

Skeletal Muscle Forkhead Box 3A (FOXO3A) 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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AMP-Activated Protein Kinase (AMPK) Activity

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CHAPTER I: INTRODUCTION

Sarcopenia

As an individual increases in age, a decrease in muscle mass and strength occurs (Rogers & Evans, 1993). This normal age-related decrease in muscle mass with advancing age is termed sarcopenia (Roubenoff, 2000), and occurs in both men and women (Hughes et al., 2001). The decrease in muscle mass associated with aging occurs more rapidly in the physically inactive than their active counterparts. In physically inactive people, there is a loss of approximately 3-5% of muscle mass each decade after the age of 30, and a parallel decline in muscle strength [reviewed by (Nair, 1995)]. There is a greater loss in fast twitch muscle fibers than in slow-twitch muscle fibers. Additionally, a decrease in muscle cell size, particularly in type II muscle fibers (or fast twitch fibers), is seen in the elderly (Rogers & Evans, 1993). In addition to this decrease in muscle mass and strength, lean body mass slowly decreases throughout adulthood. Lean body mass is highest in the third decade, after which it falls slowly for the next two decades, and then more rapidly. Body fat content also progressively rises (Forbes & Reina, 1970). Sarcopenia decreases the ability of the elderly population to perform activities of daily living. This decrease in muscle mass and strength are linked to an increased risk of falls and fractures in this population (Deschenes, 2004). This causes decreased independence and quality of life in the elderly. Sarcopenia and decreased muscle strength are also associated with increased health care costs. Decreased strength and power has been linked to several chronic afflictions that are common among the

aged. These include osteoporosis, insulin resistance, and arthritis (Deschenes, 2004). The increase in such diseases in the elderly population has contributed to a rise in health care costs. Per capita health care spending has increased dramatically between 1966 and 2001 (Altman, Tompkins, Eilat, & Gavin, 2003). It has been estimated that by the year 2010, approximately \$183 billion dollars will be spent on nursing homes by the elderly (Greenlund & Nair, 2003).

Resistance Training

Resistance training interventions have proven useful in the elderly population, especially in delaying the progression of sarcopenia. Resistance exercise has been shown to increase both strength and muscle fiber size (Frontera et al., 1988; Kryger & Andersen, 2007; Rogers & Evans, 1993). Muscle strength has been shown to increase by an average of 5% per resistance training session in an aging population (Frontera et al., 1988). However, resistance training interventions are not 100% effective. In rats, a decrease in ability of old skeletal muscle to hypertrophy as compared to young skeletal muscle has been shown (Degens & Alway, 2003). In addition, hypertrophy is decreased in aged skeletal muscle (Hortobagyi et al., 1995). This decrease in hypertrophy in the elderly population occurs in fast-twitch muscle fibers (Hortobagyi, 2005; Kosek et al., 2006; Thompson, 1994; Thomson & Gordon, 2005, 2006). Similar findings have been recorded even with the introduction of chronic overload in rats (Blough & Linderman, 2000; Thomson & Gordon, 2005). Despite resistance exercise, inhibition of gains in force production with age in humans has been shown (Korhonen et al., 2006). Additionally, not all of the muscle mass lost through the aging process can be recovered

through an intervention. Once lost, fast twitch muscle fibers cannot be recuperated with resistance training.

Protein Degradation

Muscle protein degradation (MPD) occurs continuously within prokaryotic and eukaryotic cells. It is the process by which cells are continuously broken down (Viana et al., 2008), and leads to a decrease in muscle mass and muscle fiber size if MPD is greater than muscle protein synthesis. In a young, growing individual, protein synthesis generally equals or occurs at a more rapid rate than protein degradation. This leads to an increase in muscle strength and muscle hypertrophy, especially with the aid of resistance training (Kryger & Andersen, 2007; Rogers & Evans, 1993; Trappe et al., 2000). There is an increase in protein degradation at rest in the older population (Drummond, Miyazaki et al., 2008).

Resistance Exercise Response

As a person ages, there is a shift in the balance between protein synthesis and protein degradation in response to resistance exercise training (Gordon et al., 2008). Protein degradation has also been shown to be higher both at rest and after resistance exercise in both old versus young rats (Tamaki et al., 2000) and humans (Kumar et al., 2009). Higher protein degradation in muscle cells results in greater muscle atrophy and decreased muscle strength that is associated with sarcopenia in the elderly population (Mitch & Goldberg, 1996). Moreover, a greater protein degradation response to resistance exercise in the elderly also likely contributes to the diminished muscle growth

observed in response to resistance exercise training in that population (Kosek et al., 2006).

Upstream Signaling Pathways

Protein degradation in skeletal muscle cells is regulated by a number of upstream signaling pathways. One of the main signaling pathways upstream controlling protein degradation in skeletal muscle contains atrogin-1 and muscle RING finger 1 (MuRF1), both of which are believed to be stimulated by the atrophy-related forkhead box (FOXO) transcription factors (especially FOXO1 and FOXO3A) (Zhao et al., 2007) and MuRF1 (Gordon et al., 2008). The FOXO transcription factors are a subgroup of the Forkhead family of transcription factors. The FOXO transcription factors are regulated (inhibited) by the insulin/PI3K/Akt signaling pathway (Carter & Brunet, 2007). With phosphorylation of FOXO3A at Ser^{318/321} by Akt (protein kinase B), the FOXO3A transcription factor is prevented from translocating to the nucleus of the cell and sequestered in the cytosol. The nucleus is the location where FOXO3A stimulates transcription of atrogin-1 and MuRF1 mRNA, which ultimately get translated to atrogin-1 and MuRF1 proteins and lead to muscle protein breakdown [as reviewed (Murton, Constantin & Greenhaff, 2008)]. Therefore, the phosphorylation of FOXO3A leads to its inactivation (due to lack of translocation) and helps lessen protein degradation. FOXO3A, and subsequently atrogin-1 and MuRF1, activation during resistance training increases with age (Raue et al., 2007; Nakashima & Yakabe, 2007). The increase in such upstream signaling pathways potentially leads to diminished muscle hypertrophy in aged skeletal muscle.

On the other side of the balance between protein synthesis and protein degradation is mammalian target of rapamycin (mTOR), which is an upstream signaling pathway that regulates both initiation and elongation factors controlling protein synthesis. mTOR is activated partly via phosphorylation by Akt with mechanical loading; phosphorylation of mTOR can be inhibited by the tuberous sclerosis complex (TSC2) (Inoki, Li, Zhu, Wu & Guan, 2002). Generally, mTOR is stimulated during resistance exercise in the young population (Drummond, Dreyer et al., 2008); leading to muscle hypertrophy and strength gains (Bodine et al., 2001). Akt is phosphorylated following mechanical loading of the muscle cell to activate mTOR through mTOR phosphorylation at Ser²⁴⁴⁸. Downstream, mTOR stimulates protein translation through initiation by activating 70-kDa ribosomal protein S6kinase (p70s6k) (Inoki, Li, Zhu, Wu & Guan, 2002). P70s6k has been shown to inhibit eukaryotic elongation factor 2 kinase (eEF2k) (Wang et al., 2001), which activates eEF2 (Ryazanov, Shestakova, & Natapov, 1988). Activation of eEF2 can lead to increased translational elongation, which is a process of protein synthesis in muscle cells (Horman et al., 2002). In aged muscle, however, mTOR activation is decreased following resistance training (Parkington et al., 2003). Hindering mTOR activation limits this pathway, which decreases protein synthesis and muscle hypertrophy, ultimately leading to an increase in protein degradation and lack of muscle hypertrophy in aging skeletal muscle.

AMPK

Upstream of mTOR, the FOXO transcription factors, atrogin-1, and MuRF1 lies 5'-AMP-activated protein kinase (AMPK). AMPK essentially acts as a sensor of cellular energy, responding to increases in the AMP/ATP intracellular ratio (Miranda, Tovar,

Palacios, & Torres, 2007). It attempts to maintain energy stores within the cells to create homeostasis. AMPK hinders protein synthesis (Bolster, 2002), while increasing the processes leading to muscle protein degradation (Nakashima & Yakabe, 2007). Moreover, there is a fair amount of evidence that AMPK inhibits overload-induced muscle hypertrophy (Gordon et al., 2008; Thomson & Gordon, 2005, 2006).

AMPK phosphorylation in response to resistance exercise has been shown to be elevated with age in humans (Drummond et al., 2008) and rats (Thompson and Gordon, 2005). These high levels of AMPK are thought to inhibit mTOR activation, which is lower in response to chronic overload (Thompson and Gordon, 2006) or resisted contractions in aged muscle (Parkington et al., 2003). Additionally, AMPK activation acts to stimulate protein degradation by enhancing FOXO3/FOXO1 transcription factors, and thus stimulating atrogen-1 and MuRF1 (Nakashima & Yakabe, 2007). FOXO1 and FOXO3 may be differentially regulated by AMPK (AMP-activated protein kinase). In vitro, AMPK appears to phosphorylate FOXO3 at several sites, and this occurs more efficiently than AMPK's phosphorylation of FOXO1. Moreover, AMPK can also inhibit Akt's phosphorylation of FOXO at ^{Ser318/321} and this allows FOXO3A to translocate to the nucleus, leading to upregulation of protein degradation pathway components (Greer et al., 2007). Skeletal muscle atrogen-1 mRNA content is known to be higher after resistance exercise in older vs. younger humans (Raue et al., 2007), and the muscle protein synthesis response to resistance exercise is reduced with age (Kumar et al., 2009). Thus, the elevated AMPK response to resistance exercise with age (Drummond et al., 2008), in part acting through mTOR, may be one mechanism by which these phenomena occur.

Diet, Carbohydrate Intake, and Skeletal Muscle Glycogen Content

The diet of the elderly population differs from that of their normal, young counterparts. The elderly consume fewer total calories than the young (Lieberman, Wurtman & Teicher, 1989; Wurtman et al., 1988), and do not reach the total daily energy intake recommendations (Shabayek & Saleh, 2000). Additionally, older people generally consume less carbohydrates and fats than younger individuals (Lieberman, Wurtman & Teicher, 1989; Wurtman et al., 1988).

The dietary differences between young and elderly populations, especially the decrease in carbohydrate consumption, are partially responsible for the decrease in muscle glycogen levels in the elderly (Cartee, 1994). In young individuals, a decrease in muscle glycogen is thought to increase AMPK activity, which is higher in the glycogen-depleted state than in the glycogen-loaded state during aerobic exercise (Wojtaszewski & MacDonald et al., 2003). Additionally, there has been evidence of up to a 26% decrease in muscle glycogen content following resistive leg exercise (Tesch et al., 1986), as well as a 25% loss in muscle glycogen in the biceps brachii after resistive exercise to muscular fatigue (MacDougall et al., 1999). Thus, the lower muscle glycogen content coupled with a resistance exercise-induced further reduction in glycogen content in the elderly population may accentuate the increase in AMPK activation seen in response to resistance exercise in aged skeletal muscle. This elevated AMPK activation may exacerbate FOXO3A signaling and protein degradation and attenuate muscle hypertrophy and strength gains seen in the elderly population (Thomson & Gordon, 2005).

Specific Aim

Skeletal muscle fiber atrophy has been shown to occur as age increases (Hortobagyi et al., 1995). Additionally, muscle glycogen levels are lower in the elderly population than in the young (Cartee, 1994). Low glycogen and energy levels can activate AMPK (Wojtaszewski & MacDonald et al., 2003); AMPK phosphorylation following resistance exercise is higher in aged skeletal muscle (Drummond et al., 2008). Following resistance exercise, atrogin-1 and MuRF1, which are downstream of FOXO3A, are also higher in old individuals when compared to young (Raue et al., 2007; Nakashima & Yakabe, 2007). Akt-stimulated FOXO3A phosphorylation at Ser318/321 inhibits nuclear translocation and thus inhibits FOXO3A-stimulated atrogin-1, and MuRF1 transcription. FOXO3A phosphorylation at Ser318/321 is attenuated by AMPK (Greer et al., 2007). However, the FOXO3A phosphorylation response to resistance exercise in young versus old individuals has not been examined. Thus, the specific aim of this investigation was to examine the FOXO3A Ser^{318/321} phosphorylation response to resistance exercise and its relation to muscle glycogen content and AMPK activation in young versus old subjects. It was hypothesized that FOXO3A Ser^{318/321} phosphorylation would be lower in response to acute resistance exercise in the skeletal muscles of older versus younger individuals. It was further hypothesized that this lower FOXO3A phosphorylation response would be related to lower glycogen content and higher AMPK activation in the skeletal muscles of older versus younger individuals.

CHAPTER II: REVIEW OF LITERATURE

Sarcopenia

Sarcopenia, which is the wasting away of skeletal muscle with increasing age, is a growing problem in the United States due to the increasing amount of elderly people in the population. After the age of 45, a steady decline in muscle mass is typically observed (Hughes et al., 2001). Sarcopenia has been shown to result in as much as 14% of muscle loss and 34% of muscle protein loss between the ages of 25 and 75 (Cohn et al., 1980). This clinical issue results in a decreased functional capacity and independence, as well as a decrease in the ability for the elderly to complete activities of daily living. The loss of these abilities significantly decreases the quality of life for the aging population. Additionally, body mass differences are seen between young and old subjects. Lean body mass has been observed to slowly decrease throughout adulthood. Lean body mass is highest in the third decade, after which it falls slowly for the next two decades, and then more rapidly in the elderly. Body fat content also progressively rises with increasing age (Forbes & Reina, 1970).

Skeletal muscle atrophy occurs due to both a reduction in muscle fiber size and number. This decline occurs in the fast-twitch muscle fibers rather than the slow-twitch fibers (Welle, 2002). Specifically, 20-50% reductions in fiber size of fast-twitch fibers have been observed; however, slow-twitch fibers atrophy only 1-25% (Doherty et al., 1993). A strength decline in the elderly group of between 20-40% has been reported in studies comparing young and old (70-90 years) subjects, with relative loss between

women and men being equal (Murray et al., 1985). This loss of strength strongly correlates with loss of skeletal muscle mass. An approximately 40% decrease in total muscle cross sectional area has been observed between the ages of 20 and 60 years (Doherty et al., 2002). The decrease of skeletal muscle size and strength associated with sarcopenia may be due to a number of factors, including inadequate nutrients in the diet, hormonal changes, and decreased physical activity (Boirie, 2009).

It is estimated that approximately one-quarter to one-half of the population of both men and women over the age of 65 have sarcopenia (Baumgartner et al., 1998). From a practical standpoint, sarcopenia could lead to an increased risk of falls and fractures, and eventually a loss of independence (Sayer et al., 2006). As many as 13.4% of men and 16.3% of women over the age of 60 need help getting out of the bed while 20.3% of men and 30.9% of women in this age range have difficulty walking up a flight of only 10 steps. These percentages continue to increase with age (Ostchega, Harris, Hirsch, Parsons, & Kington, 2000).

The decrease in strength associated with sarcopenia has been linked to several chronic diseases that commonly occur in the elderly population, including insulin resistance and diabetes, arthritis, and osteoporosis (Deschenes, 2004). Sarcopenia is also linked to increased health care costs. Between 1966 and 2001, per capital health care spending has increased dramatically, due in part to afflictions associated with the decreased muscle strength and mass of sarcopenia (Altman, Tompkins, Eilat, & Gavin, 2003). In 2000, health care costs related to diseases associated with the loss of muscle mass with age were around 18.5 billion dollars (Janssen, Shepard, Katzmarzyk & Roubenoff, 2004). With such a prevalence of age-related disability and cost, it is

important to examine the mechanism(s) underlying muscle atrophy with age, as well as potentially compensatory methods to decrease the effects of increased aging on muscle mass and strength.

Resistance Training

Due to the overall loss of strength and muscle fiber size in the elderly, resistance training interventions are popular and have proven beneficial to this population. This type of exercise is especially helpful in delaying the process of sarcopenia. The benefits of resistance training include increasing both the cross-sectional area of the muscle fiber and muscle strength (Frontera et al., 1988; Kryger & Andersen, 2007; Rogers & Evans; 1993). An average of a 5% increase in muscle strength has been shown per resistance training session in the aging population (Frontera et al., 1988). However, although clinically significant improvements are seen in the aging with resistance training interventions, fast-twitch hypertrophy is greatly reduced in the elderly population (Hortobagyi et al., 1995; Kosek et al., 2006; Thompson, 1994; Thomson & Gordon, 2005, 2006). This decline in the hypertrophic effect of overloading has also been shown in the fast-twitch muscle fibers of aged rats (Thomson & Gordon, 2005). Slow-twitch muscle fibers are more resistant to this loss of effect of resistance training with age than fast-twitch fibers (Trappe et al., 2000). Additionally, elderly females show less muscle hypertrophy and strength gains in response to resistance training than elderly males (Bamman et al., 2003). This decrease in the overall effect of resistance training with increasing age is a contributing factor of many of the clinical diseases and ailments often associated with the elderly, such as sarcopenia, osteoporosis, increased risk of falls, broken bones, and orthopedic surgeries.

Protein Degradation

Muscle protein degradation (MPD) is a catabolic process that occurs naturally and continuously in both prokaryotic and eukaryotic cells. MPD is the break-down and deterioration of the proteins comprising muscle cells (Viana et al., 2008). The major sites of intracellular protein degradation are the proteasomes and the lysosomes. Protein degradation is an energy-consuming process that requires ATP (Reggiori & Klionsky, 2005; Wolf & Hilt, 2004), and has been shown to be stimulated in the lysosomes by high stress situations, such as during nutrient depletion (Codogno & Meijer, 2005). This process can lead to muscle atrophy when protein degradation occurs at a higher frequency than the rate of protein synthesis. Protein degradation is increased at rest in the older population (Drummond, Miyazaki et al., 2008; Trappe et al., 2004).

Resistance Exercise Response

In the skeletal muscle fibers of young men and women, protein synthesis, which is the formation of proteins within the muscle, equals or outweighs the effects of protein degradation. This leads to maintenance or an increase in muscle fiber size and strength in the young population, especially with the aid of resistance exercise training (Kryger & Andersen, 2007; Rogers & Evans, 1993; Trappe et al., 2000). In the elderly this balance is often altered. Protein degradation has been shown to be higher even after resistance exercise in both older rats (Tamaki et al., 2000) and humans (Kumar et al., 2009) than their young counterparts. Thus, this may be one reason why there is less skeletal muscle fiber hypertrophy in the elderly population than the young with resistance exercise (Kosek et al., 2006; Kryger & Andersen, 2007).

Upstream Signaling Pathways

There are a number of upstream signaling pathways that stimulate muscle protein degradation in mammalian cells. The pathway associated with atrogin-1 and MuRF1 is one of the main signaling pathways upstream that controls muscle protein degradation (Gordon et al., 2008). Atrogin-1, which is an atrophy-related gene, is believed to be stimulated by the FOXO transcription factors in skeletal muscle cells (Nakashima & Yakabe, 2007; Zhao et al., 2007). The FOXO transcription factors are a subgroup of the Forkhead family of transcription factors and are regulated in part by the insulin/PI3K/Akt signaling pathway (Carter & Brunet, 2007). Akt (protein kinase B) phosphorylates FOXO3A at Ser^{318/321}, thus preventing the FOXO transcription factors from entering the nucleus of the cell. Instead, they are then sequestered in the cytosol. In the cell nucleus, FOXO3A stimulates the transcription of atrogin-1 and MuRF1 mRNA. This mRNA ultimately is translated into atrogin-1 and MuRF1 proteins, which lead to muscle protein breakdown [as reviewed (Murton, Constantin & Greenhaff, 2008)]. Therefore, the phosphorylation of FOXO3A leads to inactivation (due to prevention of translocation) and helps lessen protein degradation. The major contributors of protein degradation, and thus muscle fiber atrophy, are the FOXO1 and FOXO3 transcription factors (Zhao et al., 2007). The activation of FOXO3A has been shown to stimulate atrogin-1, as well as MuRF1, and to cause dramatic atrophy in the skeletal muscle of rats (Sandri et al., 2004). MuRF1 expression has been shown to increase under catabolic conditions (Bodine et al., 2001). FOXO3A, atrogin-1, and MuRF1 mRNA levels during resistance exercise training is increased in the elderly (Raue et al., 2007; Nakashima & Yakabe, 2007). Stimulation

of FOXO3 results in both activation of autophagy and lysosomal proteolysis (Zhao et al., 2007).

Autophagy and lysosomal proteolysis is mediated by a group of autophagy-specific gene kinases (ATG). LC3B, or LC3B-II (light chain 3B), which is also known as ATG 8, is a member of this family, and is the active isoform that is formed from the cleavage of cytosolic LC3-I (microtubule-associated protein 1 light chain 3) during autophagy. LC3B-II is used as a marker of increased autophagy. The processes of autophagy and lysosomal proteolysis contribute to the increase in muscle cell atrophy and strength loss associated with sarcopenia in aging skeletal muscle (Zhao et al., 2007).

On the other hand, mammalian target of rapamycin (mTOR) is an upstream signaling pathway associated with protein balance. The mTOR pathway particularly controls protein synthesis response to loading, and ultimately muscle hypertrophy (Bodine et al., 2001). mTOR is activated partly via phosphorylation by Akt with mechanical loading (Inoki, Li, Zhu, Wu & Guan, 2002). However, mTOR also suppresses protein degradation. In normal, young individuals, mTOR is stimulated during acute resistance exercise (Drummond & Dreyer et al., 2008). This leads to stimulation of protein synthesis, as well as both muscle hypertrophy and strength gains in this population (Bodine et al., 2001; Thomson & Gordon, 2006). Pharmacological blockade of mTOR signaling inhibits resistance exercise-induced protein synthesis in rats (Kubica, Bolster, Farrell, Kimball, & Jefferson, 2005) and humans (Drummond, Dreyer et al., 2008) and inhibits chronic overload-induced muscle hypertrophy (Bodine et al., 2001). In the elderly, mTOR activation is decreased (Parkington et al., 2003; Thomson & Gordon, 2006). mTOR phosphorylation can be deactivated by activation of the tuberous

sclerosis complex (TSC) pathway (Inoki, Zhu & Guan, 2003). mTOR is inhibited through TSC2 phosphorylation at Thr¹²²⁷ and Ser¹³⁴⁵. This causes TSC2 activation (Inoki, Zhu & Guan, 2003) to inhibit protein synthesis to save energy stores within the muscle cell. Additionally, mTOR deactivation increases autophagy and lysosomal proteolysis, and thus, increases muscle atrophy (Zhao et al., 2007). Protein degradation pathways are more elevated in the elderly following resistance exercise than in the young (Raue et al., 2007; Trappe et al., 2004). This can ultimately lead to an increase in sarcopenia in the elderly population.

AMPK

5'-AMP-activated protein kinase (AMPK) lies upstream of mTOR, the FOXO transcription factors, atrogen-1, and MuRF1. AMPK is has been dubbed a metabolic master switch capable of sensing cellular energy disturbances and restoring ATP stores (Winder & Hardie, 1999). A decrease in the cellular AMP/ATP intercellular ratio stimulates AMPK (Miranda, Tovar, Palacios, & Torres, 2007), which attempts to create homeostasis within the cell by maintaining energy stores. AMPK is activated by processes that either decrease production of ATP or processes within the cell that increase utilization of ATP, such as hypoxia or skeletal muscle contraction (Hardie, Hawley, & Scott, 2006). For example, AMPK activation occurs in response to low glucose levels within the cell (Salt, Johnson, Ashcroft & Hardie, 1998). Low glycogen and energy levels, even at rest or during aerobic exercise, can activate AMPK (Wojtaszewski & MacDonald et al., 2003). Once activated, AMPK inhibits energy consuming pathways. It acts to promote ATP production through glycolysis. This increases glucose uptake and fatty acid oxidation while inhibiting other energy taxing

processes within the cell (Hardie, Hawley & Scott, 2006). AMPK ultimately hinders protein synthesis, which is an energy expensive process; thus, AMPK inhibits the processes that lead to muscle hypertrophy and gains in muscle strength, while increasing the processes leading to muscle protein degradation (Nakashima & Yakabe, 2007).

There is a negative correlation between AMPK phosphorylation and hypertrophy in overloaded rat skeletal muscle (Thomson & Gordon, 2005). Additionally, AMPK activity at rest and during overload have been shown to be higher in old versus young rat muscle (Drummond et al., 2008); similar observations have been made in humans following a bout of acute resistance exercise (Drummond, Dreyer et al., 2008). This elevated AMPK activity is seen in fast-twitch muscle, but not in slow-twitch muscle fibers (Thomson & Gordon, 2005).

The high levels of AMPK phosphorylation and/or activity found in aged skeletal muscle likely act to inhibit mTOR activation through tuberous sclerosis complex (TSC2) phosphorylation. This causes TSC2 activation. The TSC pathway inhibits mTOR phosphorylation at Ser¹³⁴⁵ (Inoki, Zhu & Guan, 2003). This site is usually the location of Akt phosphorylation, which activates mTOR with mechanical loading of the muscle cell (Inoki, Li, Zhu, Wu & Guan, 2002). The activation of TSC2 and deactivation of mTOR hinders protein synthesis and thus prevents a decrease of energy stores within the cell (Parkington et al., 2003).

Additionally, AMPK activation acts to stimulate protein degradation by enhancing the FOXO3/FOXO1 transcription factors, and thus stimulates atrogin-1 and MuRF1 (Nakashima & Yakabe, 2007). AMPK can cause FOXO3A to translocate into

the nucleus of the cell, where activates the transcription of atrogin-1 and MuRF1 into mRNA. This mRNA can become atrogin-1 and MuRF1 protein, which ultimately lead to protein degradation (Greer et al., 2007). Since AMPK levels have shown to be higher in aged skeletal muscle (Drummond, Dreyer et al., 2008), this may be responsible for the increased protein degradation and decreased protein synthesis, and consequently the muscle atrophy and sarcopenia, often present in the older population. Additionally, higher AMPK levels in aged skeletal muscle may be responsible for diminished muscle fiber hypertrophy with overload after resistance training in the elderly (Kosek et al., 2006).

Diet and Carbohydrate Intake

The diet of the elderly has been shown to differ from of the normal young population. The elderly consume fewer total calories than the young (Furuyama et al., 2003; Lieberman, Wurtman & Teicher, 1989; Wurtman et al., 1988). An inverse relationship has also been shown between dietary intake and age in the elderly population (Sonn, Rothenberg & Steen, 1998). Many elderly adults do not even consume the recommended total daily energy intake (Shabayek & Saleh, 2000). This population also generally consumes significantly less carbohydrates and fats than their younger counterparts (Lieberman, Wurtman & Teicher, 1989; Wurtman et al., 1988). This decreased carbohydrate consumption is both in an absolute sense and when consumption is adjusted for differences in body weight (Wurtman et al., 1988).

AMPK and Glycogen

The diet of the elderly population, especially in respect to the decrease in carbohydrate consumption by this age group, is partially responsible for a decrease in muscle glycogen levels with increased age (Cartee, 1994). In young subjects a decrease in muscle glycogen is thought to increase AMPK activity levels, both at rest and with resistance exercise, because AMPK activity is higher in the glycogen-depleted state than in the glycogen-loaded state (Wojtaszewski & MacDonald et al., 2003) due to allosteric and phosphorylation effects (Greer et al., 2007). Additionally, there has been evidence of up to a 26% decrease in muscle glycogen content following resistive leg exercise (Tesch et al., 1986), as well as a 25% loss in muscle glycogen in the biceps brachii after resistive exercise to muscular fatigue (MacDougall et al., 1999). Muscle glycogen content following resistive exercise has not been studied in the elderly population, but it would follow that since aged skeletal muscle has lower glycogen content than young skeletal muscle, the further decrease in muscle glycogen following resistance exercise may exacerbate the already elevated AMPK phosphorylation and activity in aged muscle after resistance exercise.

Specific Aim

Muscle fiber atrophy has been shown to increase with age (Hortobagyi et al., 1995), and muscle fiber hypertrophy with resistance training or other overloading paradigms is diminished with increasing age (Kosek et al., 2006; Thomson & Gordon, 2005). This decrease in strength and fiber size may be partially due to a decrease in

protein synthesis rate, (Drummond, Dreyer et al., 2008) as well as an increase in muscle protein degradation in the elderly population (Kumar et al., 2009). Protein degradation is higher in aged skeletal muscle following resistance exercise than in young (Raue et al., 2007; Trappe et al., 2004); protein synthesis is decreased in the elderly population after resistance training (Drummond, Dreyer et al., 2009). Higher AMPK phosphorylation and activity in aged skeletal muscle in response to overload and/or resistance training has been shown in rats (Thomson et al., 2009) as well as humans (Drummond, Dreyer et al., 2008), and AMPK lies upstream of protein degradation. Nutritional differences and a decrease in muscle glycogen content with increased age have also been observed (Cartee, 1994). Additionally, a decrease in muscle glycogen leads to an increase in AMPK activity. Following resistance exercise, atrogin-1 and MuRF1 mRNA, which are downstream of FOXO3A, are also higher in old individuals when compared to young (Raue et al., 2007; Nakashima & Yakabe, 2007). Phosphorylation of FOXO3A at Ser^{318/321} is stimulated by Akt. This inhibits nuclear translocation of FOXO3A, and thus prevents FOXO3A from transcribing atrogin-1 and MuRF1 into mRNA. This process is attenuated by AMPK (Greer et al., 2007). Therefore, the specific aim of this investigation was to examine the FOXO3A Ser^{318/321} phosphorylation response to resistance exercise and its relation to muscle glycogen content and AMPK activation in young versus old subjects.

It was hypothesized that FOXO3A Ser^{318/321} phosphorylation would be lower in response to acute resistance exercise in the skeletal muscles of older versus younger individuals. It was further hypothesized that this lower FOXO3A phosphorylation

response would be related to lower glycogen content and higher AMPK activation 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) and 11 old subjects (5 male and 6 female). Young subjects ranged in age from 18 to 30 years of age, while the old subjects were between the ages of 55 and 85 years. The subjects are all healthy, lean men and women with no reported previous cardiovascular disease, diabetes, or hypertension. None of the participants were on medications that alter glucose regulation. All subjects are non-smokers. Each of the subjects was screened prior to the study to determine age, height, weight, body mass index (BMI (kg/m²)), medical history, and exercise history. Participants were recruited through the posting of flyers, word of mouth, and email to East Carolina University faculty and staff. This study was approved by the East Carolina University Institutional Review Board for the Use of Human Subjects (See Appendix A).

Table 3.1. Subject Characteristics.

	Young Adults (n=7)	Old Adults (n=11)
Age (years)	21.7 ± 2.1	67.0 ± 8.6
BMI (kg/m ²)	23.5 ± 3.05	29.0 ± 3.2
Body Fat (%)	21.9 ± 5.0	32.6 ± 4.0*
Weight (kg)	75 ± 14	85 ± 11*

Table 3.1. Body mass index (BMI) was not significantly different between groups. The young and old groups differed significantly (*) in body fat (%) and weight (kg). Significance was set at $p \leq 0.05$.

Experimental Design

The purpose of this investigation was to examine the FOXO3A Ser^{318/321} phosphorylation response to resistance exercise and its relation to muscle glycogen content and AMPK activation in young versus old subjects.

It was hypothesized that FOXO3A Ser^{318/321} phosphorylation would be lower in response to acute resistance exercise in the skeletal muscles of older versus younger individuals. It was further hypothesized that this lower FOXO3A phosphorylation response would be related to lower glycogen content and higher AMPK activation in the skeletal muscles of older versus younger individuals.

Table 3.2. Subject Visitations

Initial Visit (non-fasted)	Experimental Session (overnight fast)
	Exercise Session
1) Informed consent & medical history	- Bilateral leg extension
2) Height and weight measurements	- 3 warm-up sets (50, 70, & 90% of 10RM)
3) 4-site skinfold measurements	- 3 sets to failure (100% of 10RM)
4) 10-repetition maximum determination	Muscle Biopsy – alternating legs
	- pre-exercise, immediately post (0 min), 60 min, & 120 min post

Table 3.2. 10-repetition maximum (10RM) was determined using the leg extension machine in the FITT building at East Carolina University. The warm-up sets consisted of 10 repetitions, 5-7 repetitions, and 3-5 repetitions for each respective set (50, 70, and 90% of the estimated 10RM). The 0 minute time point was immediately (within 60-90 seconds) following the resistance exercise. 60 minutes and 120 minutes post are obtained 1 and 2 hours following the 0 minute post exercise biopsy, respectively.

Initial Visit

The first session was performed in the Fitness Instruction, Testing, and Training (FITT) building at East Carolina University. The initial visit consisted of an initial screening, baseline measurements, familiarization with the resistance exercise equipment, and determination of a 10-repetition maximum (10RM) on the Cybex leg extension machine for each participant.

During the initial visit, participants filled out an informed consent, medical history, and a 1-day dietary recall. Height and weight was obtained, followed by 4-site skinfold measurements, which was taken at the bicep, tricep, suprailiac, and subscapula. Body fat density was estimated for each participant using the Durnin and Womersley

skinfold assessment and body density formula ($[495/(1.1714 - 0.063 * \text{LOG}[\text{sum of skinfolds}] - 0.000406 * [\text{age}] - 450)]$) (Durnin & Womersley, 1974).

Following the initial assessment and measurements, the 10-repetition maximum was determined on the Cybex Leg Extension machine. Subjects were acquainted with the leg extension machine in order to reduce the likelihood of injury or cardiovascular events. Prior to the 10RM testing, participants were asked to predict the maximum amount of weight they can lift 10 times. Half of this amount was used as a warm-up. After completion of the warm-up, the weight was increased by 5-20 pounds, depending upon the performance of the individual. Each set consisted of 10 repetitions, followed by a 1-2 minute rest interval. Completion of testing occurred when subjects were unable to complete more than 10 repetitions. This amount was the participant's 10RM.

Before the end of the initial visit, subjects were given an information sheet that provided guidelines for the 1-2 week period prior to the experimental session. Subjects were instructed to refrain from exercise and donating blood throughout the duration of the study. Additionally, for the 2 days prior to the experimental session, participants were directed to refrain from drinking alcohol, moderate caffeine intake, and drink 64-oz. of water daily. Some older subjects initially chosen to be in a high carbohydrate group were counseled on food choices to increase carbohydrate intake prior to the experimental session (see "Failed Attempt at Dietary Intervention" for more details). On the night preceding the experimental session, participants were asked to fast for at least 12 hours prior to reporting to the laboratory. On the day of the experimental session, participants were instructed to drink 16-oz. of water before coming into the laboratory, and to wear comfortable exercise clothing.

Failed Attempt at Dietary Intervention

All of the young subjects and seven of the old subjects were instructed to continue with the normal eating habits throughout the study. In an initial experimental attempt to enhance muscle glycogen content in some older individuals, four old subjects were instructed to eat a diet high in carbohydrates for 3 days prior to the experimental session. Subjects were counseled on food choices and given food guidelines to assist with dietary choices during the initial visit. When given free food choices in a controlled environment, elderly subjects generally ingest approximately 30% of their total calories from carbohydrates and 50% from fat (Wurtman, 1988). Since the main goal of the study design was to enhance skeletal muscle glycogen content, the high carbohydrate diet attempted to guide the older subjects to eat 60-70% of their total calories from carbohydrates. Additionally, this diet attempted to decrease calories from fat accordingly (protein intake will remain unaltered if possible). Dietary logs for all study subjects were maintained in the 3 days prior to the experimental session. These logs were analyzed via Nutritionist Pro™ software to assess caloric intake and dietary composition.

Unfortunately, the older subjects intended to maintain a high carbohydrate diet did not successfully accomplish this, either due to low adherence or lack of nutritional choice knowledge. Dietary analysis of food logs showed no difference between the old “ad lib” group and the high carbohydrate old group. Table 3.3 presents the diet breakdown of both old groups of subjects, as well as the young subjects. Since dietary composition was virtually identical between the old “ad lib” group and the group intended for high carbohydrate, all old subjects were combined for all analyses in this study.

Table 3.3. Young vs. Old Dietary Intake

	Total kcal/day	% kcal Protein	% kcal Carb	% kcal Fat
All Young (n=7)	1608.4 ± 486.4	15.3 ± 5.4	52.9 ± 12.1	29.7 ± 6.5
All Old (n=9)	1994.4 ± 308.9	17.8 ± 2.9	47.6 ± 10.5	34.6 ± 9.5
Old “Ad Lib”* (n=5)	1994.7 ± 579.4	18.2 ± 2.7	47.0 ± 11.8	34.8 ± 11.2
Old High CHO (n=4)	1993.9 ± 137.6	17.2 ± 3.5	48.4 ± 10.4	34.4 ± 8.5

Table 3.3 represents the breakdown of dietary intake between the Young and Old Groups, and in the two old subgroups. Old “Ad Lib” are the older subjects with no dietary restrictions. The Old High CHO group was instructed to eat a high carbohydrate diet. Upon dietary analysis, it was discovered that the groups were very comparable in the percentage of carbohydrates consumed.

* The data from the Old “Ad Lib” group is a majority sub-sample of the entire group of 7. Dietary logs were not obtained from all subjects in this group.

Experimental Session

One to two weeks following the initial visit, subjects came into either the Brody School of Medicine, room 3S08, or The East Carolina Heart Institute, after an overnight fast for the exercise session. The 1-2 week waiting period was used as a recovery period in the event that muscle soreness is caused by the 10RM testing at the initial visit, as this may skew the results during the exercise session. The 10RM testing was used for the resistance exercise bout in the experimental session in an attempt to stimulate AMPK and the downstream signaling pathways. A muscle biopsy was obtained prior to exercise for pre-exercise measurements, immediately post exercise (after completion of the final

resistance exercise set and completion of preparation for the biopsy), one hour following the immediately post-exercise biopsy, and two hours after taking the immediately post-exercise biopsy. Biopsy time points are based upon the results of Drummond et al. (Drummond, Dreyer et al., 2008), which concluded that AMPK phosphorylation is greater in old versus young men by 1 hour post-resistance exercise, and continues until at least 2 hours post resistance exercise. A figure of the timeline for the experimental session is presented in Figure 3.1.

Timeline of Experimental Session

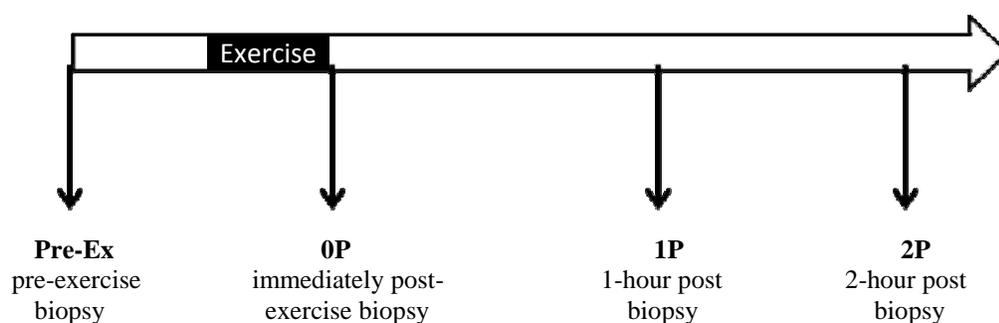


Figure 3.1. Timeline of events for the experimental session for each subject. Pre-Ex is the period prior to the exercise session. 0P is the exercise session. 1P is 60 minutes after the resistance exercise session. 2P is 120 minutes after the resistance exercise session.

Acute Resistance Exercise

The acute resistance exercise was performed on the Cybex Leg Extension machine after the pre-exercise muscle biopsy was obtained. The exercise session first consisted of a warm-up set of 50% of the predetermined 10RM. A 1.5 minute rest interval was given, followed by a second warm-up set consisting of 5-7 repetitions performed at 70% of the predetermined 10RM. Following an additional 1.5 minute rest interval, the final warm-up set consisted of 3-5 repetitions at 90% of the predetermined 10RM. The standard 1.5 minute rest interval was given, and then each participant began the three working sets using the predetermined 10RM. Each working set was performed until failure; both the first and second working set was followed by a 1.5 minute rest interval. Once a participant reached failure, they were encouraged verbally to complete 1-3 forced repetitions with the assistance of the investigator directing the exercise session.

Muscle Biopsies

All biopsy procedures were performed under aseptic conditions. The vastus lateralis muscle was located on each subject, and the area was marked and shaved if necessary. The area was sterilized with iodine swabs and draped with a sterile field. Cold spray was administered to numb the area and approximately 5 mL of local anesthetic (1% Lidocaine) was injected into the biopsy site subcutaneously along the vastus lateralis. A small incision of approximately ¼ inch was made on the skin through the fascia of the vastus lateralis muscle using a No. 11 scalpel. A 5-mm Bergstrom

needle was used for muscle biopsies. Sterile procedure and suction was then applied in order to maximize the amount of muscle extracted during each biopsy.

Immediately following each biopsy, direct pressure and ice was applied to the procedure site to subside bleeding. Once bleeding ceased, a steri strip bandage was applied to hold the incision site together, followed by a band aid, a Tegaderm patch, and a pressure wrap. This particular set-up was to allow proper healing and aid in a decreased likelihood of scarring at the biopsy site.

Preparation for the first two muscle biopsies occurred prior to exercise on each leg. Subsequent biopsies used alternating legs, with the first and third biopsies occurring on the same side. Following the pre-exercise biopsy, the participant performed the acute resistance exercise session. Directly after the resistance exercise bout, the subject was moved to a bed where the immediately post-exercise biopsy was taken. After 60 minutes, the third muscle biopsy was taken approximately 5cm proximal to the initial biopsy site. A final biopsy was taken at 120 minutes post-exercise from the vastus lateralis approximately 5cm from the second biopsy site.

The muscle tissue was divided following removal. One portion was immediately oriented for cross-sectional alignment and mounted in a mixture of tragacantham gum and OCT compound. Following embedding in the OCT mixture, samples were frozen in isopentane chilled in liquid nitrogen. The second portion of the muscle tissue was placed into a cryovial and flash frozen in liquid nitrogen for later western blot analyses. Samples were then stored at 80 degrees Celsius until they were analyzed.

Sample Analysis

Analysis of Muscle Glycogen Content

Analysis of muscle glycogen content occurred using a method modified from a combination of methods derived from Passonneau and Lauderdale (1974), Passonneau and Lowry (1993), and Roy and Tarnopolsky (1998). Specifically, 5-15 mg of each subject's muscle sample was homogenized on ice in 53.3 volumes (3 mg/160 μ L) of 0.1 M NaOH with a ground glass homogenizer. A 100- μ L aliquot of each homogenate was heated for 20 minutes at 80°C in order to destroy any free glucose. Afterwards, the pH of each sample was neutralized with 100 μ L of 0.2 M acetic acid and vortexing. Next, two 50- μ L aliquots of each sample were placed into duplicate tubes containing 200 μ L of 0.1 M sodium acetate buffer (0.05 M acetic acid; 0.05 M sodium acetate, pH 4.6-4.7). All remaining steps were performed in duplicate. To obtain free glucosyl units, glycogenolysis was accomplished by adding 10 μ L of amyloglucosidase (10 μ g/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), vortexing, and then incubating at room temperature for 1 hour. Samples were clarified of any precipitate that remained in suspension by centrifugation at 16000 x g for 5 minutes. A glucose assay that is commercially available (based upon hexokinase and glucose-6-phosphate dehydrogenase reactions; Sigma-Aldrich # GAHK20) was then performed to determine free glucosyl units in each sample. The glycogen content (μ g/mg wet weight) of the original muscle samples was then determined by comparison to a standard curve made with type III glycogen from rabbit liver (Sigma-Aldrich # G8876). This was treated in exactly the same manner as the muscle samples starting with the NaOH step.

Western Blotting Analysis for AMPK Phosphorylation, ACC, FOXO3A Protein, and MuRF1

AMPK, ACC, FOXO3A protein and phosphorylation measurements, and MuRF1 were all analyzed using the Western blot analysis method. The primary antibodies are 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-MuRF1 (CST; Cat # 4305), anti-phospho-FOXO3A (CST; Cat # 9465), and anti-phospho-Acetyl CoA carboxylase (Millipore Corporation; Temecula, CA; Cat. # 07-303). Phospho-Acetyl CoA is an *in vivo* indicator of AMPK activity. A small piece of each frozen muscle biopsy sample was homogenized using a buffer that consisted of 50 mM HEPES (pH 7.4), 0.1% Triton X-100, 4 mM EGTA, 10 mM EDTA, 15 mM $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$, 100 mM β -glycerophosphate, 25 mM NaF, 50 $\mu\text{g}/\text{ml}$ leupeptin, 50 $\mu\text{g}/\text{ml}$ pepstatin, and 33 $\mu\text{g}/\text{ml}$ aprotinin. All homogenizations were performed in ice to prevent excessive heat build-up. Heat build-up can denature proteins. Additionally, all homogenizations were performed using a ground glass homogenizer that utilizes a variable speed motor.

Assessment of the homogenates for protein concentration was carried out in triplicate using a modification of the Lowry procedure (DC Protein Assay, Bio-Rad, Hercules, CA, USA). Total muscle protein homogenates were mixed in a loading buffer (50 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 2% β -mercaptoethanol, 0.1% bromophenol blue) at a dilution of 1 mg per ml. The mixture was then boiled for 5 minutes. Proteins were separated by a 4-7.5% gradient sodium dodecyl sulfate-

polyacrylamide gel electrophoresis (SDS-PAGE). Gel electrophoresis occurred for 1.5 hours at 4°C on a PVDF membrane at 100V in a transfer buffer. The buffer contained 25 mM Tris-base pH~8.3, 192 mM glycine, and 20% methanol. Ponceau S, stained the membranes. Following staining, they were dried and scanned into a digital image. This image allows measurement of the relative total protein loaded into each label through the gray scale integrated optical density of the full length of each individual lane.

Membranes were then blocked for one hour at room temperature in blocking buffer, consisting of 5% nonfat dry milk in TBS-T (20 mM Tris-base, 150 mM NaCl, 0.1% Tween-20) pH 7.5. Following that step, the membranes were incubated in the primary antibody diluted in 1% bovine serum albumin in TBS-T overnight at 4°C. Primary antibody dilutions consisted of anti-phospho-AMPK: 1/4000; anti-AMPK: 1/1000; anti-phospho-ACC: 1/1000; anti-ACC: 1/1000; anti-FOXO3A: 1/1000; anti-MuRF1: 1/1000. Membranes were then washed 4 times with 5 minutes between each wash in TBS-T, incubated in secondary antibody in blocking buffer for an hour while at room temperature. There was then another round of 4 x 5 minutes wash periods in TBS-T.

Following the last wash, detection of the HRP activity occurred using a chemiluminescence reagent (Amersham, Piscataway, NJ) and exposure to autoradiographic film (Classic Blue Sensitive; Midwest Scientific, St Louis, MO, USA). The integrated optical densities (IODs) were quantified by densitometry and calculation of the concentration of the antigen present in each muscle as the IOD was normalized to units of total muscle protein initially loaded on the gel. Correction for the grayscale IOD of each total lane was evaluated on the image of the Ponceau stain that was previously

captured. The HRP-conjugated anti-rabbit secondary antibody was acquired from Amersham.

Statistics

An analysis of variance (ANOVA) with repeated measures was used to analyze the differences between and within groups over time for glycogen and all western blot data. Fischer's LSD post-hoc test for measurement of post-hoc differences was utilized where necessary. All correlational analyses were performed as Pearson Product-moment correlations. Subject characteristic data were analyzed using independent samples t-tests. Significance was set at an alpha level of $p \leq 0.05$.

CHAPTER IV: RESULTS

Subject Strength and Work Volume

All subjects completed both the initial visit and experimental session. Exercise performance was measured using both the total and relative volume of exercise for each group (Y and O). Total volume was determined by multiplying the total amount of weight lifted by the repetitions for each work set (excluding warm-up sets) during the experimental session. Relative volume was determined by taking the total volume and divided by each individual's fat-free mass (kg). Data are presented in Table 4.1 (below). Additionally, 10-RM (kg) values for young and old groups were broken down by gender (Table 4.2).

Table 4.1. Assessment of 10-repetition Maximum Strength and Workout Volume Between Young Adults (Y) and Old Adults (O)

	Y (n=7)	O (n=11)
Estimated 10 RM (kg)*	48 ± 10	32 ± 14
Estimated total volume (kg resistance x repetitions)	1644.6 ± 195	1131.8 ± 395
Relative volume * (kg resistance/kg FFM)	28 ± 1.9	19 ± 4.9

*A significant difference between young and old groups was found ($p < 0.05$). Total volume (kg resistance) was calculated to assess the absolute amount of weight lifted during the working sets by each subject with 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})]. Note that the total volume calculations are only an estimate, as the last 1-3 repetitions of each working set were usually assisted to varying degrees in order to help the subject complete the repetitions. Total volume was normalized to fat free mass in order to show the relative amount of weight lifted: relative volume = total volume (kg resistance) / fat free mass (kg). All data are presented as means ± SEMs.

Table 4.2. Estimated 10-RM Breakdown between Genders

	YM (n=4)	YF (n=3)	OM (n=5)	OF (n=6)
Estimated 10-RM (kg)	55.4±12.8	38.6±9.9*	40.0±8.5	27.7±9.6*

All data are presented as Means ± SEMs. *A significant main effect of gender was found in estimated 10-RM between young males (YM) and old males (OM) combined vs. young females (YF) and old females (OF) combined. Significance is set at $p \leq 0.05$.

Muscle Glycogen Content

A significant difference was seen in glycogen content between young and old subjects at the pre-exercise time point. Additionally, there was a significant main effect of time points 0P (immediately post-exercise), 1P (1-hour post-exercise) and 2P (2-hours post-resistance exercise) for both young and old groups (Figure 4.1). There was also a main effect for percent decrease in glycogen content across all post-exercise time points. The young group had a greater drop in glycogen content following the acute resistance exercise bout than the old group.

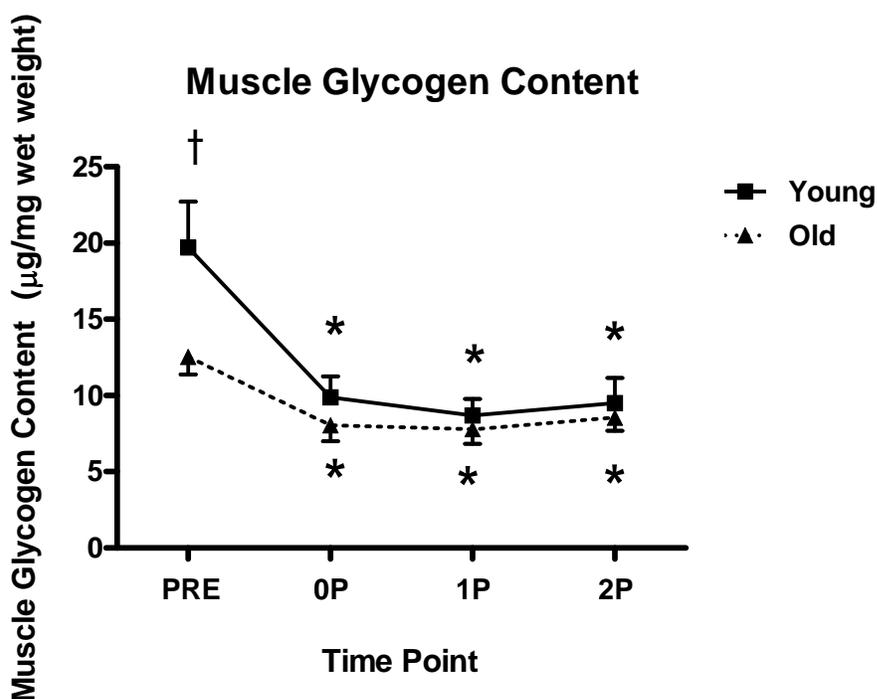


Figure 4.1. Mean \pm SEM glycogen content in young and old subjects. PRE is the pre-exercise muscle biopsy, 0P is the immediately post-exercise muscle biopsy, 1P is the 1 hour post-exercise muscle biopsy, 2P is the 2 hour post-exercise muscle biopsy.

†Significant ($p < 0.05$) difference between young and old groups at PRE time point.

*Significantly lower than PRE within young and old groups independently. There was

also a main effect for percent decrease in glycogen content in young vs. old of time point 0P, 1P, and 2P (data not shown).

AMPK Western Blotting

A significant increase was found in AMPK phosphorylation at Thr¹⁷² versus PRE (pre-exercise) in old subjects at the 0P (immediately post-resistance exercise) time point, but this was not observed in the young group (Figure 4.2). No difference was found in total AMPK between young and old subjects or at any time point (Figure 4.3). A significant main effect time point for phospho/total AMPK ratio and post-hoc analysis showed that time point 0P was elevated versus PRE for young and old groups combined (Figure 4.4).

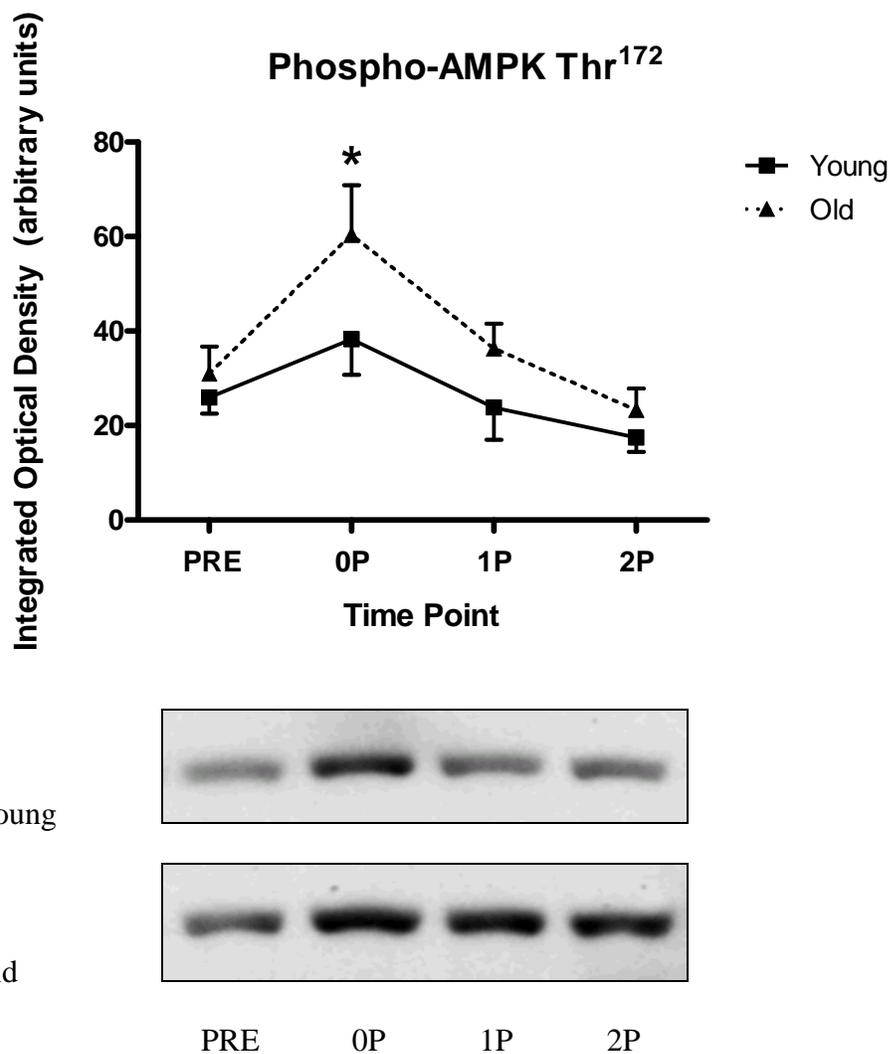


Figure 4.2. Mean \pm SEM phospho-AMPK in young and old subjects and representative blots. PRE is the pre-exercise muscle biopsy, 0P is the immediately post-exercise muscle biopsy, 1P is the 1 hour post-exercise muscle biopsy, 2P is the 2 hour post-exercise muscle biopsy. * Phospho-AMPK was significantly ($p \leq 0.05$) elevated above PRE in old subjects at the 0P time point.

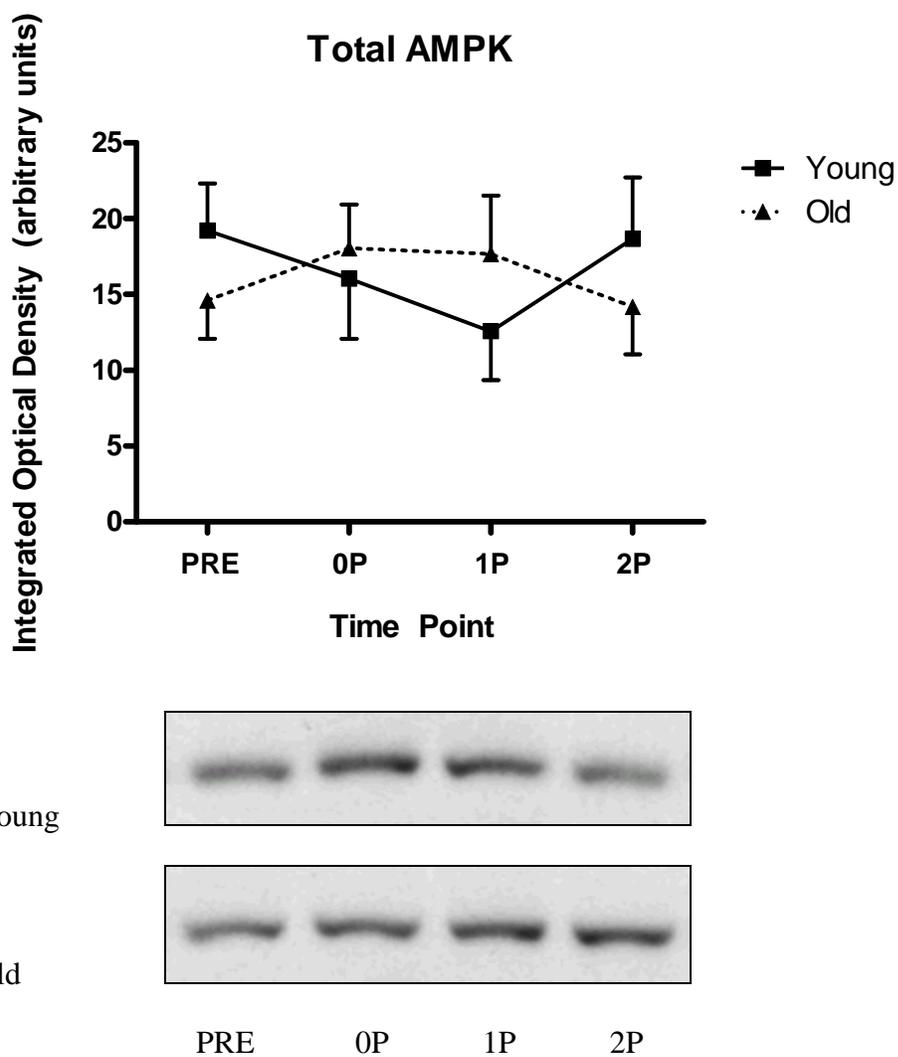


Figure 4.3. Mean \pm SEM phospho-AMPK in young and old subjects and representative blots. PRE is the pre-exercise muscle biopsy, 0P is the immediately post-exercise muscle biopsy, 1P is the 1 hour post-exercise muscle biopsy, 2P is the 2 hour post-exercise muscle biopsy. Total AMPK showed no significant differences between young and old groups, or across any time points.

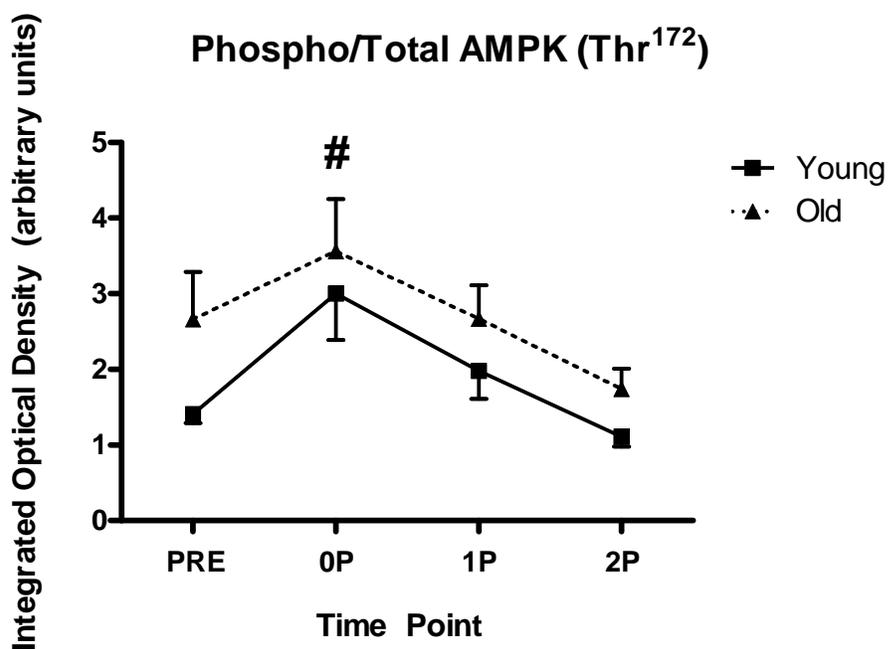


Figure 4.4. Mean \pm SEM phospho/total AMPK ratio in young and old subjects. PRE is the pre-exercise muscle biopsy, 0P is the immediately post-exercise muscle biopsy, 1P is the 1 hour post-exercise muscle biopsy, 2P is the 2 hour post-exercise muscle biopsy. # Phospho/total AMPK ratio showed a significant main effect time point, and post-hoc analysis showed that time point 0P was elevated vs. PRE for young and old groups combined.

ACC Western Blotting

Phospho-acetyl CoA carboxylase (ACC) at Ser⁷⁹ showed a significant main effect time point, and post-hoc analysis showed that time points 0P (immediately post-exercise) and 1P (1 hour post-exercise) were elevated versus pre-exercise in young and old groups combined (Figure 4.5). The same findings were consistent for total ACC (Figure 4.6) and the phospho/total ACC ratio (Figure 4.7).

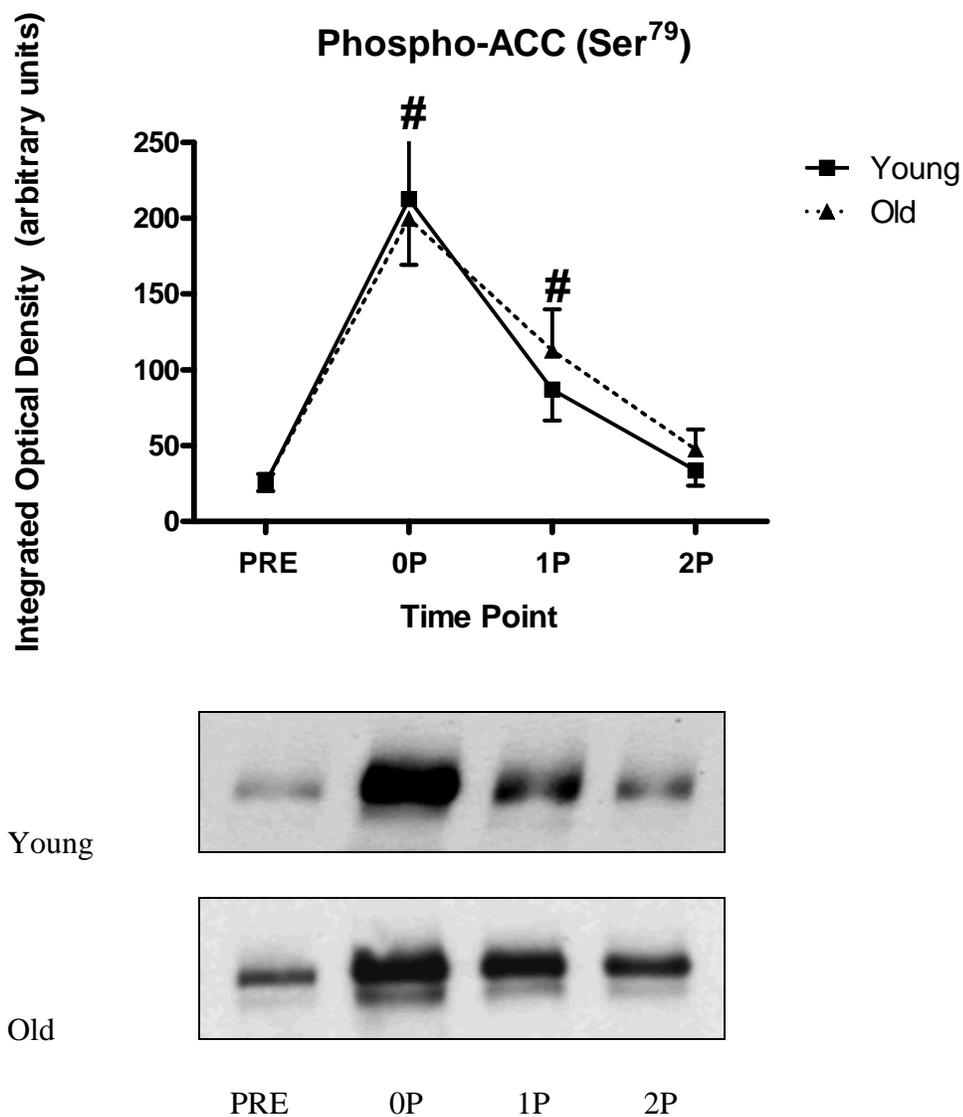


Figure 4.5. Mean \pm SEM phospho-ACC in young and old subjects and representative blots. PRE is the pre-exercise muscle biopsy, 0P is the immediately post-exercise muscle biopsy, 1P is the 1 hour post-exercise muscle biopsy, 2P is the 2 hour post-exercise muscle biopsy. # Significant ($p \leq 0.05$) main effect of time points, and post-hoc analysis showed that time points 0P and 1P were elevated vs. PRE for young and old groups combined.

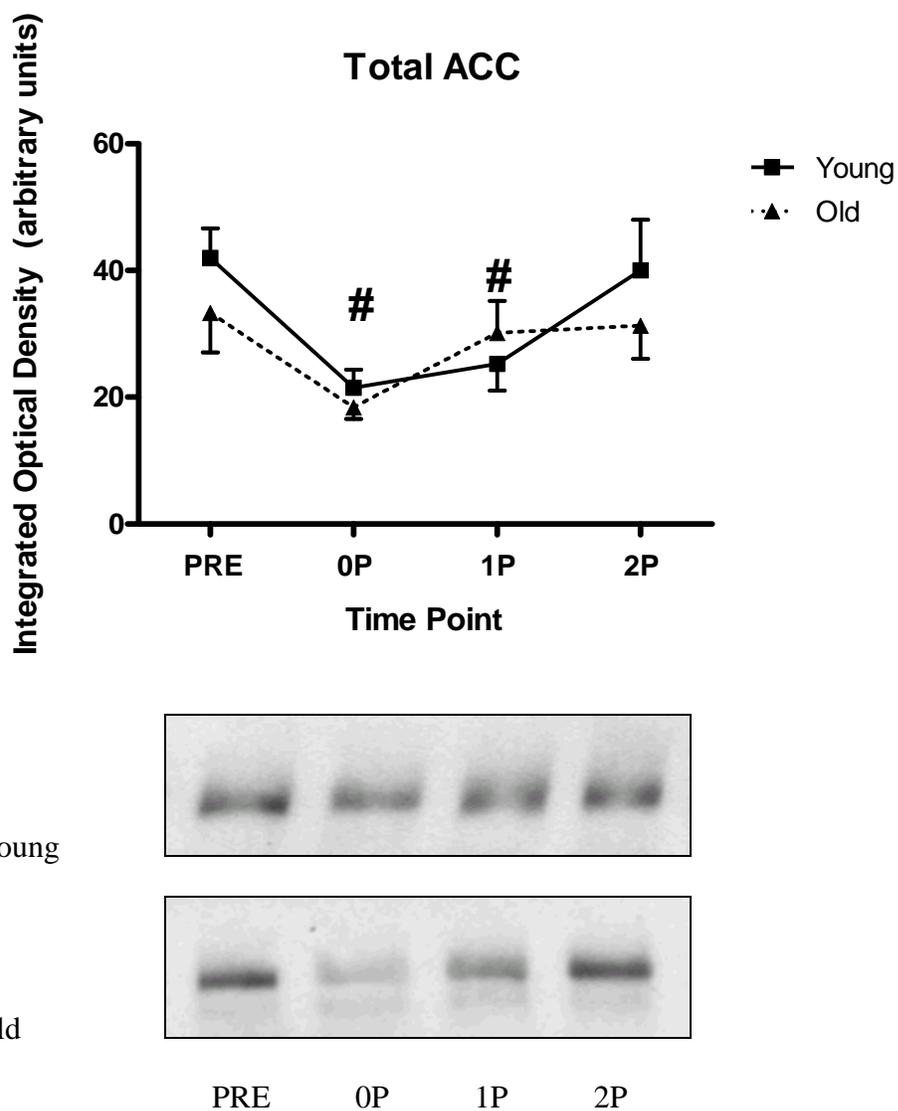


Figure 4.6. Mean \pm SEM total ACC in young and old subjects and representative blots. PRE is the pre-exercise muscle biopsy, 0P is the immediately post-exercise muscle biopsy, 1P is the 1 hour post-exercise muscle biopsy, 2P is the 2 hour post-exercise muscle biopsy. # Significant ($p \leq 0.05$) main effect of time points, and post-hoc analysis showed that time points 0P and 1P were decreased vs. PRE for young and old groups combined.

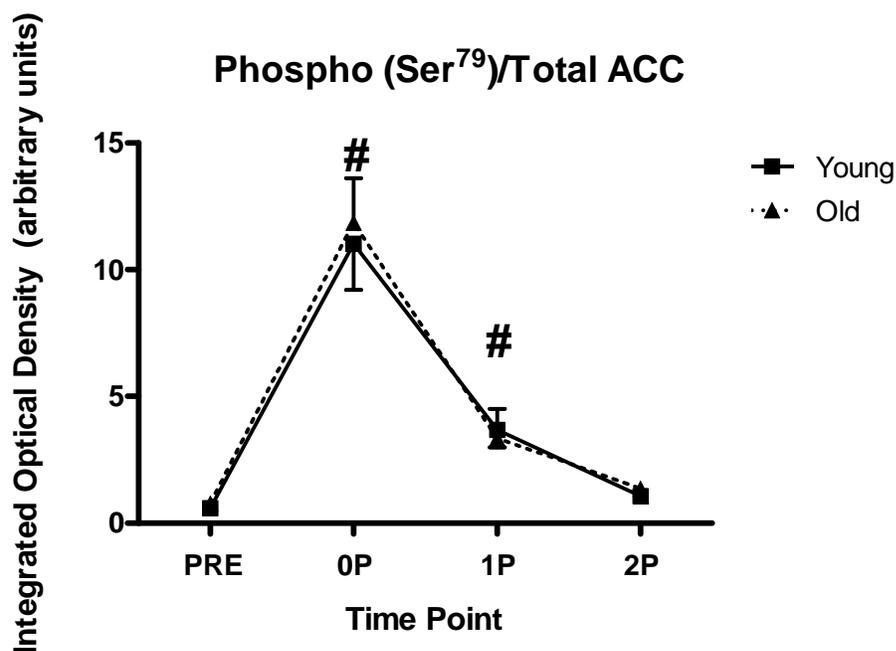


Figure 4.7. Mean \pm SEM phospho (Ser⁷⁹)/total ACC ratio in young and old subjects. PRE is the pre-exercise muscle biopsy, 0P is the immediately post-exercise muscle biopsy, 1P is the 1 hour post-exercise muscle biopsy, 2P is the 2 hour post-exercise muscle biopsy. # Significant ($p \leq 0.05$) main effect of time points, and post-hoc analysis showed that time points at 0P and 1P were elevated vs. PRE for young and old groups combined.

FOXO3A Western Blotting

There were no significant differences observed in phospho-FOXO3A at Ser^{318/321} pre- to post-exercise or between young and old subjects at any of the time points (Figure 4.8). The percent change from baseline (pre-exercise) of phospho-FOXO3A at Ser^{318/321} showed a p-value of 0.17 for the main effect of young versus old subjects across all time points (Figure 4.9).

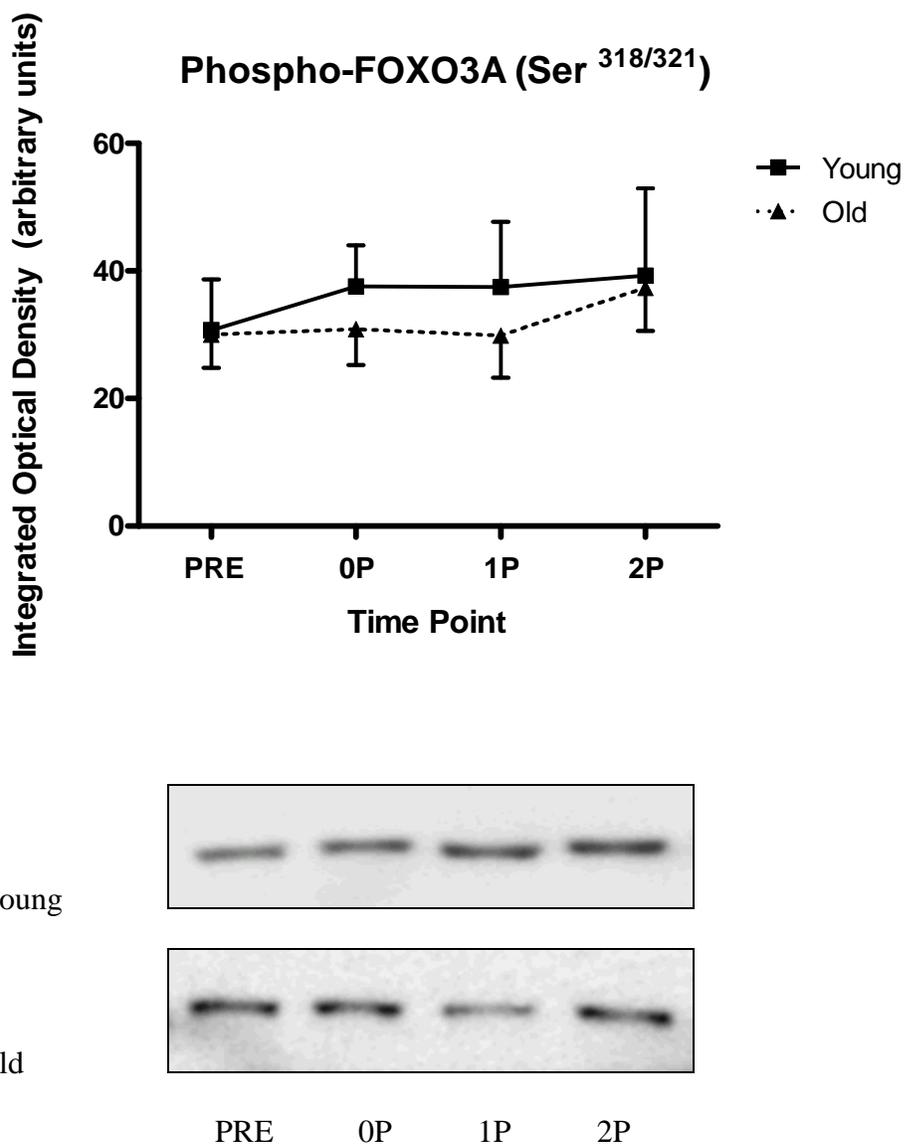


Figure 4.8. Mean \pm SEM phospho-FOXO3A at Ser^{318/321} in young and old subjects and representative blots. PRE is the pre-exercise muscle biopsy, 0P is the immediately post-exercise muscle biopsy, 1P is the 1 hour post-exercise muscle biopsy, 2P is the 2 hour post-exercise muscle biopsy. Phospho-FOXO3A was not significantly different between age groups or across any time points.

Phospho-FOXO3A (Ser^{318/321}) Percent Change from Baseline

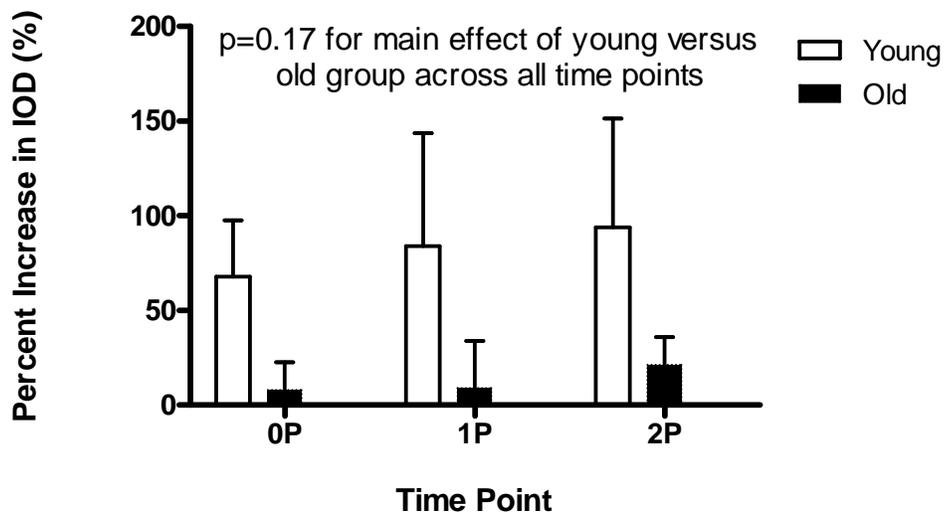


Figure 4.9. Mean \pm SEM for percent change of FOXO3A at Ser^{318/321} from baseline (PRE) in young and old subjects. PRE is the pre-exercise muscle biopsy or baseline, 0P is the immediately post-exercise muscle biopsy, 1P is the 1-hour post-exercise muscle biopsy, 2P is the 2-hour post-exercise muscle biopsy. $p = 0.17$ for main effect of young vs. old group across all time points.

MuRF1 Western Blotting

No significant differences were seen in total MuRF1 in response to exercise or between young and old subjects at any time point (Figure 4.10). For MuRF1 percent change from baseline (pre-exercise), there was a p-value of 0.155 for the main effect of young versus old subjects across all time points. In addition, there was a significant main effect time point for total MuRF1, and post-hoc analysis showed that time point 2P (2 hours post-exercise) was decreased versus 0P (immediately post-exercise) for both young and old groups (Figure 4.11).

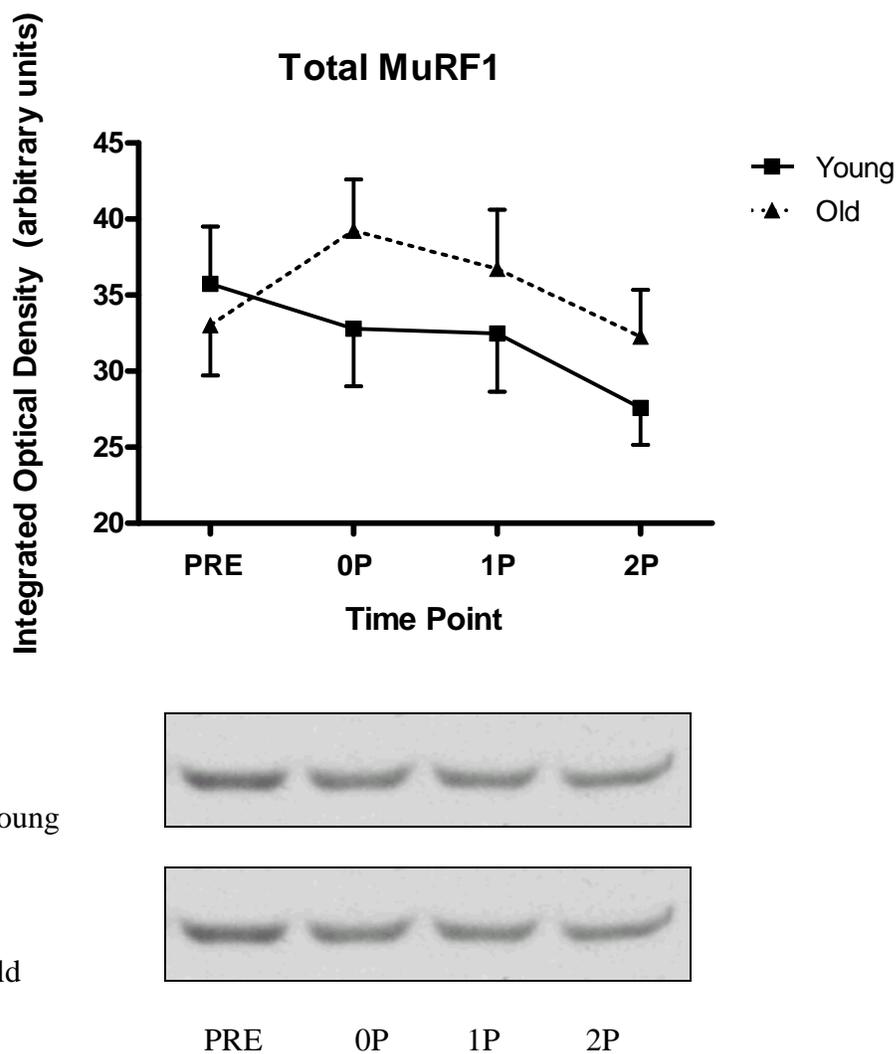


Figure 4.10. Mean \pm SEM total MuRF1 in young and old subjects and representative blots. PRE is the pre-exercise muscle biopsy, 0P is the immediately post-exercise muscle biopsy, 1P is the 1 hour post-exercise muscle biopsy, 2P is the 2 hour post-exercise muscle biopsy. No significant differences were seen in total MuRF1 between young and old subjects, or across any time points.

MuRF1 Percent Change from Baseline

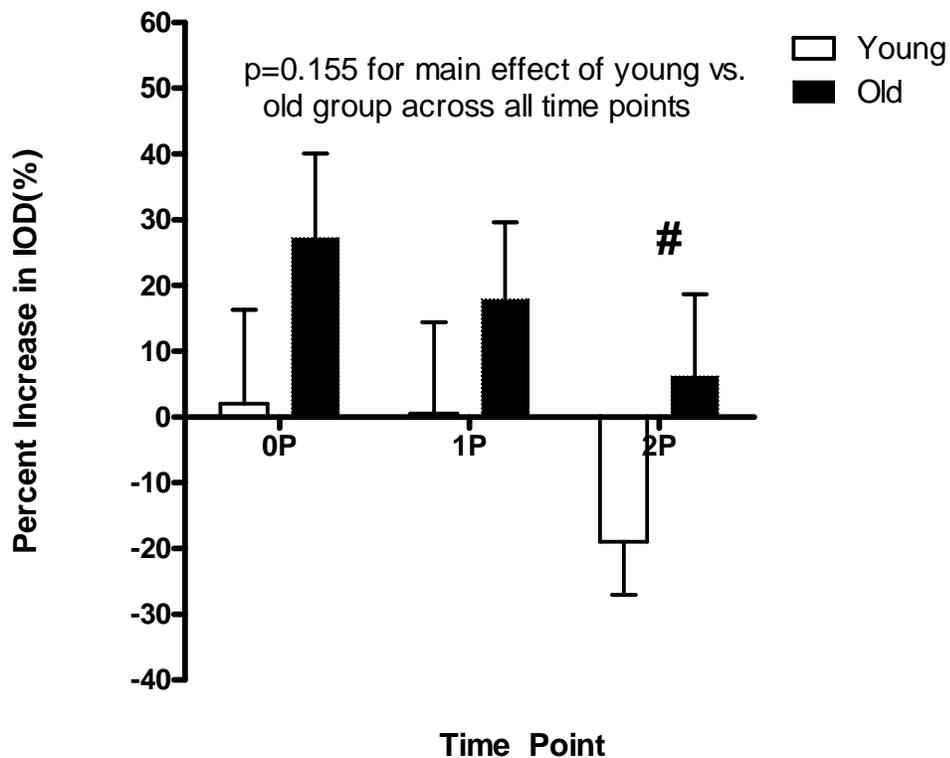
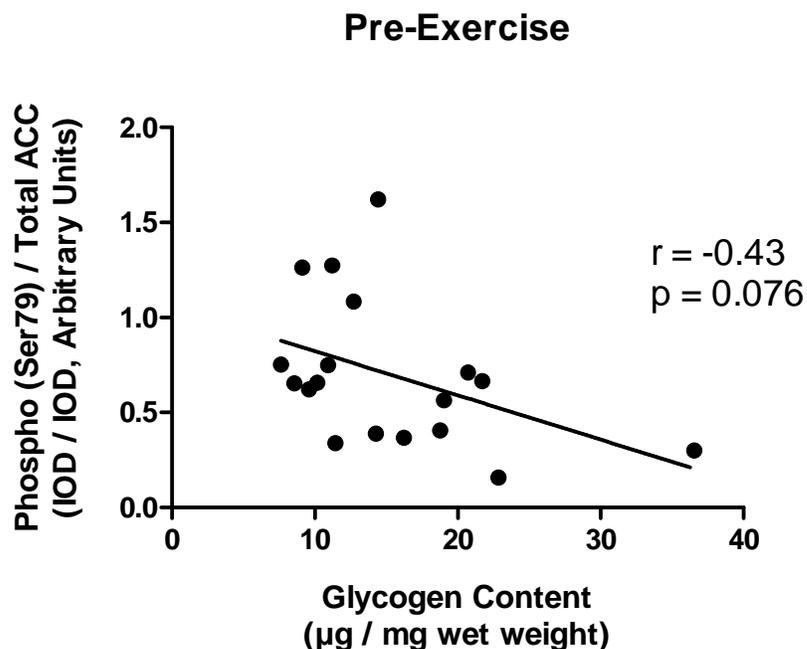


Figure 4.11. Mean \pm SEM percent change of MuRF1 from baseline (PRE) in young and old subjects. PRE is the pre-exercise muscle biopsy or baseline, 0P is the immediately post-exercise muscle biopsy, 1P is the 1 hour post-exercise muscle biopsy, 2P is the 2 hour post-exercise muscle biopsy. # Significant ($p \leq 0.05$) main effect at time point 2P versus 0P in young and old groups combined. $p = 0.155$ for main effect of young vs. old across all time points.

Association of AMPK Activity and Phospho-FOXO3A with Glycogen Content

We found a significant, or close to significant, relationship between glycogen content and both FOXO3A phosphorylation and the phospho/total ACC ratio at most timepoints (Figs 4.12-4.15). We chose to examine the relationship of glycogen content with ACC phosphorylation status rather than AMPK phosphorylation because it is a better indicator of AMPK activity (Park et al., 2002). Higher muscle glycogen content was associated with lower phospho/total ACC ratio and higher FOXO3A phosphorylation. P-values were all very close or below the significant value of $p \leq 0.05$ at the PRE, 0P, and 1P timepoints for phospho/total ACC ratio, and at the 0P, and 1P timepoints for FOXO3A phosphorylation. Thus, by increasing muscle glycogen content, AMPK activity may be decreased, while phospho-FOXO3A at Ser^{318/321} may be accentuated, potentially lessening the effect on the protein degradation pathway.



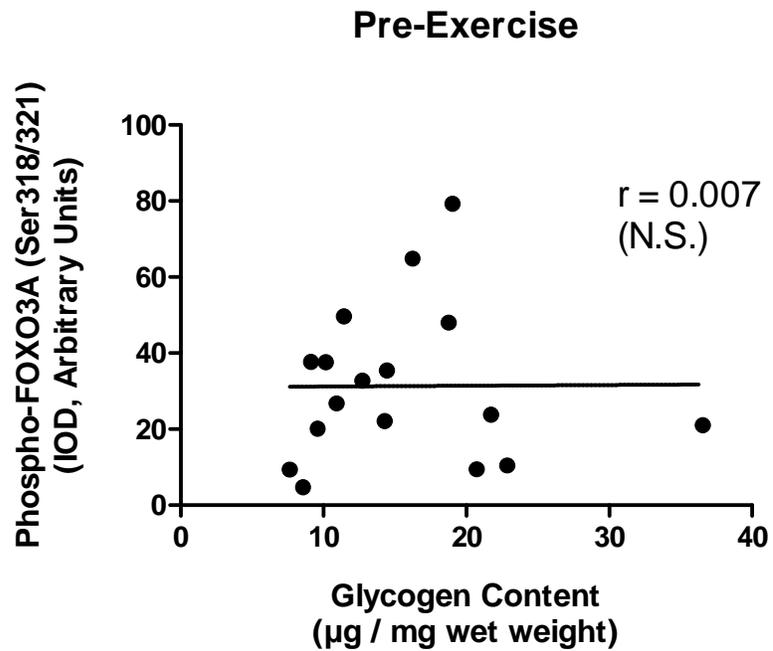


Figure 4.12. Relationship between glycogen content and the phospho/total ACC ratio (top) and between glycogen content and phospho-FOXO3A (bottom) at the pre-exercise time point. Significance is set at $p \leq 0.05$.

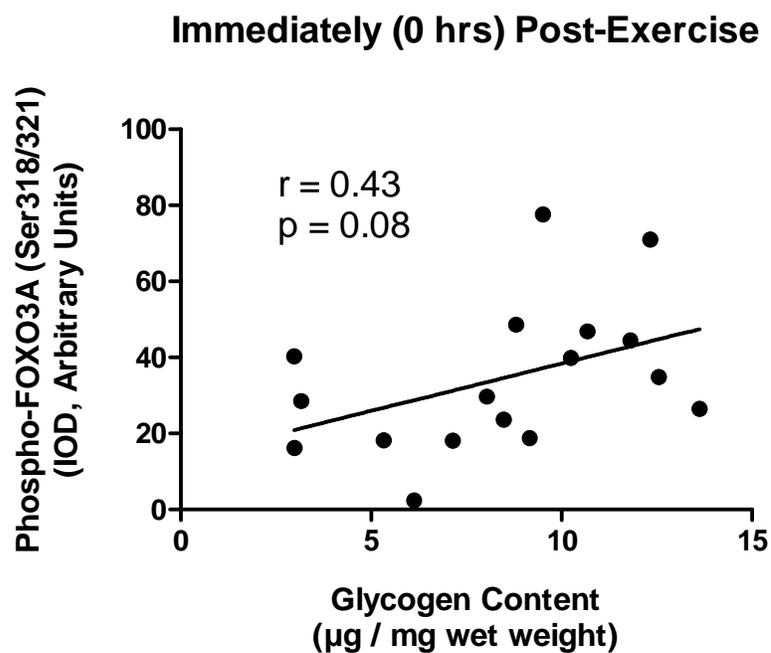
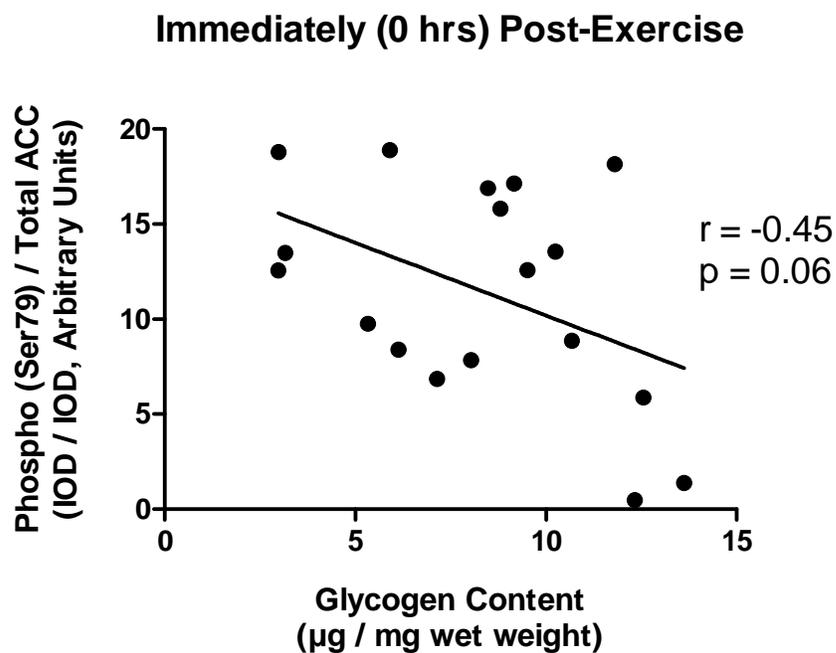


Figure 4.13. Relationship between glycogen content and the phospho/total ACC ratio (top) and between glycogen content and phospho-FOXO3A (bottom) at the immediately post-exercise time point. Significance is set at $p \leq 0.05$.

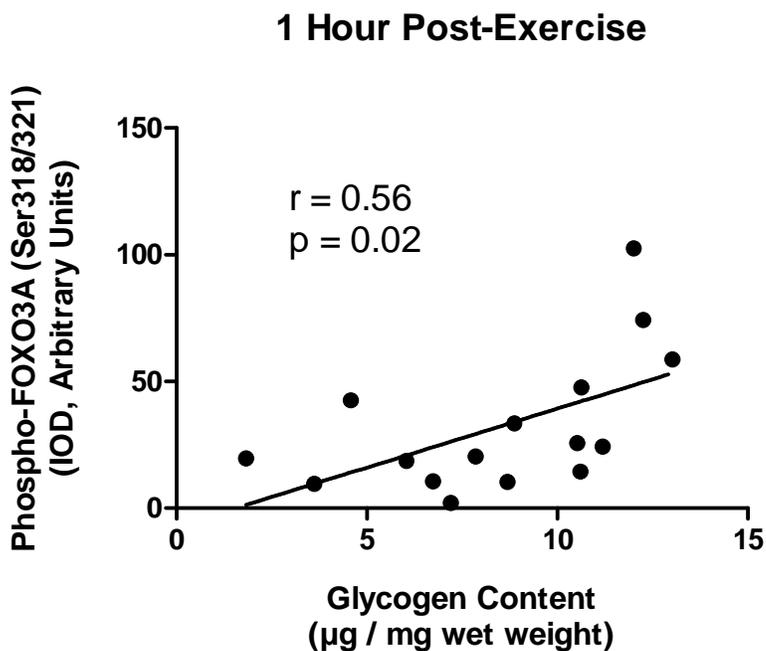
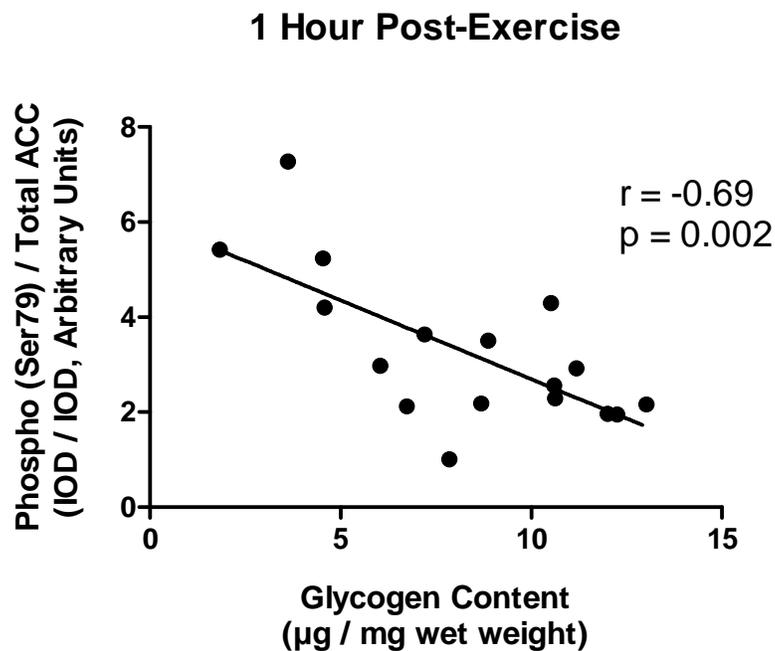


Figure 4.14. Relationship between glycogen content and the phospho/total ACC ratio (top) and between glycogen content and phospho-FOXO3A (bottom) at the one-hour post-exercise time point. Significance is set at $p \leq 0.05$.

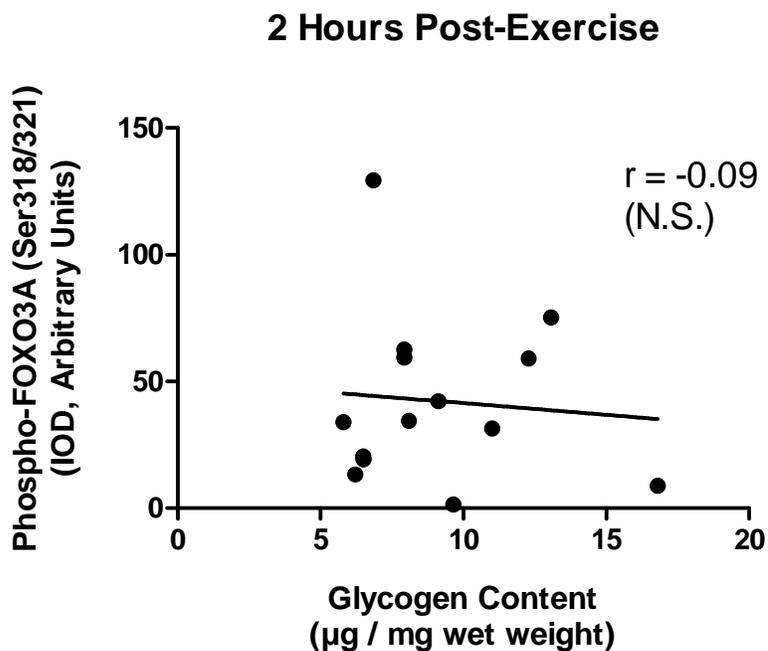
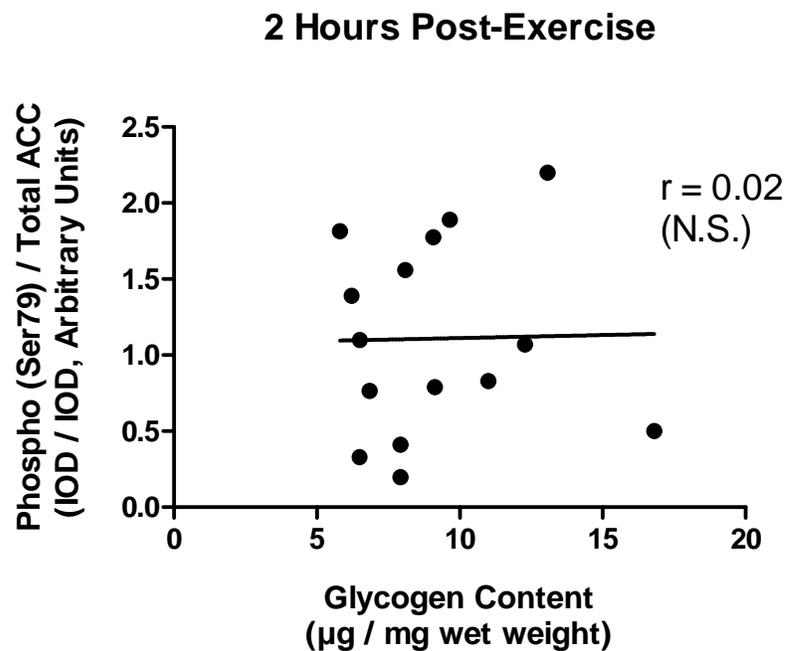


Figure 4.15. Relationship between glycogen content and the phospho/total ACC ratio (top) and between glycogen content and phospho-FOXO3A (bottom) at the two-hour post-exercise time point. Significance is set at $p \leq 0.05$.

To further investigate the potential effect of pre-exercise muscle glycogen content on the phospho-FOXO3A response to resistance exercise, subjects were grouped into either a high glycogen ($>16 \mu\text{g}/\text{mg}$) or low glycogen ($<12 \mu\text{g}/\text{mg}$) group regardless of age. The high glycogen group consisted of 5 young and 2 old subjects, while the low glycogen group consisted of 2 young and 6 old subjects. A significant main effect was observed in phospho-FOXO3A (Ser^{318/321}) between high and low glycogen content across all time points (Figure 4.16). There was also a significant main effect observed in the phospho-FOXO3A (Ser^{318/321}) percent change from baseline across all post-exercise time points in high and low glycogen content groups (Figure 4.17).

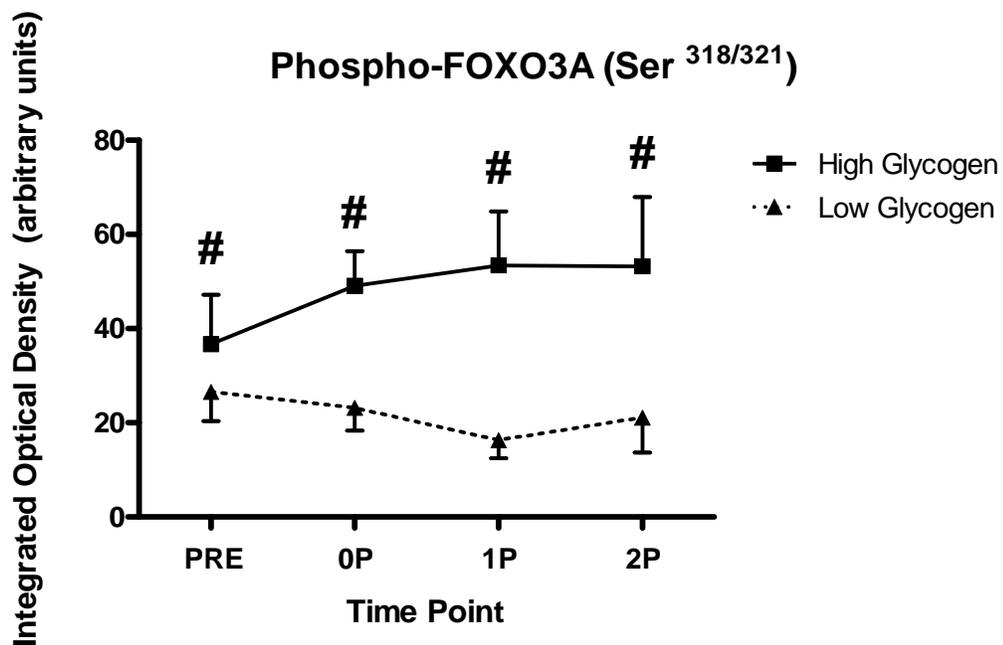


Figure 4.16. Mean \pm SEM phospho-FOXO3A (Ser^{318/321}) in high muscle glycogen vs. low muscle glycogen groups. PRE is the pre-exercise muscle biopsy, 0P is the immediately post-exercise muscle biopsy, 1P is the 1 hour post-exercise muscle biopsy, 2P is the 2 hour post-exercise muscle biopsy. # Significant ($p \leq 0.05$) main effect of high glycogen group vs. low glycogen group across all time points.

Phospho-FOXO3A (Ser^{318/321}) Percent Change from Baseline

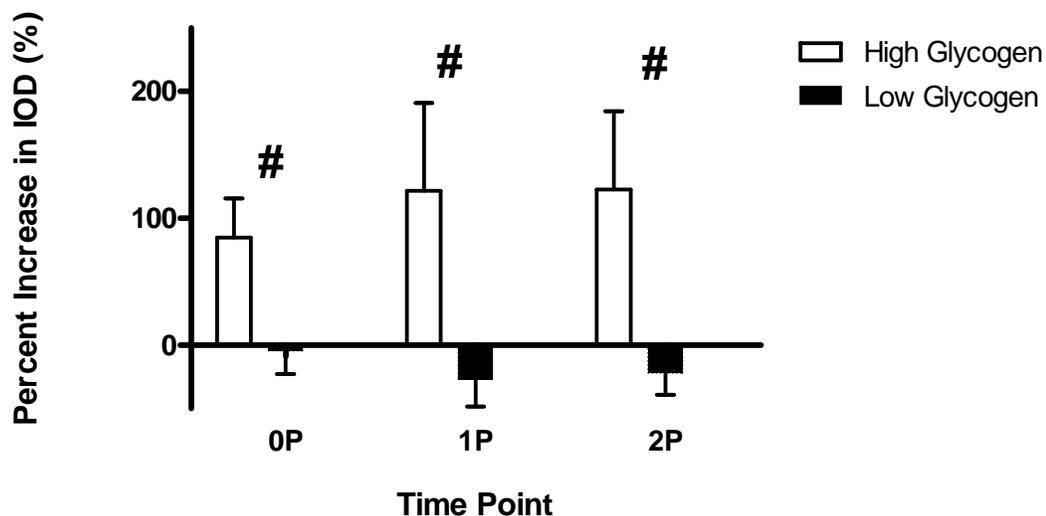


Figure 4.17. Mean \pm SEM for percent change of FOXO3A at Ser^{318/321} from baseline (PRE) in high vs. low glycogen groups. PRE is the pre-exercise muscle biopsy or baseline, 0P is the immediately post-exercise muscle biopsy, 1P is the 1-hour post-exercise muscle biopsy, 2P is the 2-hour post-exercise muscle biopsy. # Significant ($p \leq 0.05$) main effect of high glycogen group vs. low glycogen group across all post-exercise time points. Significance is set at $p \leq 0.05$.

Association of AMPK Activity and FOXO3A

We also examined the relationship between AMPK activity (as indicated by phospho/total ACC ratio at Ser⁷⁹) and phospho-FOXO3A (Ser^{318/321}) pre-exercise (Figure 4.18), immediately post-resistance exercise (Figure 4.19), 1-hour post-exercise (Figure 4.20), and 2-hours post-exercise (Figure 4.21). The observed associations were very weakly negative (non-significant) across all four time points.

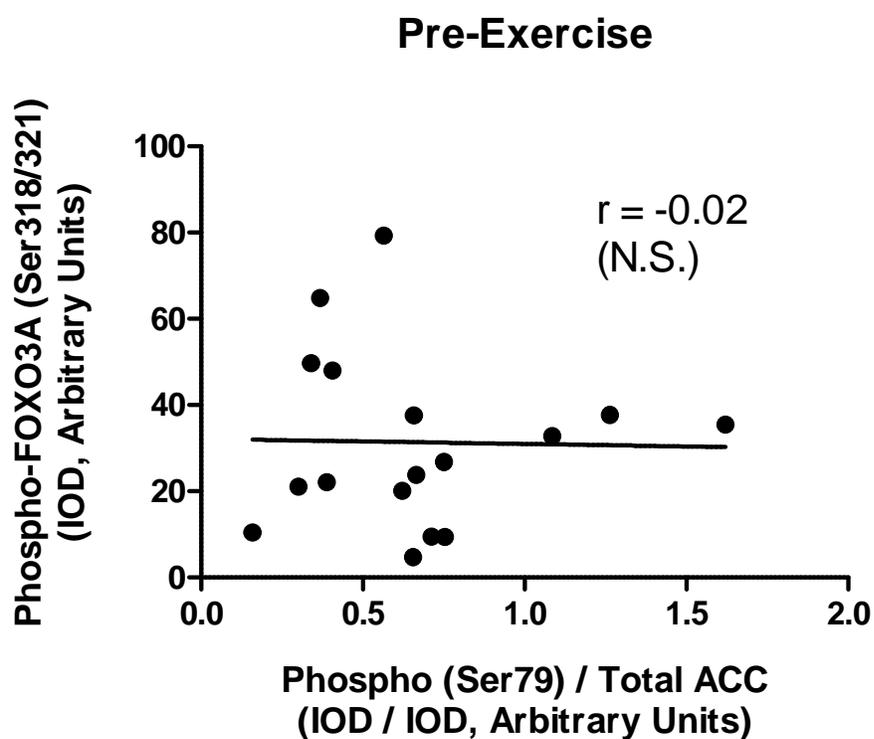


Figure 4.18. Relationship between the phosphor/total ACC ratio and phospho-FOXO3A pre-exercise. Significance is set at $p \leq 0.05$.

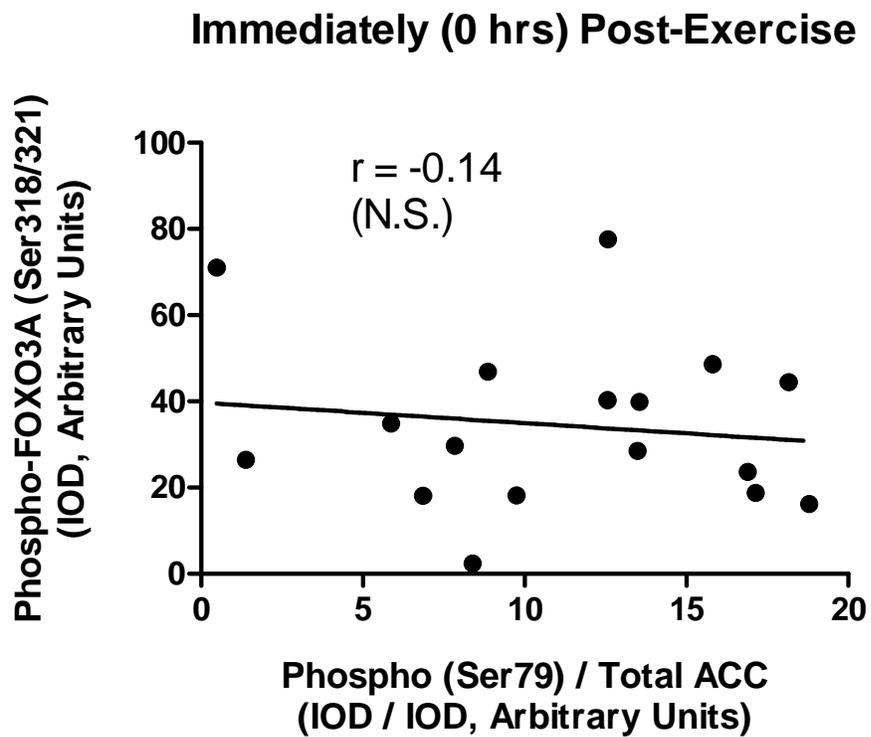


Figure 4.19. Relationship between the phospho/total ACC ratio and phospho-FOXO3A immediately post-resistance exercise. Significance is set at $p \leq 0.05$.

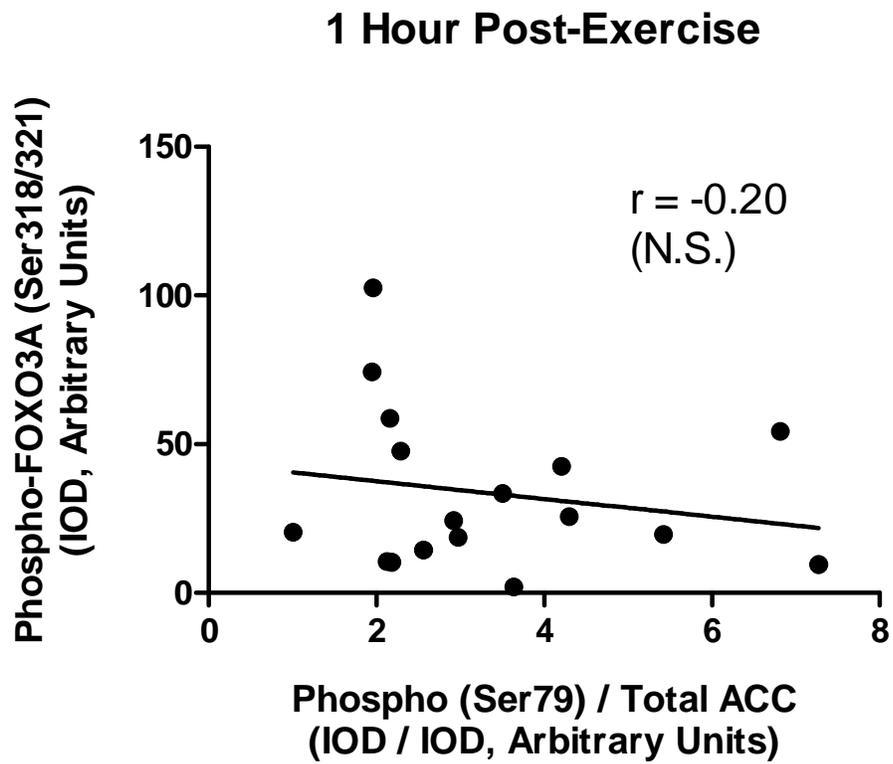


Figure 4.20. Relationship between the phospho/total ACC ratio and phospho-FOXO3A 1-hour post-exercise. Significance is set at $p \leq 0.05$.

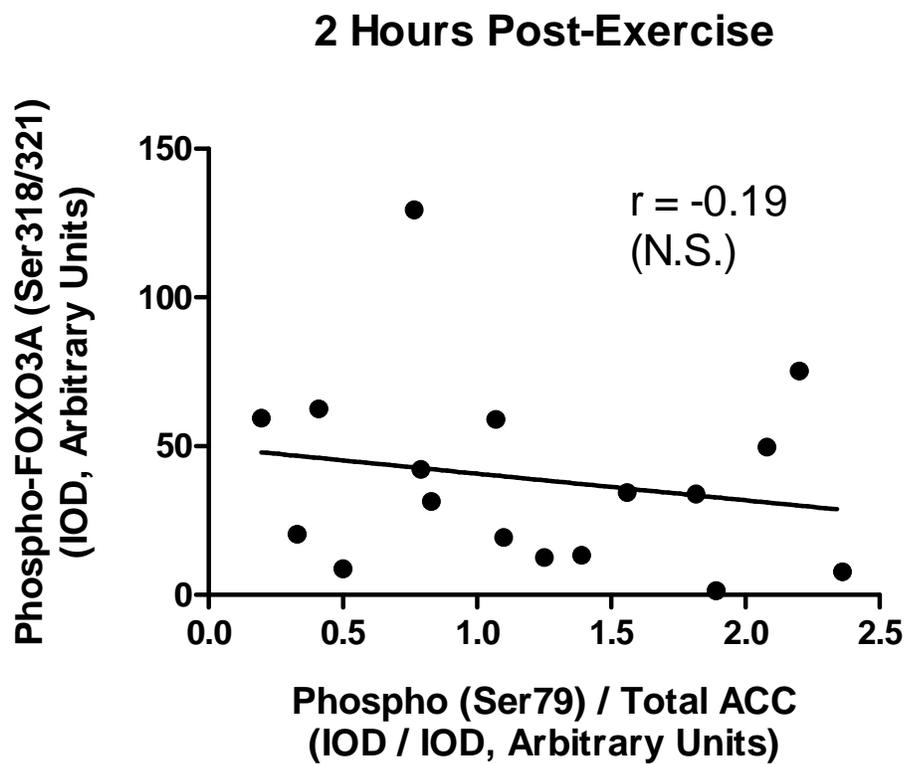


Figure 4.21. Relationship between the phospho/total ACC ratio and phospho-FOXO3A 2-hours post-exercise. Significance is set at $p \leq 0.05$.

CHAPTER V: DISCUSSION

The specific aim of this study was to examine the FOXO3A Ser^{318/321} phosphorylation response to resistance exercise and its relation to muscle glycogen content and AMPK activation in young versus old subjects. We hypothesized that FOXO3A phosphorylation at Ser^{318/321} would be lower in response to acute resistance exercise in the skeletal muscles of older versus younger individuals. It was further hypothesized that this lower FOXO3A phosphorylation response would be related to lower glycogen content and higher AMPK activation in the skeletal muscles of older versus younger individuals.

As hypothesized, AMPK phosphorylation at Thr¹⁷² was elevated in old immediately post-resistance exercise (0P), but not in young. Nevertheless, although there was a significant main effect in the phospho/total AMPK ratio immediately post-resistance exercise in both young and old groups, there were no differences between age groups for ratio. Additionally, there were no differences between young and old groups at any time point in phospho-acetyl CoA carboxylase (ACC), which is a downstream marker of AMPK activity (Hawley et al., 1996). However, there was a significant increase (main effect) at 0P (immediately post-resistance exercise) and 1P (one hour post-exercise) for both young and old groups compared to pre-exercise. This significant increase was also seen in the phospho/total ACC ratio immediately post-resistance exercise and one hour post-resistance exercise in both the young and old group, with no significant differences between age groups across any time points for phospho/total ACC ratio.

The present AMPK phosphorylation results are in partial agreement with some previously published literature. Research conducted by Drummond et al. (Drummond, Dreyer et al., 2008) showed a significant exercise-induced increase in AMPK phosphorylation in older subjects at 1 hour and 3 hours following an acute bout of resistance exercise, while younger subjects had no such increase. While our results show a significant elevation of AMPK phosphorylation in old subjects immediately post-resistance exercise, the phosphorylation of AMPK fell to levels comparable to young subjects by one hour post-exercise. Drummond et al. found phospho-AMPK levels to remain elevated for a longer period of time (3 hours post-resistance exercise), but did not assess the phospho/total AMPK ratio. A number of factors could have contributed to this difference. Drummond et al. (Drummond, Dreyer et al., 2008) used 8 working sets during the acute resistance exercise session, while this study used 3 working sets. This higher and prolonged workload could have contributed to a higher and prolonged AMPK phosphorylation response to the leg extension exercise. This possibility is supported by data from Thompson and Gordon (Thompson and Gordon, 2005), who found a higher AMPK phosphorylation in old muscle when compared to young both at rest and after chronic overload in rats.

Although the current study found a small age difference in the AMPK phosphorylation response to resistance exercise, the ACC phosphorylation response was similar between age groups. In fact, AMPK phosphorylation is not the best indicator of AMPK activity. Glycogen and AMP can bind to AMPK and influence activity by impacting the phosphorylation of AMPK, as well as by allosterically influencing AMPK activity independent of phosphorylation (McBride et al., 2009; Sanders et al., 2007).

ACC is phosphorylated by AMPK at Ser⁷⁹ (Tong et al., 2009) and is considered a better indicator of true in vivo AMPK activity (Park et al., 2002). This may explain why Drummond et al. (Drummond, Dreyer et al., 2008) found no phospho-AMPK increase with resistance exercise in young in contrast with their old subjects, while other data from the same laboratory (Dreyer et al., 2006) have shown an increase in AMPK activity after acute resistance exercise in young subjects. Likewise, the current investigation found a somewhat accentuated AMPK phosphorylation response to resistance exercise in old subjects compared to young, while ACC phosphorylation, an indicator of AMPK activity, was elevated equally in both age groups and in a more prolonged fashion than AMPK phosphorylation. ACC phosphorylation, and phospho/total ACC ratio both showed a significant main effect at the immediately post-exercise and the one-hour post-exercise time points when compared to the pre-exercise values in young and old groups combined. These data indicate that AMPK activation may not have differed between young and old individuals in the current investigation.

A number of factors may have contributed to the lack of difference observed in phospho-ACC between age groups in the current study. Significant differences may not have been seen between the young and old groups because the older subjects were healthier than the average older adult. Thus, this group of old subjects may not be representative of the general older adult population. In conjunction with a healthier than average older group, it is possible that the young group is not a true representation of the young adult population. The delimitations of the current study excluded individuals who participated in regular physical activity. Therefore, the young subjects in the current study may not adhere to as healthy of a lifestyle as the older subjects. Also, there may

have been gender differences in strength and 10-RM that could have played an impact on ACC phosphorylation. However, since male and female subjects characteristics were not differentiated in the current study, these differences could have been diminished in the group averages. In addition, potential differences in phospho-ACC between age groups may have occurred only in fast-twitch fibers and thus been “masked” by homogenization with slow-twitch fibers. Muscle atrophy and diminished muscle hypertrophy occur in fast-twitch muscle fibers, but not slow-twitch fibers (Rogers & Evans, 1993); however, both fiber types are mixed in the muscle biopsy samples taken from the subjects. Thompson and Gordon (Thompson and Gordon, 2005) observed the highest AMPK and ACC results only in fast twitch muscle fibers, but not slow-twitch. Therefore, the mixture of fiber types in the sample could have also played a role in the lack of observable differences between phospho-ACC between young and old groups in the current investigation.

Another possible explanation for the lack of differences seen between AMPK and ACC (AMPK activity) may be fasting status. All of our subjects came into the experimental session following a 12-hour fast. A study by Combaret et al. (Combaret et al., 2005) found that proteolysis was elevated in rat skeletal muscle with increased age; however, the highest effects in proteolysis were seen following a meal. Also, Drummond et al. (Drummond, Dreyer et al., 2008) required their subjects to ingest an essential amino acid and carbohydrate supplement 1 hour following the resistance training exercise. Their use of non-fasted subjects may have had an impact on AMPK phosphorylation because AMPK is partially regulated by internal energy stores. Thus, some age-related differences are postprandial. Therefore, differences in AMPK and AMPK activity may

primarily occur following a meal as opposed to the fasted state. This may also have been a reason why we saw no age-related differences in FOXO3A in the current investigation. FOXO3A activation may be elevated during the fasting state, and may therefore have already been near maximal during the experiment. We may have seen different results had we not required subjects to be fasted during the experimental session.

Additionally, glycogen is known to affect AMPK activity both by phosphorylation and independent of phosphorylation (McBride et al., 2009). Muscle glycogen content before and after resistance exercise in young versus old subjects has not been previously examined. There was a significant difference observed in glycogen content between the old and young groups at the pre-exercise time point. This is in line with previous data, which shows a decrease in resting muscle glycogen levels with age (Cartee, 1994). Additionally, the results of the current study showed a significant post-exercise decrease for both young and old groups. This is also consistent with published literature, as Tesch et al. showed a significant decrease in muscle glycogen content following resistive leg exercise in young subjects (Tesch et al., 1986).

In this study, glycogen dropped to a greater degree in young than in old subjects after exercise and was statistically equal at every time point thereafter. This difference in glycogen loss may be a result of the different skeletal muscle fiber types found in young and old adults. Previous research shows that age-related muscle loss occurs in the fast-twitch muscle fibers as opposed to slow-twitch fibers (Welle, 2002). As a result, older individuals have lower percentage fast-twitch fibers than young individuals [as reviewed (Andersen, 2003)]. Fast-twitch muscle fibers store and utilize more glycogen, which may be linked to the sharper decline in glycogen content from pre-exercise to immediately

post-exercise in the young subjects. In addition, the similar glycogen content seen in the current study after exercise may have had a significant effect on ACC phosphorylation (i.e. AMPK activity) to produce a similar ACC phosphorylation in young and old subjects. Indeed, there was a significant, or near significant, negative correlation between glycogen content and ACC phosphorylation status before, immediately post-, and one-hour post-resistance exercise (Figures 4.12, 4.13, and 4.14).

ACC phosphorylation (or AMPK activity) may have been impacted in the current study by the diet of the participants. The older subjects in this study consumed total calories that were approximately 24% higher than the young subjects, which is not consistent with past research. A study by Wurtman et al. (Wurtman et al., 1988) found that elderly individuals generally consume fewer total calories than their young counterparts. The similarity between young and old subjects in the present study may have been a result of the low total calories consumed by the young subjects. The young subjects averaged approximately 1,600 kcal per day, which is lower than would be expected in the young, healthy population (Wurtman et al., 1988). Overall, the variance in diet from the expected norms may have partially caused the lack of difference observed in ACC phosphorylation (AMPK activity) in this study.

FOXO3A phosphorylation (Ser^{318/321}) showed no significant difference in response to resistance exercise or between young and old groups across time points in the current study. However, there was a non-significant ($p = 0.17$) pattern for the young group to have a higher FOXO3A phosphorylation response to exercise than the old group. Additionally, in contrast to the negative correlation between glycogen content and phospho/total-ACC, phospho-FOXO3A showed a significant, or near significant, positive

correlation with muscle glycogen content over two post-resistance exercise time points. As previously mentioned, Akt phosphorylates FOXO3A, which sequester FOXO3A in the cytosol, block nuclear translocation, and ultimately blocks FOXO3A transcriptional activity. However, AMPK activity inhibits this phosphorylation of FOXO3A by Akt, allowing FOXO3A to translocate into the nucleus of the cell. Glycogen, on the other hand, blocks AMPK activity, allowing FOXO3A to be phosphorylated by Akt [as reviewed (Murton, Constantin & Greenhaff, 2008)]. Therefore, phospho/total ACC, reflecting AMPK activity, would be negatively correlated with muscle glycogen content, while phospho-FOXO3A would be positively correlated with glycogen content.

A high positive relationship of phospho-FOXO3A with glycogen content was observed in the current study; thus, it was decided to separate the groups into high versus low glycogen content regardless of age. A significant effect was observed when the groups were split in this fashion. The high glycogen group showed an increase in FOXO3A phosphorylation, while the low glycogen group showed no change or a slight decrease in FOXO3A phosphorylation in response to resistance exercise. The high and low glycogen groups were split based on pre-exercise (baseline) glycogen content. These results may provide practical implications. Increasing glycogen content through diet may suppress the protein degradation pathway following resistance exercise. Therefore, these results support altering nutritional intake to increase glycogen as a way to potentially decrease protein degradation regardless of age.

A study performed by Nakashima and Yakabe (Nakashima and Yakabe, 2007) showed that AMPK activation stimulates FOXO3A. However, this study looked at the effect of AMPK on FOXO3A mRNA and total protein, while the current study looked at

FOXO3A phosphorylation. FOXO3A phosphorylation is a more acute, and therefore quicker, response, than creating a new protein. FOXO3A phosphorylation is activated by Akt at Ser^{318/321}. This particular site was shown in a study by Tong et al. (Tong et al., 2007) to cause FOXO3A to be phosphorylated in the cytoplasm and excluded from the nucleus of the cell. This sequestering of FOXO3A by Akt decreases the effects of the protein degradation pathway.

A strong association was not observed in the current study between phospho-FOXO3A (Ser^{318/321}) and AMPK activity (as measured by the phospho (Ser⁷⁹)/total ACC ratio). This argues against our hypothesis that a lower FOXO3A phosphorylation response would be related to higher AMPK activation, and suggests that a factor other than AMPK may more strongly mediate protein degradation pathway(s) following resistance exercise. Based on previous research, this factor may be Akt. Creer et al. (Creer et al., 2005) observed a higher phospho-Akt response to resistance exercise in a high vs. a low glycogen state. This would have theoretically resulted in a higher FOXO3A (Ser^{318/321}) phosphorylation, which was not measured in that study but is a finding of the current study under conditions of high pre-exercise muscle glycogen content. Thus, with respect to the current study, we postulate that there was a higher Akt phosphorylation in response to resistance exercise under the high muscle glycogen condition, which may explain why the higher FOXO3A (Ser^{318/321}) phosphorylation in that condition was not strongly related to lower AMPK activation. In the same accord, our observed trend (albeit a weak one; $p=0.17$), of a higher FOXO3A (Ser^{318/321}) phosphorylation response in younger vs. older subjects agrees with the results of Drummond et al., (Drummond, Dreyer et al., 2008), who found that the phospho-Akt

response to resistance exercise is diminished with increased age when compared to young subjects. The potential link between glycogen content, Akt, and FOXO3A in response to resistance exercise, as well as how age may affect these responses, is a topic for future endeavors.

There was a significant main effect seen in MuRF1 in the current study between young and old groups at 2-hours post-resistance exercise. We measured total MuRF1 as an additional measure of protein degradation pathway activation in old versus young individuals; however, we did not expect to observe a response. Nakashima and Yakabe (Nakashima and Yakabe, 2007) found that AMPK stimulates MuRF1 mRNA in skeletal muscle. This study was performed on C2C12 myotubes. Additionally, the time points in their study were different than the current study (6 hours and 24 hours as opposed to immediately post-exercise, 1-hour post- and 2-hours post-exercise). Based on the findings of the current study and previous literature, it is reasonable to believe that the stimulation of MuRF1 by AMPK takes time; thus, the reason we found a significant main effect at 2 hours post-exercise is unclear.

The current study provides instance for a number of future research endeavors. There were no significant differences between age groups shown in ACC, FOXO3A phosphorylation, and total MuRF1. However, although no significant differences were observed between age groups, a high association was found between phospho/total ACC and phospho-FOXO3A with muscle glycogen content. Studying nutritional differences on AMPK and the other markers of protein degradation would be beneficial, since previous literature shows AMPK phosphorylation differences between age groups following essential amino acid ingestion and carbohydrate supplement (Drummond,

Dreyer et al., 2008). Additionally, it may prove beneficial to separate the muscle sample by fiber type (fast-twitch versus slow-twitch) to analyze protein degradation changes on the individual fiber types within old and young subjects.

In summary, this investigation examined the FOXO3A Ser^{318/321} phosphorylation response to resistance exercise and its relation to muscle glycogen content and AMPK activation in young versus old subjects. AMPK phosphorylation was increased in old, but not young, men and women, immediately following resistance exercise. In addition, glycogen content was significantly lower in old subjects compared to young prior to the resistance exercise. In both young and old groups, glycogen content decreased and remained decreased immediately post-exercise, as well as 1- and 2-hours post-exercise. Relationships were observed between glycogen content and both phospho/total ACC ratio and FOXO3A phosphorylation. Higher glycogen content across all subjects was associated with a lower phospho/total ACC ratio (indicative of AMPK activity) and higher phospho-FOXO3A content. Moreover, higher glycogen content prior to exercise was associated with significantly higher FOXO3A phosphorylation before, and in response to, resistance exercise, as well as a greater phospho-FOXO3A percent change from baseline with exercise. These data indicate a strong association between glycogen content, AMPK activity, and FOXO3A signaling regardless of age. However, a relationship between FOXO3A phosphorylation and AMPK activity was not observed, suggesting that another factor may play a role in activating the protein degradation pathway following resistance exercise in older individuals.

Practical Application

Future research is needed to determine whether older adults with higher progression of sarcopenia show different results in the relationship between glycogen content and AMPK phosphorylation, ACC, and other markers of protein degradation. It may be pertinent to suggest to young and old individuals alike to keep glycogen content high as a method to minimize activation of the protein degradation pathway in response to resistance exercise.

CHAPTER VI: REFERENCES

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APPENDIX A: ECU INSTITUTIONAL REVIEW BOARD APPROVAL DOCUMENT

UMCIRB #:

UNIVERSITY AND MEDICAL CENTER INSTITUTIONAL REVIEW BOARD
REVISION FORM

RECEIVED

JAN 20 2010

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 Beaufort County Hospital
 Pitt County Memorial Hospital, Inc Carteret General Hospital
 Heritage Hospital Boice-Willis Clinic
 Other

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:

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

Briefly describe and provide a rationale for this revision

- 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.
- 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 12/31/2010
 Approval by expedited review according to category 457FR46.110
 See separate correspondence for further required action.

Signature L. Wiley Nifong, MD

Print

Date 1/21/2010

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

<u>Medication</u>	<u>Dosage</u>	<u>Reason</u>	<u>Times taken per day</u>

5. **Family History**

	Age if alive	Age of death	Cause of death
Father	_____	_____	_____
Mother	_____	_____	_____

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

Age of occurrence	Relationship
--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 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: Hope Tharrington/Jen Macesich

Telephone #: (252) 883-2001/ (919) 606-2853

EXPERIMENTAL SESSION INSTRUCTIONS**Human Performance Laboratory**

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

1. Do not drink alcohol.
2. If you consume caffeine, do so only in moderation.
3. 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 East Carolina Heart Institute 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 Hope Tharrington/Jen Macesich.