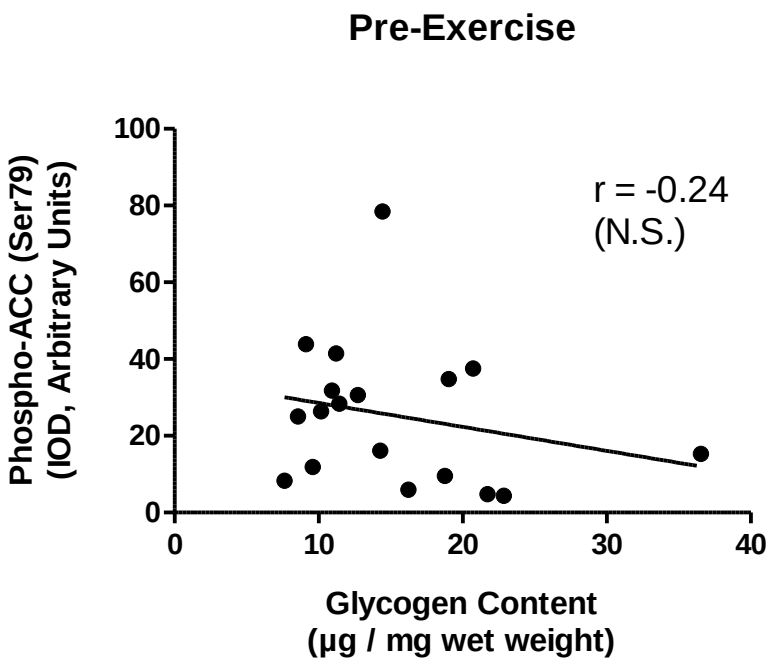

Figure 4.8. Relationship between glycogen content and phospho-ACC (Ser79) in the vastus lateralis muscles of young and old subjects prior to an acute bout of resistance exercise.

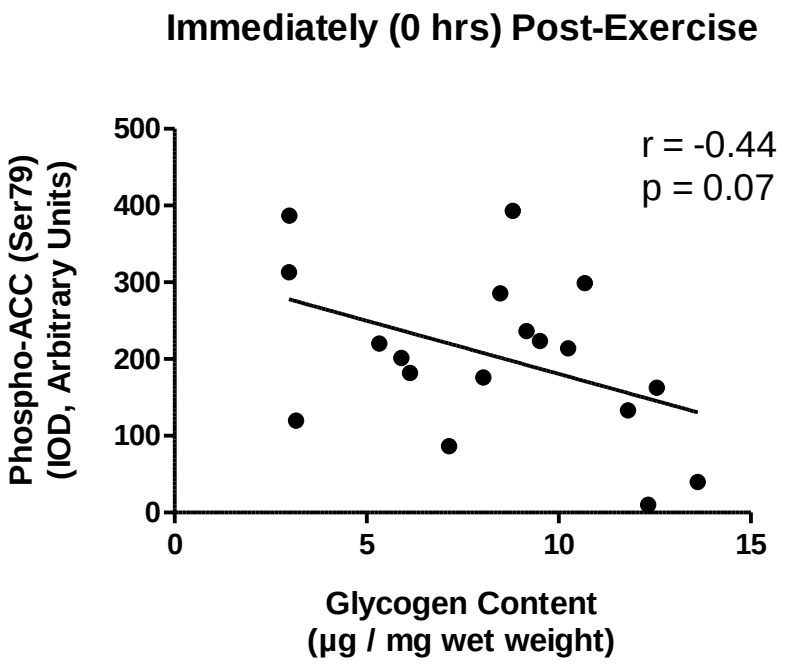

Figure 4.9. Relationship between glycogen content and phospho-ACC (Ser79) in the vastus lateralis muscles of young and old subjects immediately after an acute bout of resistance exercise.

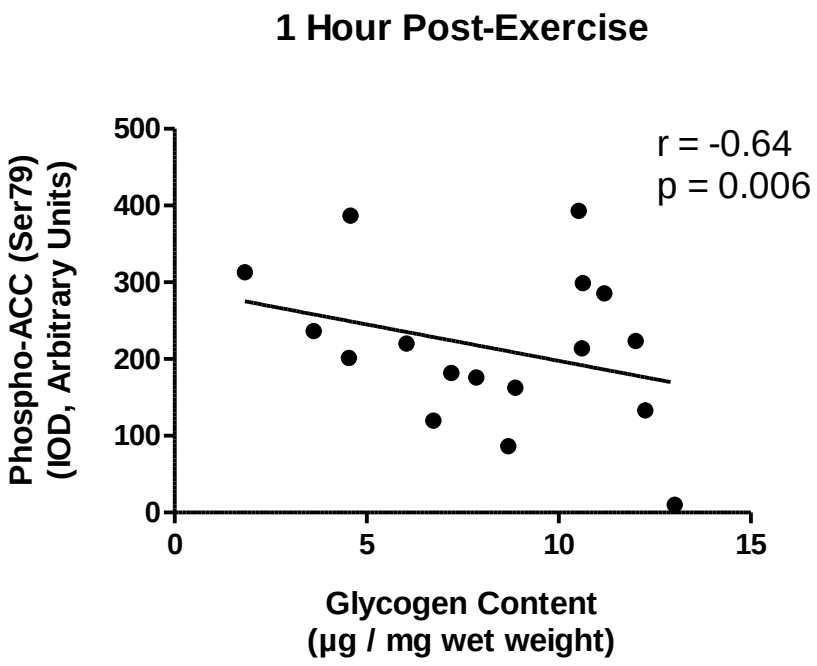

Figure 4.10. Relationship between glycogen content and phospho-ACC (Ser79) in the vastus lateralis muscles of young and old subjects 1 hour after an acute bout of resistance exercise.

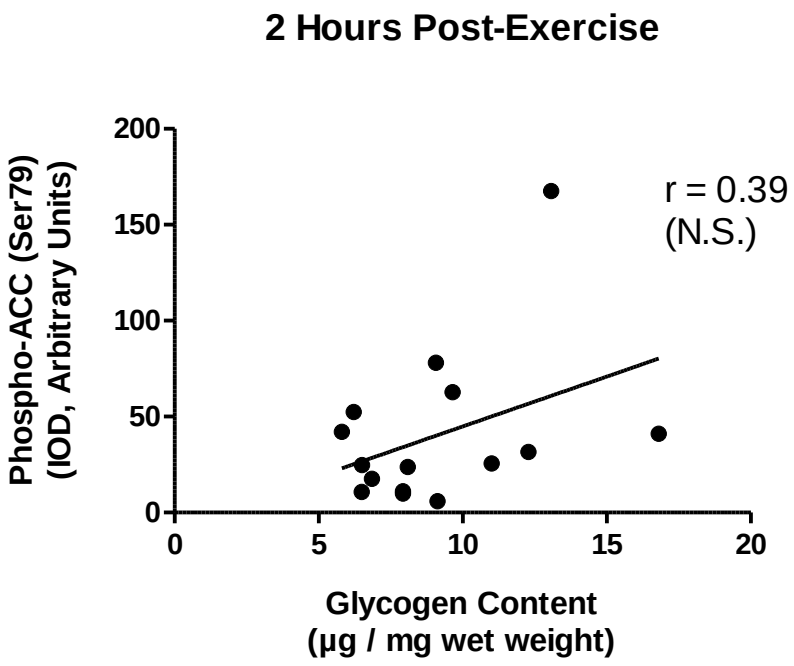

Figure 4.11. Relationship between glycogen content and phospho-ACC (Ser79) in the vastus lateralis muscles of young and old subjects 2 hours after an acute bout of resistance exercise.

eEF2

eEF2 phosphorylation at Thr^{56} was not significantly different between age groups, but a main effect of time point was significant at all post exercise time points compared to the PRE time point for both the young and old groups combined (Figure 4.12). Likewise, total eEF2 did not have significant differences between the age groups, but there also was a significant main effect of time point at all post exercise time points compared to pre exercise (Figure 4.13). Similar findings were seen with the ratio of phospho/total eEF2, exhibiting no significant differences between the age groups. There was, however, a significant main effect of all time points versus the PRE time point for phospho/total eEF2 (Figure 4.14).

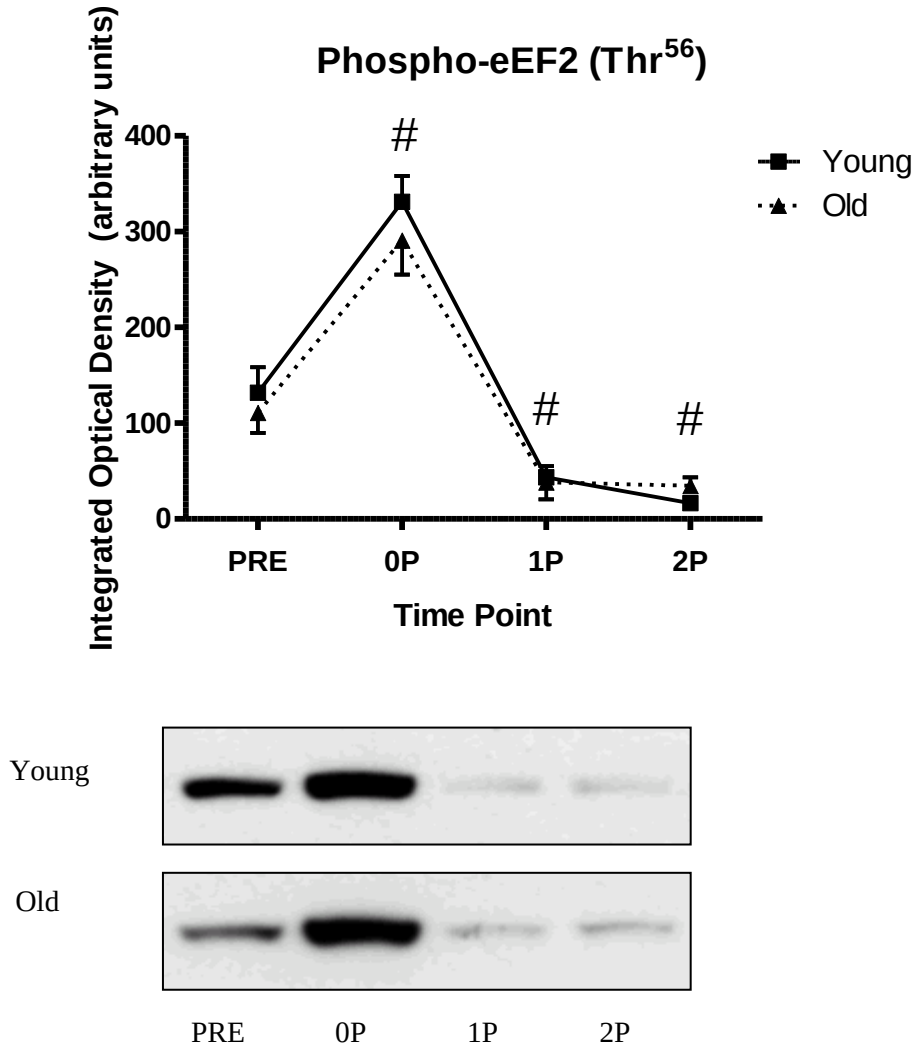

Figure 4.12. Mean ± SEM phospho-eEF2 (Thr56) in young and old subjects and the representative western blots. PRE is the pre exercise muscle biopsy, 0P is the immediately post exercise muscle biopsy, 1P is the one hour post exercise muscle biopsy, and 2P is the two hour post exercise muscle biopsy. Phospho-eEF2 had a significant main effect of time point at 0P,1P and 2P versus the PRE time point for both young and old groups combined. Significance is set at $p \leq 0.05$.

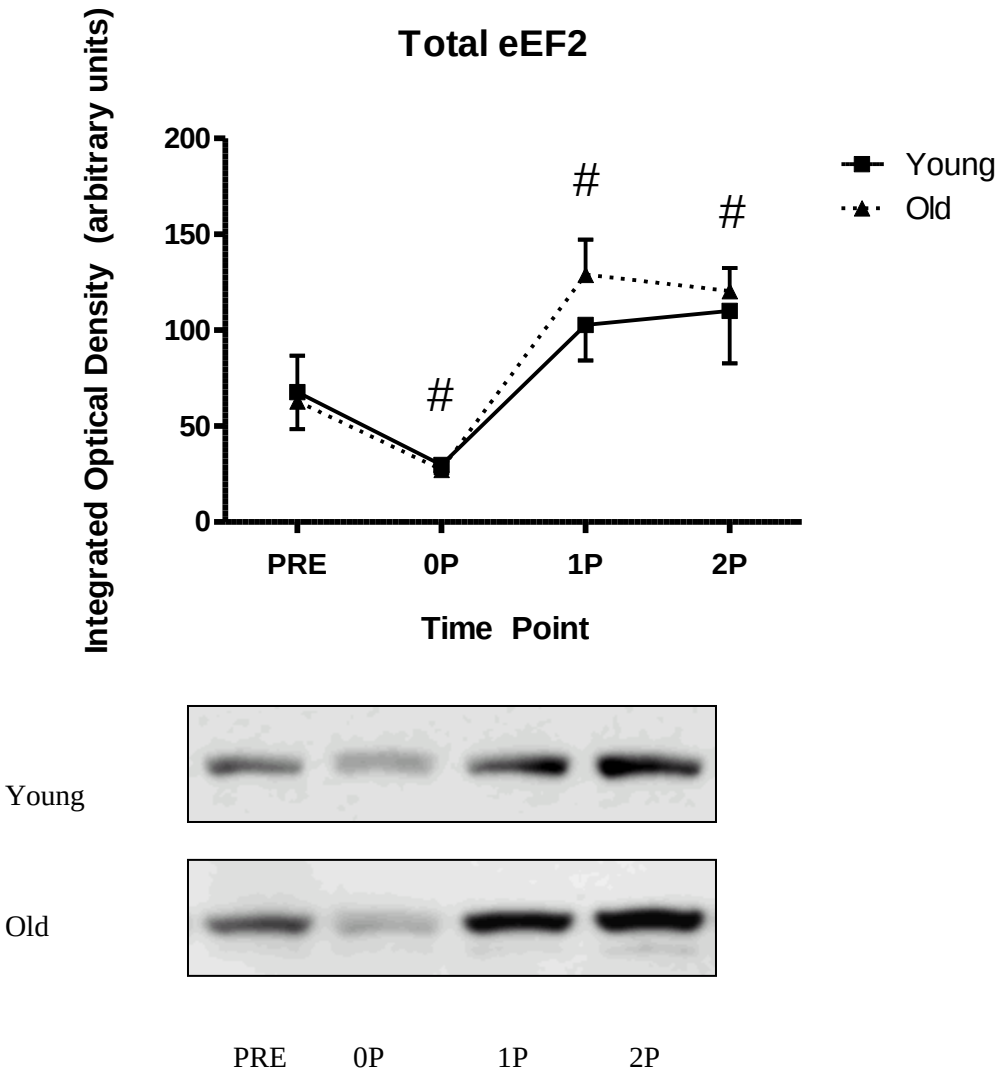

Figure 4.13. Mean ± SEM total eEF2 in young and old subjects and the representative western blots. PRE is the pre exercise muscle biopsy, 0P is the immediately post exercise muscle biopsy, 1P is the one hour post exercise muscle biopsy, and 2P is the two hour post exercise muscle biopsy. Total eEF2 had a significant main effect of time point at 0P, 1P and 2P versus the PRE time point for both young and old groups combined. Significance is set at $p \leq 0.05$.

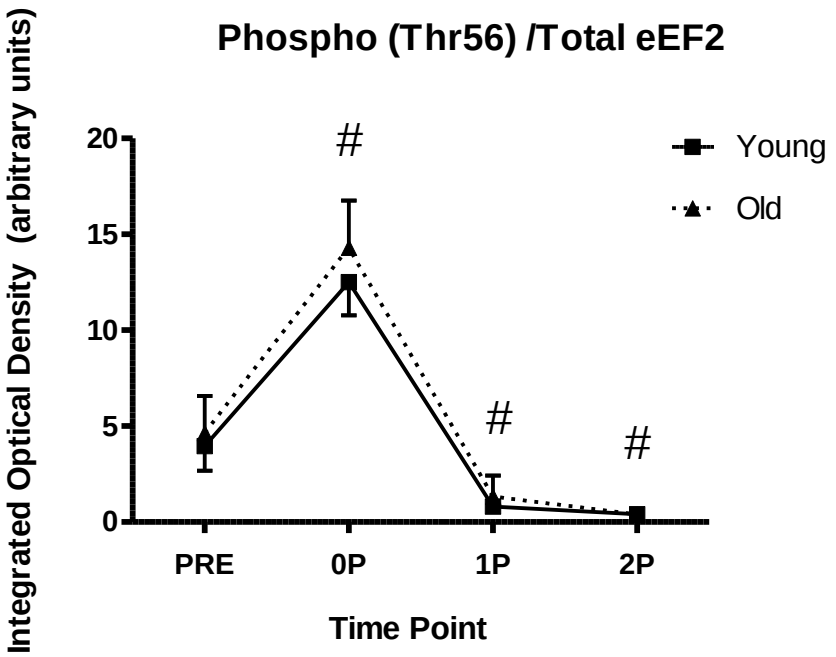

Figure 4.14. Mean ± SEM phospho/total eEF2 (Thr56) in young and old subjects. PRE is the pre exercise muscle biopsy, 0P is the immediately post exercise muscle biopsy, 1P is the one hour post exercise muscle biopsy, and 2P is the two hour post exercise muscle biopsy. Phospho/total eEF2 had a significant main effect of time point at 0P, 1P and 2P versus the PRE time point for both young and old groups combined. Significance is set at $p \leq 0.05$.

Phospho-ACC (Ser79) Correlations with Phospho-eEF2 (Thr56)

The relationship between phospho-ACC and phospho-eEF2 content was examined at the PRE, 0P, 1P, and 2P time points. Under baseline conditions, or at the PRE exercise time point, no significant relationship existed between the phosphorylation status of ACC at Ser^{79} and the phosphorylation status of eEF2 at Thr^{56} (Figure 4.15). At the 0P and 1P time points, significant or near-significant correlations of 0.49 ($p=0.048$) and 0.65 ($p=0.005$) indicated a positive relationship between phospho-ACC and phospho-eEF2 at those time points (Figures 4.16 and 4.17). This indicates that within at least the first hour after a bout of resistance exercise, the phosphorylation of ACC at Ser^{79} is correlated with an increase in phosphorylation of eEF2 (Thr^{56}) phosphorylation (an indication of less activity for eEF2 at those time points). The relationship between phospho-ACC (Ser79) and phospho-eEF2 (Thr^{56}) was lost by 2 hours post-exercise.

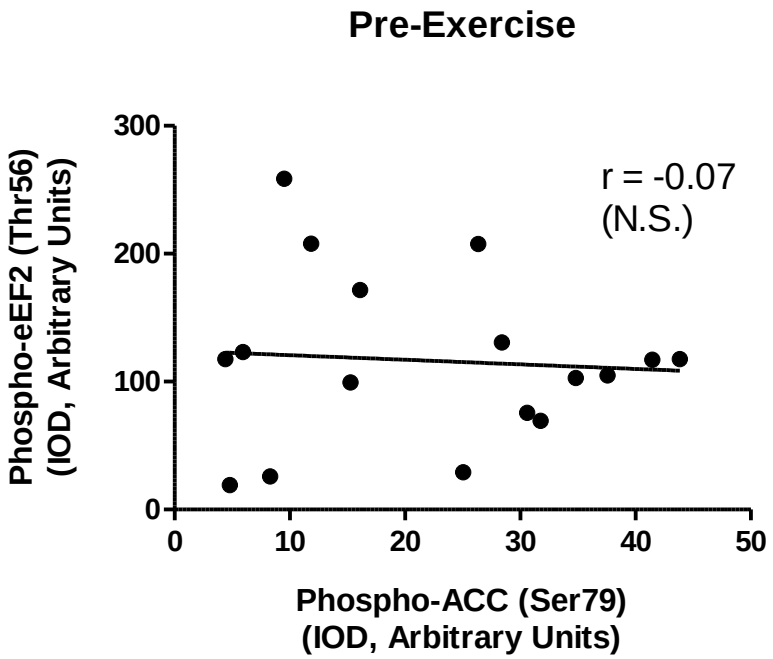

Figure 4.15. Relationship between phospho-ACC (Ser79) and phospho-eEF2 (Thr56) in the vastus lateralis muscles of young and old subjects prior to an acute bout of resistance exercise.

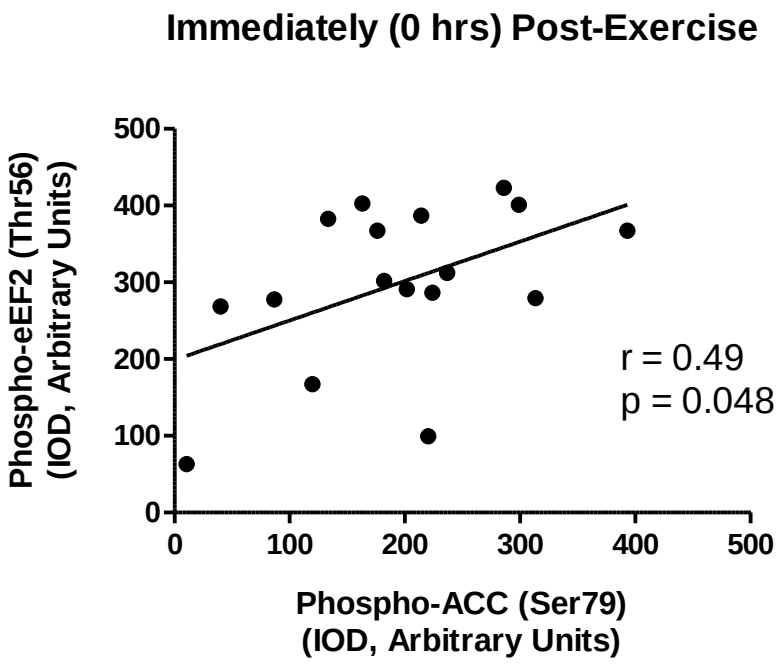

Figure 4.16. Relationship between phospho-ACC (Ser79) and phospho-eEF2 (Thr56) in the vastus lateralis muscles of young and old subjects immediately after an acute bout of resistance exercise.

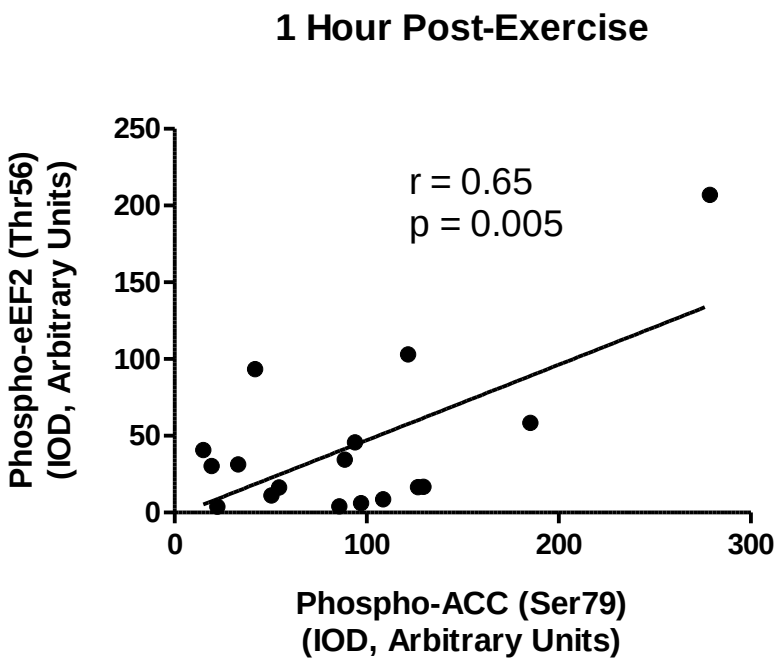

Figure 4.17. Relationship between phospho-ACC (Ser79) and phospho-eEF2 (Thr56) in the vastus lateralis muscles of young and old subjects 1 hour after an acute bout of resistance exercise.

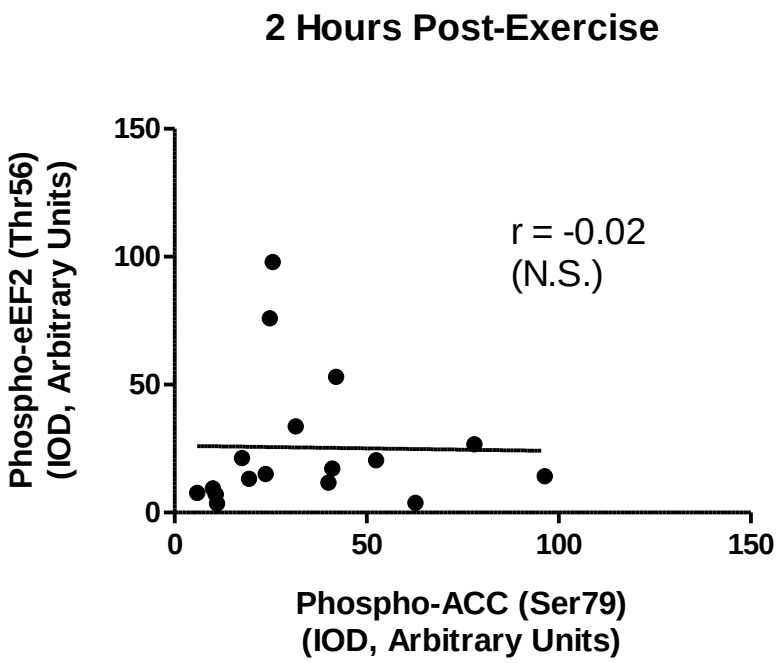

Figure 4.18. Relationship between phospho-ACC (Ser79) and phospho-eEF2 (Thr56) in the vastus lateralis muscles of young and old subjects 2 hours after an acute bout of resistance exercise.

CHAPTER V: DISCUSSION

The aim of this study was to examine the eEF2 phosphorylation response to resistance exercise and its relation to muscle glycogen content and AMPK activation in old versus young subjects. We hypothesized that inhibitory eEF2 Thr56 phosphorylation would be higher in response to acute resistance exercise in the skeletal muscles of older versus younger individuals. We further hypothesized that this higher eEF2 phosphorylation response would be related to higher AMPK activation, and that higher AMPK activation would be related to lower muscle glycogen content, in the skeletal muscles of older versus younger individuals. At rest, the old had significantly lower muscle glycogen levels compared to the young men and women. After the resistive leg extensions, both groups had a significant decrease in the level of muscle glycogen content, with no difference between age groups at the post-exercise time points. AMPK phosphorylation was significantly increased from the baseline measurement after the resistive leg extensions within the old group, but not in the young group. However, ACC phosphorylation (indicating in vivo AMPK activity) showed no difference between age groups, with a significant main effect increase for both groups after the acute bout of exercise up to one hour post-exercise. Likewise, there was a significant main effect of eEF2 phosphorylation after the acute bout of resistance exercise, but again, no significant differences were seen between the groups. AMPK activity (as assessed by phospho-ACC, which is a better indicator of AMPK activity) was negatively correlated with muscle glycogen content immediately and up to one hour after the exercise session, and eEF2 phosphorylation was positively correlated with AMPK activity during that same time period.

The primary findings of the current study could have larger implications that help understand the specific portion of the protein synthesis signaling pathway on which we

concentrated for this investigation. After a bout of resistance training, an increase in AMPK activity may be related to a decrease in post-exercise glycogen content in fasted individuals. Wojtaszewski et al. compared the effects of muscle glycogen content to AMPK activity after a bout of exercise and observed a similar relative decrease in glycogen content after an exercise session (Wojtaszewski et al., 2003). That same study indicated that while exercising during times of high muscle glycogen content AMPK activity was reduced, and the opposite was true for low muscle glycogen content (Wojtaszewski et al., 2003). Elevated AMPK phosphorylation as observed in aged rat fast-twitch muscle during overload is associated with a diminished response in various indicators of the protein synthesis pathway, specifically eEF2 phosphorylation (Thomson & Gordon, 2006). In this study, regardless of age, higher muscle glycogen content was associated with lower AMPK activity (as assessed by phospho-ACC content) at 0P and 1P, and this lower AMPK activity was associated with lower inhibitory phosphorylation of eEF2 at those same time points. These findings indicate the possibility that higher muscle glycogen content may result in lower AMPK activation and consequently lower inhibitory eEF2 phosphorylation in response to a resistance training session in the muscles of both younger and older individuals, thereby potentially enabling greater translation elongation, protein synthesis, and muscle growth regardless of age. Since muscle glycogen may play a role in AMPK activity after an acute bout of resistance training, the fact that glycogen content was similar between young and old at all post-exercise time points could explain why no differences were also seen in ACC or eEF2 phosphorylations between the age groups.

Previous research has indicated that AMPK activation in response to aerobic exercise was significantly lower under higher levels of muscle glycogen (Wojtaszewski et al., 2003). This indicates that AMPK may be in part regulated by the available fuel status in the muscle in the

form of glycogen. Similarly, the present study found a negative relationship between muscle glycogen content and AMPK activity, (as assessed by phospho-ACC level and phospho/total ACC ratio) immediately following resistance exercise and remaining for at least an hour after exercise. Thus, one potential contributor to increased AMPK activity in the hour following exercise may be the amount of glycogen present within the muscle. Nevertheless, while muscle glycogen content may play a role, it cannot be the only cause for the increase in AMPK activity in this investigation due to the fact that while glycogen content remains low 2 hours after exercise phospho-ACC content returns to baseline. In this accord, it is known that another (and primary) mechanism resulting in AMPK activation is the imbalance in AMP/ATP ratio (Winder & Hardie, 1999), which can increase in response to a session of resistance training (Hardie & Sakamoto, 2006). Consequently, AMPK phosphorylates (an activation mechanism) and activates intermediates that block protein synthesis in attempt to replenish ATP and conserve energy (Hardie & Sakamoto, 2006).

It has previously been observed that old rats have a significantly greater decreases in muscle glycogen levels when compared to their young counterparts (Cartee, 1994; Hopp, 1996). In contrast, the present study in humans noticed no significant differences in muscle glycogen content between the ages other than at baseline. The old have a significantly lower baseline measurement of muscle glycogen, but both groups decrease to amounts that are indistinguishable. Previous research in humans has also indicated a significant difference exists in AMPK phosphorylation between old and young men at various time points after an exercise bout (Drummond, Dreyer et al., 2008). Although the present study found that the older subjects tended to have higher levels of phospho-AMPK across all timepoints, this was not statistically significant. However, there was a significant increase from baseline in phospho-AMPK within

the old group immediately after an acute bout of resistance training, an effect not observed in the young group. Drummond and Dryer, (2008), utilized only men, while the current study used both women and men, although they later concluded that sex does not play a role in the responses of protein synthesis to an acute bout of exercise (Dreyer et al.). This is in correspondence with our findings, in that no significant differences were observed between the genders (data not shown). They also observed the phosphorylation of AMPK to be elevated at one and three hours after the resistance exercise bout (Drummond, Dreyer et al., 2008). Once again this differs from the present study in that the differences were most pronounced immediately after the exercise, with no significant differences at one or two hours post. It is unclear as to why the present study did not see similar results extending past the 1P time point. It can be speculated that the essential amino acid ingestion performed by subjects in the Drummond et al. study may have played a role in the signaling responses of AMPK and other variables in that study.

AMPK is directly responsible for the phosphorylation of ACC at Ser^{79} (Park et al., 2002), which is used as a marker of in vivo AMPK activity (Hawley et al., 1996). After inducing one week of overload conditions in rats, ACC and AMPK exhibit similar phosphorylation responses (Thomson & Gordon, 2005). However, this is not necessarily always the case. For example, AMP can enhance the activity of AMPK via allosteric binding in the absence of changes in AMPK phosphorylation (Winder & Hardie, 1999). A prime example of this is after a high frequency exercise stimulus and AICAR, an AMPK activator, the phosphorylation of ACC was higher compared to AMPK phosphorylation (Thomson et al., 2009). In the present study, phospho-AMPK was only increased at the 0P time point, and only in old; however, phosphorylation of ACC (Ser79) remained significantly elevated (main effect) at the 1P time

point for both age groups. Therefore, AMPK activity may not be able to be completely inferred by AMPK phosphorylation alone, and could be influenced by other factors, such as the allosteric influence of AMP. It must also be noted that other aspects of metabolism during the post-exercise recovery period (such as lactic acid, fat oxidation, etc.) may have influenced the variables being measured, but those metabolic aspects were not assessed in this investigation.

Dreyer et al. observed a significant decrease in the phosphorylation of eEF2, and thus theoretically more eEF2 activity, during the post exercise recovery period at both one and two hours compared to baseline in young human subjects (Dreyer et al.). The current study witnessed the same pattern in phosphorylation status of eEF2, and no differences with age. A previous study has also indicated no significant difference in eEF2 phosphorylation between the age groups after resistance exercise, but subjects ingested an EAA/CHO supplement after the exercise session in that study (Drummond, Dreyer et al., 2008). Thus, we postulated that eEF2 phosphoryaltion would differ with age if an EAA ingestion were not influencing mTOR signaling. However, this was not the case, as eEF2 phosphorylation responded similarly in both young and old subjects in the current study under normal (non-EAA) conditions. It is important to point out that this is the first study on human subjects that compared the effects of eEF2 in young and old individuals after an acute bout of resistance training, while under normal conditions. Previous studies have observed a significant difference in eEF2 phosphorylation status between young and old rats (Fick & Gordon, 2007; Thomson & Gordon, 2006), but such results did not translate to humans under the conditions of the current investigation.

While this study only examined the recovery period for two hours, Drummond et al. have indicated that this same elevated AMPK phosphorylation response in old (but not young) subjects can be seen up to three hours after an exercise bout (Drummond, Dreyer et al., 2008).

While the current investigation also observed an elevated AMPK phosphorylation response only in old subjects, AMPK activity was elevated immediately, and for at least one hour, after exercise in both young and old subjects. Similarly, research by Dreyer et al. indicated that AMPK activity was significantly increased immediately, and at one hour after a resistance exercise in young men and women, with the phosphorylation of eEF2 being significantly reduced at 1 and 2 hours post-exercise (Dreyer et al., 2006). The current study also observed a very similar suppression of eEF2 phosphorylation at those same time points. In both studies, the highest AMPK activity (immediately post-exercise) corresponded to an elevated eEF2 phosphorylation. In fact, AMPK activity was significantly correlated with eEF2 phosphorylation immediately and 1 hour post-exercise in the current study. Collectively, the above results indicate that AMPK may partially control eEF2 phosphorylation in response to resistance exercise regardless of age, especially immediately after (and perhaps during) exercise. There are multiple mechanisms by which AMPK activation may up-regulate eEF2 phsophorylation. One possibility is suppression of mTOR-p70s6k signaling, which would suppress inhibitory phosphorylation of eEF2 kinase (thus resulting in increased eEF2 phosphoryaltion) (Thomson & Gordon, 2006). Another is a direct effect of AMPK on eEF2 kinase (Fick & Gordon, 2007). Studies have indicated a difference in the activity in p70s6k with age status, old having lower activations (Fick & Gordon, 2007; Kumar et al., 2009; Thomson & Gordon, 2006). Since p70s6k is located upstream from eEF2, it theoretically can alter the response of eEF2 (Thr56). While the present study did not measure p70s6k, we initially would have anticipated finding a difference between ages with p70s6k activity. However, the present study found no age differences in eEF2 phosphorylation, therefore, either p70s6k did not affect eEF2

phosphorylation differently between age groups, or other compensatory mechanisms came into play.

The phosphorylation status of eEF2 is incredibly influential in the translation elongation segment of protein synthesis. The body decreases protein synthesis during the exercise bout and in turn increases it during the recovery period (Phillips et al., 1997). In the present study, the increase in total eEF2 coupled with decrease in phospho-eEF2 is evidence that the muscle is encouraging translation elongation, and thereby protein synthesis. The significant increase (main effect) of the phosphorylation of eEF2 at the 0P time point is indicative of less activity, and likewise, the significantly lower (main effect) levels observed during recovery indicate increased activity. Protein synthesis, being the energy expensive pathway that it is (Thomson & Gordon, 2005), could also account, in part, for the decrease in muscle glycogen content during the recovery period. Once again, because the glycogen levels do not change from 0P to 1P, while the P-eEF2 has a huge change to favor protein synthesis, it is likely that glycogen is not the only factor, and that the acute changes in AMP (and subsequent changes in AMPK activation) are also a prime contributor.

In the current study there were no differences between the dietary intakes of young and old subjects on any of the components. In other studies it has been shown that not only do older individuals consume fewer calories, but also fewer calories come from carbohydrates (Lieberman et al., 1989). It is possible that the discrepancy between the present and previous findings is due to individual variability in dietary log completion. However, another possible reason for the difference between these findings in dietary intake could be due to the fact that Lieberman et al. adjusted their dietary data for differences in body weight (Lieberman et al., 1989). Once the current study normalized for body weight, there still were no differences on any

of the dietary components between the age groups. We can further speculate that differences between the current study and the findings of Lieberman et al. could be attributed to various factors, including geographic location, time of year, financial burden of the individual, or time constraints influencing food choices.

There were significant differences in strength between the young and old subjects. The older subjects had significantly lower 10RM scores, both on and absolute basis as well as normalized to fat free mass. These findings are dissimilar to the findings of Drummond and Miyazaki, 2008, who that found no significant differences between strength between old and young subjects when compared to lean body mass (Drummond, Miyazaki et al., 2008). The difference between their findings and ours could be a result of the subjects that were selected for participation. The young subjects in the current study may not have been motivated to put forth a maximal effort. They also may not have participated with concern for the outcome of the study, but more for the financial compensation that resulted from participation, once again leading to decreased effort. While the total work volume in the current study was not significantly different, it is nevertheless reduced by 27% with age. The lack of statistical difference in total volume between older vs. younger individuals may be due to higher percentage of slow twitch muscle fibers in age (Hortobagyi et al., 1995), potentially leading to more endurance and thus higher repetitions.

The initial aim of this study intended to explore potential differences in AMPK activity, eEF2 phosphorylation and muscle glycogen content between age groups, and the only variable for which was found a significant difference was muscle glycogen content at baseline. This lack of differences in any the previously mentioned measures may be attributed to numerous causes. For example, the present study recruited healthy older individuals whom were relatively free

from medications at an advanced age. This sample of individuals may be healthier and therefore not representative of the normal aged population. Secondly, the young subjects recruited were all inactive individuals and could have been unfamiliar with exercise and therefore might have not given their true maximum effort. These subjects may have felt exercise to be uncomfortable and therefore exercised below that of which they were capable. Lastly, all subjects were fasted, so the difference between the young and old subjects may not be pronounced in a fasted state (Li & Goldberg, 1976).

Some limitations of this study should be addressed for future studies. First, the subjects underwent a brief dietary counseling and were provided a dietary guide to help them make appropriate high carbohydrate, low fat choices. After analyzing the diets there was no significant difference between diets of the "normal" and "high carbohydrate" individuals. It is possible that while each individual indicated that they understood the directions of the high carbohydrate diet, they may not have been sure of how to change their diet so drastically. If the individuals would have been provided the same food, we could have eliminated subject error and determined whether a three day high carbohydrate diet would in fact decrease the activation of AMPK in the elderly. Second, as previously mentioned, the subjects were requested to fill out a diary of everything they consumed for the three days prior to reporting to the lab. This could have had an effect on what and how much the individuals ate. The older individuals could have overcompensated in their diet to attempt to fulfill the requirements of the study.

While the present study did not successfully accomplish a high carbohydrate diet, other methods of increasing muscle glycogen content via diet in conjunction with resistance training would be beneficial to investigate. Manipulation of dietary intake may be a cost-effective

solution to combat the negative consequences of age associated muscle atrophy and diminished overload-induced hypertrophy.

In summary, we show a relationship between lower muscle glycogen content and elevated AMPK activity immediately and for at least one hour following resistance exercise in fasted young and old men and women. Moreover, higher AMPK activity was associated with higher eEF2 phosphorylation (and presumably inhibited eEF2 activity) during that same post-exercise time period. Although it has also been previously observed that elevated AMPK phosphorylation in aged muscle is associated with a diminished eEF2 response (Thomson & Gordon, 2006), we saw no difference in the responses of AMPK activity or eEF2 phosphorylaton between age groups in the current investigation. Nevertheless, regardless of age, the current results indicate that lower glycogen content could play a role in enhancing AMPK activation, which in turn could enhance post-exercise eEF2 phosphorylation (and thus suppress eEF2 activity) in fasted individuals regardless of age. Additional research should be conducted to determine whether a state of high glycogen content is capable of eliciting lower AMPK and ACC phosphorylation levels. It would also be beneficial to include older individuals with varying health statuses to determine whether a lower functioning individual, with a more accelerated level of sarcopenia, can elicit greater differences between the ages. Also, more studies should be conducted to determine the correlational significance between protein synthesis intermediaries (such as mTOR, p70S6k and eEF2) and muscle glycogen content.

Practical Application

Potentially, individuals with advanced stages of sarcopenia could benefit merely from the ingestion of a diet higher in carbohydrates and kcals, or other strategies that may enhance muscle

glycogen content. Not only would this combat the age-related deterioration in muscle, but also could prevent the loss of mobility, due to lack of strength, in later years. It is possible that keeping muscle glycogen at a high level, independent of age, could help promote the activation of the protein synthesis pathway and muscular strength.

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APPENDIX A: ECU INSTITUTIONAL REVIEW BOARD APPROVAL DOCUMENT

UMCIRB #:

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JAN 20 2010

UMCIRB

UNIVERSITY AND MEDICAL CENTER INSTITUTIONAL REVIEW BOARD
REVISION FORM

UMCIRB #: 08-0706 **Date this form was completed:** 1/20/10

Title of research: Age-related Changes in Skeletal Muscle Signaling after Acute Heavy Resistance Exercise

Principal Investigator: Scott E. Gordon, Ph.D.

Sponsor: Human Performance Lab

Fund number for IRB fee collection (applies to all for-profit, private industry or pharmaceutical company sponsored project revisions requiring review by the convened UMCIRB committee):

Fund	Organization	Account	Program	Activity (optional)
		73059		

Version of the most currently approved protocol: 11/17/08
Version of the most currently approved consent document: 12/2/08

CHECK ALL INSTITUTIONS OR SITES WHERE THIS RESEARCH STUDY WILL BE CONDUCTED:

- ☒ East Carolina University
- ☐ Pitt County Memorial Hospital, Inc
- ☐ Heritage Hospital
- ☐ Other
- ☐ Beaufort County Hospital
- ☐ Carteret General Hospital
- ☐ Boice-Willis Clinic

The following items are being submitted for review and approval:

- ☒ Protocol: version or date 1/20/10
- ☒ Consent: version or date 1/20/10
- ☐ Additional material: version or date

Complete the following:

1. Level of IRB review required by sponsor: ☐ full ☒ expedited
2. Revision effects on risk analysis: ☐ increased ☒ no change ☐ decreased
3. Provide an explanation if there has been a greater than 60 day delay in the submission of this revision to the UMCIRB.
4. Does this revision add any procedures, tests or medications? ☐ yes ☒ no If yes, describe the additional information:
5. Have participants been locally enrolled in this research study? ☒ yes ☐ no
6. Will the revision require previously enrolled participants to sign a new consent document? ☐ yes ☒ no

Briefly describe and provide a rationale for this revision

1. One of the testing locations has been changed from Brody School of Medicine Room 3S-08 to East Carolina Heart Institute Room 2379. All appropriate equipment and supplies will be moved to the new location, and all procedures and medical support will remain exactly the same. There is no increased risk. The forms have been changed to reflect the new location.
2. I would like to add LaDonna Lynn Maddy to the research team (IRB training completion date approximately 1/12/10).

Principal Investigator Signature Scott E. Gordon **Print** Scott E. Gordon **Date** 1/20/10

Box for Office Use Only

The above revision has been reviewed by:
☐ Full committee review on _____ ☑ Expedited review on 1/21/2010

The following action has been taken:
☑ Approval for period of 1/21/2010 to 12/5/2010
☑ Approval by expedited review according to category 45CFR46.110
☐ See separate correspondence for further required action.

Signature | Print | Date

APPENDIX B: INFORMED CONSENT DOCUMENT

Principal Investigator: Scott E. Gordon, Ph.D.
Institution: Human Performance Laboratory
Address: 363 Ward Sports Medicine Building
Telephone #: (252) 737-2879

This consent form may contain words that I do not understand. I should ask the study doctor or the study coordinator to explain any words or information in this consent form that I do not understand.

INTRODUCTION

I have been asked to participate in a research study being conducted by Scott E. Gordon, Ph.D. and his associates. This research is designed to determine the response of several molecules in muscle after one resistance exercise (strength training) session, and if this response changes with age. All of these molecules and cells are involved with muscle hypertrophy (growth).

We will study 10 younger (18-45 years) and 20 older inactive adults (46-85 years), at rest and during and after a resistance exercise session for the legs. Inactive is defined as not having participated in any regular form of exercise for the past six months (less than 30 minutes per day, one day per week). Studies will take place in the Human Performance Laboratory and Brody School of Medicine at East Carolina University.

PLAN AND PROCEDURES

Prior to any testing, I will report to the Human Performance Laboratory Fitness, Instruction, Testing, and Training (FITT) Building to first be allowed to read and sign this Informed Consent for research as then fill out a medical history questionnaire and 3-day food record. I will be allowed to complete this process on the day of my first visit. On this day, I will then undergo determination of my height and body weight, and percent body fat. I will also undergo strength testing and familiarization with the resistance exercise test. One to two weeks later, I will report to the East Carolina Heart Institute Room 2379 in a fasted state for the resistance exercise testing session, four blood samples from a forearm vein, and 4 biopsies of the thigh muscles.

The following section is an outline of the experimental visits and the procedures to be accomplished on each visit. Note that more detailed descriptions of each procedure immediately follow this section. There will be 2 visits for a total of approximately 5 hours of total participation time spread over approximately 2 weeks:

First Visit (Human Performance Lab FITT Building) (1.5 hours):

1.) Thorough interview in person for informed consent, health history questionnaire, and 3-day food record.
2.) Determination of height, weight, and skinfold thickness (fat pinch) for percent body fat.
3.) Determination of the maximum weight I can lift 10 times (10 repetition maximum, also called a 10 RM) for the seated leg extension exercise.

4.) Counseling on food choices for those individuals undergoing the high carbohydrate diet, and instructions for keeping dietary logs.

Second Visit (East Carolina Heart Institute Room 2379) (7-14 days after initial visit) (3.5 hours):

1.) I will report to the East Carolina Heart Institute Room 2379, in the morning after an overnight fast (not having eaten after midnight the night before).
2.) Four small blood samples will be obtained during this visit from a forearm vein (before, and after exercise).
3.) Four thigh muscle biopsies (tissue samples) will be performed during this session, two on each leg.
4.) A resistance exercise (strength training leg extension) bout of approximately 15 minutes focused on the thigh muscle group will be performed. The bout will consist of 6 sets of leg extension exercise.

Detailed Description of the Procedures to be Used During this Study:

- Body composition screening. My height and weight will be measured on my first visit. My body fat will be estimated by measuring skinfold thicknesses (fat pinch) with a skinfold caliper at four sites: biceps, triceps, back, and waist. I may feel a slight pinch or squeeze from the caliper at the skinfold sites, but no known risks are associated with this procedure.
- Strength testing. An exercise test to determine my strength levels and familiarize me with the resistance exercise protocol will be performed during my first visit (after the informed consent). As part of my familiarization and subject characterization, I will be assessed for maximal strength by 10-repetition maximum (10-RM) testing. This will entail determining the maximum amount of weight that I can lift in ten repetitions for the leg extension exercise. This procedure will consist of me initially lifting lighter weights and progressing to the heaviest weight that I can lift. An adequate amount of rest will be provided between repetition attempts. All exercises will be performed on Cybex weight machines. This session will also serve as a familiarization session to make me comfortable with the resistance exercise to be performed during the experimental session. I will be examined for proper exercise technique during this session and instructions or modifications will be provided if necessary. During this session, my tolerance for the heavy resistance exercise protocol will also be assessed.
- High Carbohydrate Diet. If I am one of the older subjects that is randomly selected to do the high carbohydrate diet, I will be instructed to eat a diet high in carbohydrates for the 3 days prior to my second visit to the laboratory. I will be counseled on food choices and given food guidelines to assist with dietary choices during the initial visit. The goal is for me to eat approximately 65-70% of my total calories from carbohydrates while attempting to decrease my calories from fat accordingly (protein intake will remain unaltered). Some examples of high CHO food choices that are low fat but not low in protein are most vegetables, very lean meats, skim (non-fat) milk, etc. Dr. Kimberly B. Heidal, PhD, MHS, RD, LDN, from the ECU Dept. of Nutrition is a team member for this study and has provided guidelines and dietary instructions to help accomplish this goal. There is no anticipated risk to me while I undergo this level of carbohydrate consumption for 3 days.

- Leg resistance exercise workout. During my second visit to the laboratory, I will perform a 15-minute resistance exercise bout focused on the quadriceps (thigh) muscle group. The bout will consist of 6 sets of leg extension exercise. The first 3 sets will be warm-up sets performed at 50% (8 repetitions), 70% (6 repetitions), and 90% (4 repetitions) of my previously determined 10-RM weight. The fourth through sixth sets will be performed at 100% of the 10 RM weight and will be performed until I am no longer able to perform them on my own (approximately 10 repetitions). I will rest for 90 seconds between all sets
- Fasting blood draws. I will not have anything to eat 12 hours prior to my second visit to the lab so that blood can be drawn from my forearm vein by a needle. During the second visit to the lab, blood will be drawn before, and after the resistance exercise workout described above. Four total blood samples of 5 milliliters each will be obtained during this study. The total amount of blood obtained will be 20 milliliters, which is approximately 1/25 of a pint.
- Muscle Biopsies. I will undergo four muscle biopsies (tissue samples) to determine the levels of several molecules in muscle after one resistance exercise (strength training) bout. These biopsies will be obtained immediately before, immediately after, and 1 and 2 hours after the resistance exercise bout in visit # 2. For this procedure, I will have a small amount of anesthesia (3 cc of 1% Lidocaine) injected in a ½ inch area under the skin of my thigh. A small (1/4 inch) incision will then be made through the skin, fat and fibrous tissue that lies over the muscle. A biopsy needle (about ½ the width of a pencil) will then be inserted ½ to 1 inch into the muscle. A small piece of muscle (½ the size of an eraser on the end of a pencil) will then be clipped out with the biopsy needle. The needle will be withdrawn and the muscle sample immediately preserved by freezing. Dr. Robert Hickner, Ph.D. or Dr. Timothy Gavin, Ph.D. will perform the muscle biopsies. These investigators have performed a total of over 500 muscle biopsies.

POTENTIAL RISKS AND DISCOMFORTS

There are certain risks and discomforts that may be associated with this research, including those listed below.

- The general performance of muscular exercise and physical effort can entail the potential hazards of injury from overexertion and/or accident. The possibility of cardiopulmonary (heart and lung) overexertion is slight. It will be minimized by screening, selection, and monitoring procedures which are designed to anticipate and exclude the rare individual for whom exercise might be harmful. It is questionable whether it is possible to overexert the heart by voluntary physical effort unless there is some underlying disease. Nevertheless, there are a number of disorders, some of which can readily escape clinical detection, where strenuous exercise may be potentially hazardous or may cause disability. Some of these, such as aneurysms (blood vessel ruptures) in the brain, solitary pulmonary cysts (small sacs of fluid in the lung), or alveolar blebs (small lung lesions), are rare and not readily diagnosed in the absence of symptoms. For these disorders, a history of tolerance to prior physical effort must suffice. For other, more common conditions such as ischemic heart disease (low blood and oxygen flow to the heart), several risk factors can be identified through the preliminary medical history and physician screening process.

- The risks specifically associated with resistance exercise are very low, and this study will be planned to avoid injury to the musculoskeletal (muscle and bone) system. Possible risks include the possibility of strains or pulls of the involved muscles, delayed muscle soreness 24 to 48 hours after exercise, muscle spasms (cramping), and, in extremely rare instances, muscle tears. Such risks are very low. Dizziness and fainting may also occur infrequently. I understand that every effort will be made by the researchers to make this investigation safe for my participation through proper instruction of the techniques and proper warm-up prior to exercise and testing. Furthermore, risks will be reduced by close supervision by experienced personnel to ensure that I utilize proper form.
- The total amount of blood drawn (1/25 of a pint) is negligible. There is an extremely small risk of local hematoma (bruising) or infection associated with insertion of venipuncture needles. In obtaining blood samples from a vein with a needle, the risks to me are of local discomfort, syncope (faintness), and hematomas (bruising). Thrombosis (blood clot in the vein), embolism (a blood clot that has come loose and may lodge itself in an artery), and infections are potential risks but are of very rare occurrence. Risks will be reduced or eliminated by having investigators who are trained and proficient in phlebotomy (puncturing veins with needles) use aseptic techniques. Furthermore, I will be in a seated position while blood is being obtained. All blood samples will be drawn in the laboratory under aseptic conditions with biohazard protection for the investigators and myself.
- Robert Hickner, Ph.D. or Dr. Timothy Gavin, Ph.D. will perform all biopsies, and Dr. Walter Pofahl, M.D. will provide medical coverage for biopsies performed in this investigation. There is a small risk of hematoma (bruising) or infection around the biopsy site, as well as muscle cramping, mild muscle tenderness and occasional bruising. The risk will be minimized by using sterile procedures and applying pressure to the biopsy site for 10 minutes, or until bleeding has stopped if longer than 10 minutes. A steri-strip (bandage) will be applied over the incision and will remain in place for at least 4 days to close the incision during healing. A pressure wrap will be placed around the biopsied thigh and will remain in place for 8 hours following the biopsy. There is an extremely remote risk of allergic reaction to the Lidocaine anesthesia. This risk will be minimized by using subject's who have had prior exposure to Lidocaine or Novocaine anesthesia. This precaution should eliminate this risk.
- The procedures and circumstances encompassed by this protocol provide for a high degree of safety. Every attempt will be made by the investigators to minimize any risks of this study to me. This includes familiarization, technique instruction and practice, supervision by experienced personnel, screening, and individualized testing and monitoring. The investigators will employ a close interaction with the physician in their clinical unit during this study. My safety will be enhanced in this study with individualized supervision during all laboratory visits. I will be asked to immediately alert a member of the research team if I have any injury or health problem. These factors should dramatically contribute to a reduction, if not an elimination, of any potential risks associated with this study.
- There is the potential risk that the results, especially if unfavorable or difficult to understand, may lead to my anxiety. However, I understand that the investigators are available to answer any of my questions or concerns regarding such matters, even after termination of the study.

- **To my knowledge, I am not allergic to "caine-type" anesthetics. For example, I have not had an allergic reaction to an injection at the dentist's office. To my knowledge, I do not possess any condition which would result in excessive bleeding and I do not have known heart disease, i.e., had a heart attack.**
- I am aware that there are unforeseen risks involved with this and all research studies.

POTENTIAL BENEFITS

There are potential benefits to society. The results of this study will help to determine the response of several muscle growth-related molecules and cell types in muscle after one resistance exercise (strength training) session, and if this response changes with age. The benefits of this study far outweigh the risks.
There are potential benefits to subjects. I will gain information about my blood sugar (glucose) and insulin levels, which may be indicators of health status due to the importance of blood glucose regulation. These blood glucose and insulin values will be available to me at any time if I request them. Furthermore, if my blood glucose and insulin values fall outside of the normal clinical range, I will be contacted by the investigators and advised to consult my personal physician. I will obtain information about my percent body fat and body mass index (BMI, or weight/height squared), which is also an important indicator of risk for metabolic diseases such as diabetes or heart disease. I will also gain information on my muscle fiber type (slow-twitch or fast-twitch), which is important component of athletic ability characteristics.

I will be paid a total of $200.00 compensation upon completion of the entire study.

SUBJECT PRIVACY AND CONFIDENTIALITY OF RECORDS

Only the investigators associated with this study will have access to the data obtained. The identity of the subjects will be protected by numeric coding. The data will be stored in the office of the Principal Investigator, or in a locked storage room. No identifying information will be released.

TERMINATION OF PARTICIPATION

My participation in this research study may be terminated without my consent if the investigators believe that these procedures will pose unnecessary risk to myself. I may also be terminated from participation if I do not adhere to the study protocol.

COST AND COMPENSATION

I will be paid $50.00 for my time and inconvenience for each muscle biopsy for a maximum of $200 for completion of the entire study. There are no costs to me for participation in this study.

The policy of East Carolina University does not provide for compensation or medical treatment for subjects because of physical or other injury resulting from this research activity. However, every effort will be made to make the facilities of the School of Medicine available for treatment in the event of such physical injury.

VOLUNTARY PARTICIPATION

I understand that my participation in this study is voluntary. Refusal to participate will involve no penalty or loss of benefits to which I am otherwise entitled. Furthermore, I may stop participating at any time I choose without penalty, loss of benefits, or without jeopardizing my continuing medical care at this institution.

RESEARCH PARTICIPANT AUTHORIZATION TO USE AND DISCLOSE PROTECTED HEALTH INFORMATION

The purpose of the information to be gathered for this research study is to better understand the response of several molecules in muscle after one resistance exercise (strength training) session, and if this response changes with age. The individuals who will use or disclose my identifiable health information for research purposes include Dr. Scott Gordon, Dr. Timothy Gavin, Dr. Robert Hickner, Dr. Walter Pofahl, and Mr. Bradley Harper. Individuals who will receive my identifiable health information for research purposes also include Dr. Scott Gordon, Dr. Timothy Gavin, Dr. Robert Hickner, Dr. Walter Pofahl, and Mr. Bradley Harper. The type of information accessed for this research study includes 1) general medical history (including family health history, medications, nutrition, physical activity levels and body weight history), 2) body composition information, blood levels of insulin, glucose, and other compounds related to muscle hypertrophy and metabolism, and 3) muscle fiber type percentage as well as growth-related molecules in my thigh muscle. The information will be used and disclosed in such a way as to protect my identity as much as possible; however, confidentiality cannot be absolutely guaranteed. Someone receiving information collected under this Authorization could potentially re-disclose it, and therefore it would no longer be protected under the HIPAA privacy rules (federal rules that govern the use and disclosure of my health information). There is not an expiration date for this Authorization.

I may not participate in this study if I do not sign this Authorization form. I may revoke (withdraw) this Authorization by submitting a request in writing to Dr. Scott Gordon. However, the research team will be able to use any and all of the information collected prior to my request to withdraw my Authorization.

To authorize the use and disclosure of my health information for this study in the way that has been described in this form, I must sign below and date when I signed this form. A signed copy of this Authorization will be given to me for my records.

_____

Participant's Name (print) Signature Date

_____

Authorized Representative Name (print)-----Relationship Signature Date

_____

Person Obtaining Authorization Signature Date

If I have questions related to the sharing of information, I am advised to call Scott Gordon at 252-737-2879. I may also telephone the University and Medical Center Institutional Review

Board at 252-744-2914. In addition, if I have concerns about confidentiality and privacy rights, I may phone the Privacy Officer at Pitt County Memorial Hospital at 252-847-6545 or at East Carolina University at 252-744-2030.

PERSONS TO CONTACT WITH QUESTIONS

The investigators will be available to answer any questions concerning this research, now or in the future. I may contact the primary investigators Scott E. Gordon, Ph.D. at 252-737-2879 (weekdays) or 252-321-7655 (nights and weekends). Also, if questions arise about my rights as a research subject, I may contact the Chairman of the University and Medical Center Institutional Review Board at phone number 252-744-2914 (weekdays).

CONSENT TO PARTICIPATE

I certify that I have read all of the above, asked questions and received answers concerning areas I did not understand, and have received satisfactory answers to these questions. I willingly give my consent for participation in this research study. (A copy of this consent form will be given to the person signing as the subject or as the subject's authorized representative.)

_____

Participant's Name (Print)

_____

Signature of Participant Date Time

WITNESS: I confirm that the contents of this consent document were orally presented, the participant or guardian indicates all questions have been answered to his or her satisfaction, and the participant or guardian has signed the document.

_____

Witness's Name (Print)

_____

Signature of Witness Date

PERSON ADMINISTERING CONSENT: I have conducted the consent process and orally reviewed the contents of the consent document. I believe the participant understands the research.

_____

Person Obtaining Consent (Print)

_____

Signature of Person Obtaining Consent Date

_____

Principal Investigator's Name (Print)

_____

Signature of Principal Investigator Date

FUTURE TESTING OF BLOOD/MUSCLE SAMPLES

Upon termination of this study, the blood and muscle samples collected for this study will be stored for up to 7 years to research scientific questions specifically related to age-related changes in molecules regulating muscle mass in response to resistance exercise. I understand that I have the right to decline consent for this storage beyond termination of the present study, and that this declination of consent would not exclude me from participation in the present study. I will continue to be the owner of the samples and retain the right to have the sample material destroyed at any time during this study by contacting the study principal investigator Scott Gordon, Ph.D. at 252-737-2879. During this study, the samples will be stored with number identifiers only; however, the number identifier will be linked to a specific name and will be kept on file in the possession of the principal investigator. The linked file will be stored password protected on the Principal Investigator's computer with CD backup. No other individuals will have access to these identifying materials unless the principal investigator is required by law to provide such identifying information. Data will not be publicly available and participants will not be identified or linked to the samples in publication. If a commercial product is developed from this research project, I will not profit financially from such a product. Furthermore, there are no plans for me to profit financially from such a product.

CONSENT TO PARTICIPATE IN FUTURE TESTING OF BLOOD/MUSCLE SAMPLES

I certify that I have read all of the above, asked questions and received answers concerning areas I did not understand, and have received satisfactory answers to these questions. I willingly give my consent for participation in this research study. (A copy of this consent form will be given to the person signing as the subject or as the subject's authorized representative.)

_____

Participant's Name (Print)

_____

Signature of Participant Date Time

WITNESS: I confirm that the contents of this consent document were orally presented, the participant or guardian indicates all questions have been answered to his or her satisfaction, and the participant or guardian has signed the document.

_____

Witness's Name (Print)

_____

Signature of Witness Date

PERSON ADMINISTERING CONSENT: I have conducted the consent process and orally reviewed the contents of the consent document. I believe the participant understands the research.

_____

Person Obtaining Consent (Print)

_____

Signature of Person Obtaining Consent Date

_____

Principal Investigator's Name (Print)

_____

Signature of Principal Investigator Date

APPENDIX C: PERSONAL HISTORY FORM

PERSONAL HISTORY FORM **(version 2-17-09)**

Technician _____ Contract _____ ID _____

PLEASE PRINT AND FILL OUT COMPLETELY

1. **Name**: _____ Date: _____
   Phone#: (home) _____ (work) _____
   Address: _____
   City: _____ State _____ Zip _____
   e-mail address (if available):_____
2. **Employer**: _____
   Occupation: _____

3. Date of Birth: _____ Sex**: _____** Age: **_____** Race: _____

4. **General Medical History** Circle one

Any medical complaints presently? (if yes, explain) .... yes no
_____
_____
_____

Any major illnesses in the past? (if yes, explain) ..... (date) _____ yes no
_____
_____
_____

Any hospitalization or surgery? (if yes, explain) ..... (date) _____ yes no
_____
_____
_____

Have you ever had an EKG (electrocardiogram) ? ..... (date) _____ yes no

Are you diabetic? ....If yes, at what age did you develop diabetes: _____ yes no

Are you currently taking any medications? ..... yes no

Medication	Dosage	Reason	Times taken per day

5. **Family History**

	Age if	Age of	Cause of

	alive	death	death
Father	_____	_____	_____
Mother	_____	_____	_____

Do you have a family history of: (Blood relatives only: give age of occurrence if applicable)

	Relationship	Age of occurrence
--High blood pressure ..... yes no	_____	
--Heart attack.....yes no	_____	
--By-pass surgery.....yes no	_____	
--Stroke.....yes no	_____	
--Diabetes.....yes no	_____	
--Gout.....yes no	_____	
--Obesity.....yes no	_____	

6. **Tobacco History** (check one)

_____ None
_____ Quit months/years ago
_____ Cigarette
_____ Snuff
_____ Chewing tobacco
_____ Pipe

Total years of tobacco use? _____

Cigarette history
_____ 1-10 daily
_____ 11-20 "
_____ 21-30 "
_____ 31-40 "
_____ more than 40

Snuff history
_____ < 0.5 cans daily
_____ 0.5-2.5 cans "
_____ > 2.5 cans "

Chewing history
_____ < 0.5 pouches daily
_____ 0.5-2.5 pouches "
_____ > 2.5 pouches "

7**. Weight History**

What do you consider a good weight for you? _____ Weight at age 21? _____
Weight since age 21? _____ Weight one year ago? _____
Weight now? _____

8. **Cardio-Respiratory History**

Any heart disease now?..... yes no

Any heart disease in the past?..... yes no

Heart murmur?..... yes no

Occasional chest pains?..... yes no

Chest pains on exertion?..... yes no

Fainting?..... yes no

Daily coughing?..... yes no

Cough that produces sputum?..... . yes no

High blood pressure?..... yes no

Shortness of breath --

at rest..... yes no
lying down..... yes no
sleeping at night..... yes no
after 2 flights of stairs..... yes no

9. **Muscular History**

Any muscle injuries or illnesses now?..... yes no

Any muscle injuries in the past?..... yes no

Muscle pain at rest?..... yes no

Muscle pain on exertion?..... yes no

10. **Bone-Joint History**

Any bone or joint (including spinal) injuries or illnesses now?..... yes no

Any bone or joint (including spinal) injuries or illnesses in the past?..... yes no

Ever had painful joints?..... yes no

Ever had swollen joints?..... yes no

Flat feet?..... yes no

11. **Menstrual History** (Women only)

Are you post-menopausal (e.g., not had menstrual flow for at least one year)? ... yes no

Have you had a hysterectomy? ..... yes no

If you have had a hysterectomy, were the ovaries removed? ……..... yes no

If pre-menopausal: On what date did your last period start (beginning of flow)? _____
Are your periods regular? ..... yes no
Approximately how many days apart are your periods? _____
Are you on any hormonal supplements, such as a birth control pill or
estrogen replacement therapy? ..... yes no
If so, what? _____

12. **Nutritional Survey**

How many times do you usually eat per day? _____

What time of day do you eat your largest meal? _____

How many times per week do you usually eat...

____ Hamburger	____ Sausage	____ Bacon
____ Beef	____ Pork	____ Cheese
____ Shellfish (shrimp, oysters, scallops, clams, etc.)		
____ Fish	____Poultry	____ Fried Foods
____ Breads	____ Cereals	____ Vegetables
____ Eggs	____ Desserts	____ Ice Cream
____ Other		

How many servings per week do you usually consume?

____ Whole milk	____ Coffee
____ Low-fat milk (2% milk fat)	____ Tea
____ Skim milk (non-fat)	____ Soft drinks
____ Buttermilk	____ Other

13. **Physical Activity Survey**

a. Compared to a year ago, how much regular physical activity do you currently get? (Check One)

____ much less	____ somewhat less	____ about the same
____ somewhat more	____ much more	

b. For the last three months, have you been exercising on a regular basis?..... yes no

c. What type of exercise or physical activity do you currently do or have done regularly in the past?

(For example: walking, swimming, weight lifting, gardening, etc.)

_____
_____
_____

d. On the average, how many days per week do you exercise? _____

e. How long do you exercise each time? For how many minutes? _____

f. How hard do you exercise on a scale from 1 to 5: with 1 being easy and 5 being very hard?
____ 1 ____ 2 ____ 3 ____ 4 ____ 5

g. Do you ever check your heart rate (pulse) to determine how hard you are exercising?
____ yes ____ no

h. What aerobic activity or activities would you prefer in a regular exercise program for yourself?

____ Walking and/or running	____ Tennis	____ Bicycling
____ Racquetball	____ Swimming	____ Basketball
____ Aerobic dance	____ Stationary cycling	____ Soccer
____ Stair climbing	____ Rowing	_____Other

14. **Alcohol History**
Do you ever drink alcoholic beverages? Yes ____ No ____
If yes, what is your approximate intake of beverages per week?
Beer _____ Wine _____ Mixed Drinks _____

15. **Sleeping Habits**
Do you ever experience insomnia (trouble sleeping)? Yes ____ No ____
If yes, approximately how often?_____
How many hours of sleep do you usually average per night?_____

16. **Education**
Please indicate the highest level of education completed.

____ Grade School	____ Junior High	____ High School
____ College	____ Graduate	____ Postgraduate

Please indicate degree earned (i.e. B.A., M.S., Ph.D.)_____

17. **Motivation or reason for participating in the testing program?**
____ General health and fitness evaluation
____ Medical evaluation prior to starting and exercise program
____ Baseline for weight loss
____ Required by supervisors or employers
____ Other _____

18. **Family Physician**
Name: _____
Address:_____
Phone: _____
Should it be necessary, may we send a copy of your results to your physician?

19. **Insurance** I, _____understand that this evaluation is not reimbursable under Medicare and the cost of the evaluation must be paid by me.

Signature: _____
Date: _____

APPENDIX D: DIETARY LOG FOR A TYPICAL DAY

Date _____ **Subject ID # _____**

Dietary Log for a Typical Day

Meal	Time of day	Serving Size	Food Item	Prepared by:

Please list all other vitamins, minerals, and supplements that you normally take in a day:

_____

_____

APPENDIX E: EXPERIMENTAL SESSION INSTRUCTIONS

Age-related Changes in Skeletal Muscle Signaling after Acute Heavy Resistance Exercise

Principal Investigator: Scott E. Gordon, Ph.D.
Telephone #: (252) 737-2879
Sub Investigators: Jen Macesich & Hope Tharrington
Telephone #: (919) 606-2853 & (252) 883-2001

EXPERIMENTAL SESSION INSTRUCTIONS

Human Performance Laboratory

For three full days prior to session (Start Date: _____):

1. Follow the high carbohydrate diet instructions.
2. Completely fill out diet log.
3. Do not drink alcohol
4. If you consume caffeine, do so only in moderation.
5. Drink at least 64 oz. of water per day (i.e., eight 8-oz. glasses).

On day of experimental session (Date _____):

1. Drink 16 oz. of water before reporting to the laboratory.
2. **Do not eat or drink anything but water for the 12 hours prior to reporting to the laboratory!!
3. **Do not exercise before the experimental session!!
4. Report to the Brody School of Medicine, room 3S08 at _____.
5. Wear exercise clothes, specifically shorts and athletic shoes, to the experimental session.

For the duration of the experiments:

1. Do not engage in exercise.
2. Do not donate blood or plasma.
3. If you begin taking new medications, please notify Jen Macesich or Hope Tharrington.

APPENDIX F: DIETARY INTERVENTION GUIDELINES

The goal for this 3-day diet is to lower fat intake, increase carbohydrate intake, and keep protein intake approximately the same as your normal intake.

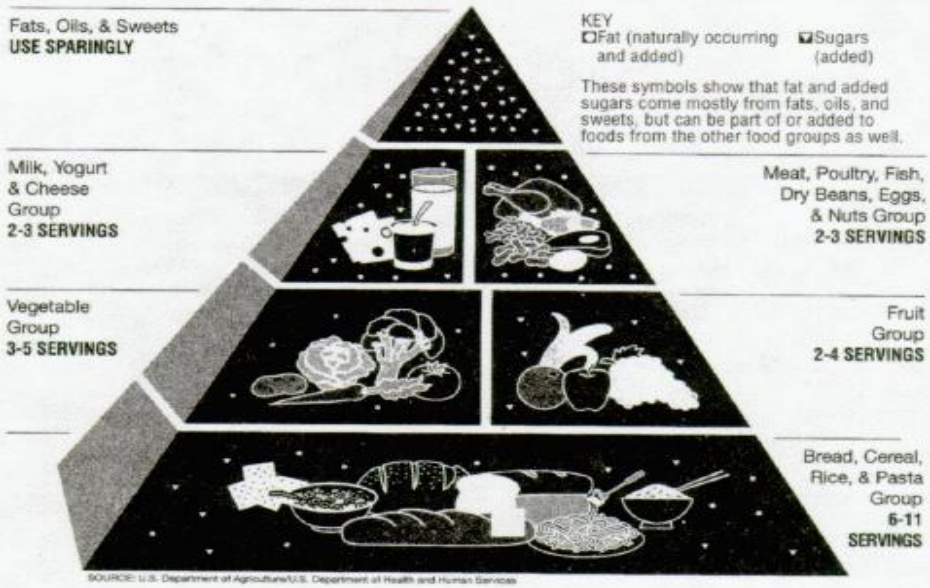
Total calories should also remain approximately the same as your normal intake.

In the 3 days before your next visit, please try to make the following food choices (also see attached Food Guide Pyramid):

- **Increase your choices from the Bread/Cereal/Rice/Pasta Group.**
- **Increase your choices from the Fruit Group.**
- **Increase your choices from the Vegetable Group.**
- **Reduce (or choose non-fat or low fat) choices from the Milk/Yogurt/Cheese Group.**
  - **-Choose skim (non-fat) milk or 1% fat milk instead of 2% or whole milk.**
  - **-Choose cheese or yogurt products that are labeled as non-fat or low fat.**
- **Choose only lean meats from the Meat/Poultry/Fish/Beans/Eggs/Nuts Group.**
  - **-Choose chicken, turkey or fish instead of pork or beef (red meat, hamburger, etc.).**
  - **-For chicken and turkey: Choose white meat (breast meat) instead of dark meat (legs and wings).**
  - **-For chicken and turkey: Remove all skin.**
  - **-Avoid processed or fried meats such as bacon, sausage, hot dogs, salami, kielbasa, and pepperoni.**
  - **-Avoid creamy or oily sauces/toppings on all meats.**
  - **-Avoid nuts or nut products (i.e., peanut butter).**
  - **-Beans or legumes are a good choice as a side dish when available.**
- **Reduce your choices from the Fats/Oils/Sweets Group.**

Also, please try to:

- **Avoid fried foods in general.**
- **Substitute fruit juice instead of milk (unless it is skim milk or 1% fat milk).**
- **Avoid creamy or oily dressings on salads and avoid creamy or oily sauces on pasta (unless they are labeled as non-fat or low-fat).**
- **Avoid a lot of butter, margarine, or sour cream on baked potatoes or other foods on which you place toppings.**
- **Substitute fruit spreads (jams, jellies, applesauce, etc.) instead of butter, margarine, or cream cheese.**
- **Substitute Pam or other light cooking spray instead of oils when cooking.**
- **Be careful: Many "sweet" items (such as cakes, ice cream, and candy bars) are also high in fat.**



What Counts as One Serving?

Grain Products Group (bread, cereal, rice, and pasta)

1 slice of bread
1 ounce of ready-to-eat cereal
½ cup of cooked cereal, rice, or pasta

Vegetable Group

1 cup of raw leafy vegetables
½ cup of other vegetables, cooked or chopped raw
¾ cup of vegetable juice

Fruit Group

1 medium apple, banana, or orange
½ cup of chopped, cooked, or canned fruit
¾ cup of fruit juice

Milk Group (milk, yogurt, and cheese)

1 cup of milk or yogurt
1½ ounces of natural cheese
2 ounces of processed cheese

Meat and Beans Group (meat, poultry, fish, dry beans, eggs, and nuts)

2 to 3 ounces of cooked lean meat, poultry, or fish
½ cup of cooked dry beans or 1 egg counts as 1 ounce of lean meat
2 tablespoons of peanut butter or ⅓ cup of nuts count as 1 ounce of meat

Fats, Oils, and Sweets

Limit calories from these, especially if weight loss is needed

Note: The amount eaten may be more than one serving. For example, a dinner portion of spaghetti would count as two or three servings of pasta.

How Many Servings Are Needed?

Teenaged boys and active men need the highest number of servings shown. Women and some older adults need the lowest number of servings shown. Children, teenaged girls, active women, and most men need a number of servings somewhere in the middle of those shown.