Abstract

Cleaved Caspase-3 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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April, 2011 
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In addition to suppressing protein synthesis and activating protein degradation in skeletal muscle and other cell types, 5’-AMP-activated protein kinase (AMPK) is known to stimulate nuclear apoptosis in non-muscle cells through cleaved (activated) caspase-3, one of the final steps in the apoptosis cascade. Although it is unknown whether AMPK stimulates caspase-3 cleavage or nuclear apoptosis in skeletal muscle cells in vivo, AMPK activity and nuclear apoptosis are elevated at rest in aged rat skeletal muscle. AMPK phosphorylation and activity are also higher in old vs. young rats and humans in response to overload or resistance exercise. Furthermore, older individuals display lower muscle glycogen content, a condition known to accentuate AMPK activity at rest and during aerobic exercise. We hypothesized that skeletal muscle cleaved caspase-3 content would be higher after acute resistance exercise in older versus younger individuals. Seven young (21.7 ± 2.1 yrs) and 11 old (67.0 ± 8.6 yrs) subjects performed an acute bout of leg extension resistance exercise. Muscle biopsies were obtained
pre-exercise (PRE), immediately post-exercise (0P), 1-hour post-exercise (1P), and 2-hours post-exercise (2P). Glycogen content was measured in muscle samples, as were the phosphorylations (via western blot) of AMPK and acetyl-CoA carboxylase (ACC; a marker of AMPK activity). Procaspase-3 and cleaved (activated) caspase-3 contents were also assessed via western blot. AMPK phosphorylation was significantly \((p < 0.05)\) increased in old, but not young, subjects immediately post-exercise. In both age groups, AMPK activity (assessed by ACC phosphorylation) was elevated vs. PRE at the 0P and 1P time points, and cleaved caspase-3 content was elevated vs. PRE at the 0P, 1P, and 2P time points. However, there was no effect of exercise on procaspase-3 content in either age group, and no differences between age groups in AMPK activity, procaspase-3 content, or cleaved caspase-3 content at any time point. There were significant, or close to significant, relationships between glycogen content and AMPK activity at time points PRE, 0P, and 1P regardless of age. However, no significant correlations between AMPK activity and cleaved caspase-3 content were observed at any time point. In summary, these data indicate that cleaved caspase-3 (e.g., caspase-3 activity) increases in response to acute resistance exercise in both young and old subjects. However, exercise-induced AMPK activation may not be the mechanism by which this occurs.
Cleaved Caspase-3 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

A Master’s Thesis in Exercise and Sport Science
East Carolina University

A Partial Fulfillment of the Requirements for the Degree Master of Science in Exercise & Sport Science (Exercise Physiology Concentration)

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April, 2011
Mentor: Dr. Scott E. Gordon
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Acknowledgements

I would like to first and most importantly acknowledge my mentor Dr. Scott Gordon for his informative guidance on this research topic. In addition, I would like to thank Dr. Joseph Houmard, Dr. Tim Gavin, and Dr. Mary Farwell for serving on my thesis committee.

Dr. Robert Hickner and Dr. Tim Gavin were also contributory in obtaining muscle biopsies from the subjects. In addition, Dr. Rengfei SHI assisted with western blot analysis techniques. This research could not have been accomplished without their assistance and expertise.

Lastly, I would like to acknowledge my fellow graduate students, Hope Tharrington, Jennifer Macesich, Christopher Courson, and Gregory Honeychuck, as well as undergraduate students, Blake Shephard, Aubrey Taylor, and LaDonna Maddy, for their assistance on this research project.
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CHAPTER 1
INTRODUCTION

Sarcopenia

Sarcopenia is the age-related loss in skeletal muscle mass (Evans & Rosenberg 1991). Sarcopenia is a process that begins in young adulthood and continues with aging (Evans, 1995 & 1997). Research has shown that sarcopenia is a major factor in age-related decreases in muscle strength (Evans, 1993). As an individual ages, there is a shift in overall body composition toward a higher level of fat mass (Borkan, Hultz, & Gerzoff, 1983). This, in association with muscle loss, can lead to increases in many disorders: including hypercholesterolemia, atherosclerosis, hyperinsulinemia, insulin resistance, type 2-diabetes, and hypertension (Depres et al., 1990). Aging is associated with declines in physical activity (PA), which have an effect on muscle atrophy and fat accumulation (Bortz, 1982). Sarcopenia is also associated with greater losses in fast twitch fibers (type II fibers) (Rogers & Evans, 1993). According to 2004 estimates, 45% of the older population is sarcopenic (Janssen, Baumgartner, & Ross, 2004). In 2000, the estimated direct healthcare cost resulting from sarcopenia was $18.5 billion. Sarcopenia accounts for about 1.5% of total healthcare expenditure within the United States (Janssen et al., 2004).

Resistance Training

Effective resistance training programs have proven to be an effective intervention for
delaying sarcopenia. Resistance training has shown increases in both strength and muscle fiber size in the elderly population (Kryger & Anderson, 2007; Rogers & Evans, 1993). Being able to maintain or even prevent sarcopenia is vital for all older individuals in order to perform most daily activities (Evans & Campbell 1993). Multiple studies have demonstrated that similar results arise from resistance training interventions for both the young and older populations. Both aerobic and resistance training have proven benefits and are highly recommended, but only resistance training can prevent or even reverse sarcopenia (Evans, 2004). However, it has been shown that resistance training does not appear to be as effective in an older population when compared to younger population. This is particularly true with fast-twitch muscle fibers, which are more apt to age-related atrophy (Welle, 1996). An investigation by Thomson and Gordon found that fast-twitch skeletal muscle hypertrophy is greatly reduced with age (Thomson & Gordon, 2005 & 2006). Furthermore, inhibition of gains in force production with age has also been shown (Korhonen, 2006).

Protein Synthesis and Degradation

Protein synthesis is the formation of proteins within the muscle and eventually leads to increases in fiber size and mass (Kryger & Andersen, 2007). Protein degradation is a biological process by which the breakdown of cells occurs continuously; leading to decreases in both muscle fiber size and mass when it outweighs synthesis (Viana et al., 2008). As a person ages, the balance between protein synthesis and degradation is altered in response to acute resistance exercise (Gordon et al., 2008). In older populations, there is an increase in protein degradation at rest (Drummond et al., 2008) and after resistance exercise (Kumar et al., 2009). It has also been
shown that protein synthesis decreases in old when compared to young individuals at rest. Mechanically induced protein synthesis within fast-twitch muscles may also be tapered with age. (Welle et al., 1995 & 1996a).

**Nuclear Apoptosis**

Apoptosis is a biological process that follows a specific plan and design that ultimately leads to programmed death in mono-nucleated cells (Kerr, Wyllie, & Currie, 1972). Apoptosis involves controlled processes that regulate specific genes. These genes coordinate events that trigger certain signaling pathways leading to the execution and implementation of cellular apoptosis. Three signaling pathways have been identified, including mitochondria-mediated, ligand-mediated, and endoplasmic reticulum stress-mediated (Danial, Korsmeyer, 2004). Apoptosis was previously proposed to only be responsible for the destruction of intact cells; therefore, most investigations have primarily dealt with mitotic single nucleated cell types. Research involving apoptotic components regarding postmitotic multinucleated cells, such as skeletal myofibers, is not entirely developed. It has been hypothesized that there is a link of apoptotic events working at the nuclear level with multinucleated skeletal myofibers and this process has been termed “nuclear apoptosis” (Allen, Roy, & Edgerton, 1999). Signaling pathways concerning muscle myofibers can be classified into either caspase-dependent or caspase-independent cascade of apoptotic markers (Siu, 2009). Apoptotic markers are divided into two primary types of apoptotic proteins, which are termed pro-apoptotic and anti-apoptotic respectively (Quadrilatero et al., 2009). It is these proteins that may be affected by acute exercise and age (Siu, 2009).
Apoptosis may play a definitive role in the development of sarcopenia. Muscle growth depends on the balance between the addition of myonuclei via satellite cells versus apoptosis of pre-existing nuclei (Allen, Roy, & Edgerton, 1999). Additionally, the apoptotic response to exercise may also affect muscle growth in older individuals. Aged skeletal muscle may lead to an increased level of these apoptotic events (Leeuwenburgh et al., 2005). According to present investigations, there are multiple apoptotic proteins that have been proven to be responsive to aging. These include Bax, Bcl-2, multiple caspases, and other apoptotic mediators such as cytochrome c and apaf-1 (apoptotic protease activating factor). Bax is a pro-apoptotic protein and Bcl-2 is an anti-apoptotic protein (Duronio, 2008). Both Bax and selective caspase (caspase-8 and -9) activities are increased in gastrocnemius and plantaris of aged rats when compared to young adult rats (Alway et al., 2002 & 2003). Caspase-3 is one of the effector caspases that leads directly to cell destruction (Kocturk et al., 2007). Procaspase-3 (32kD) acts as the inactive form, where as cleaved caspase-3 (19kD) is the active form (Siu, 2009). Most previous literature refers to the 32kD form of caspase-3 as caspase-3 or total caspase-3; however, for the purposes of this document, it will be referred to as procaspase-3. In addition, the 19kD or activated form of caspase-3 will be referred to as cleaved caspase-3.

The apoptotic signaling response to resistance exercise in aged skeletal muscle is unknown. However, because acute resistance is not fully effective in older individuals (Welle, 1996, Thomson & Gordon, 2005, 2006, & Korhonen, 2006), and apoptotic signaling is elevated in older skeletal muscle (Leeuwenburgh et al., 2005), we hypothesize that pro-apoptotic signaling will be greater in response to resistance exercise in aged skeletal muscle. According to
Kocturk et al., cleaved caspase-3 activity increased when stimulated by a bout of strenuous running in young rats (Kocturk et al., 2007).

**AMPK**

5’-adenosine monophosphate (AMP)-activated protein kinase (AMPK) is an enzyme that acts as an intracellular sensor and is activated in response to elevated AMP/adenosine triphosphate (ATP) and inorganic phosphate (Pi)/phosphocreatine (PCr) ratios. In skeletal muscle, AMPK is also activated by low glycogen content (Wojtaszewski et al., 2003). AMPK attempts to maintain energy stores within the cells to create homeostasis (Hardie, 2004). AMPK may suppress protein synthesis and activate protein degradation. Evidence also suggests AMPK phosphorylation in response to resistance training has been shown to cause a prolonged elevation in association with age in humans (Drummond et al., 2008).

The elevated AMPK activation in aged skeletal muscle may result from lower glycogen content. The caloric intake between elders and their young counterparts differ significantly. The elderly consume much fewer carbohydrates and fats, as well as having an overall lower daily caloric intake. The elderly and young differ in both amount of calories and macronutrients consumed during meals and snacks (Wurtman, 1988). A decrease in carbohydrate consumption leads to a decrease in muscle glycogen levels in the elderly (Cartee, 1994). This decrease in muscle glycogen may lead to increases in AMPK levels at rest and after resistance exercise. It has also been shown that AMPK activity is higher in a glycogen-depleted state when compared to a glycogen-loaded state (Wojtaszewski et al., 2003). Therefore, increases in AMPK may cause protein degradation, decreased hypertrophy, and decreased strength gains among the
elderly (Thomson & Gordon, 2005). AMPK inhibits overload-induced hypertrophy in fast-twitch muscle (Gordon et al., 2008).

AMPK’s role in nuclear apoptosis is still somewhat vague based on present research. Although AMPK’s role in multinucleated skeletal muscle cells is still being investigated, it does seem to play a role in apoptotic signaling in mononucleated muscle cells (Dyck & Lopaschuk, 2006; Niesler, Myburgh, & Moore, 2007). AMPK’s role in skeletal muscle fibers may be more associated with fiber atrophy, as opposed to fiber death (Dirks et al. 2006). It has been proposed that AMPK is pro-apoptotic in aged skeletal muscle and may play a role in age-related increase in apoptotic nuclei (Dyck & Lopaschuk, 2006). Investigations have shown that activation of AMPK (AICAR or other common activator) can lead to increases in procaspase-3 activity in non-muscle cells (Sai et al., 2006; Riboulet-Chavey et al., 2008; Jung et al., 2010). In addition, an investigation by Kim et al. found that inhibition of AMPK suppresses cleaved caspase-3 (e.g. activated caspase). Therefore, AMPK activation may be a positive regulator of apoptosis (Kim et al., 2009). We already have preliminary data from the Gordon laboratory that shows both Bax and procaspase-3 contents were increased with 1 week of AMPK stimulation (AICAR) (Gordon Unpublished).

Specific Aim

Advancing age is associated with skeletal muscle fiber atrophy and is specific to the fast-twitch muscle fibers (Evans & Rosenberg 1991; Rogers & Evans, 1993). Furthermore, fast-twitch skeletal muscle hypertrophy is reduced with age (Thomson & Gordon, 2005 & 2006). Muscle glycogen levels are also lower within the elderly population (Cartee, 1994). An
investigation by Wojtaszewski et al. found that depleted muscle glycogen levels activate AMPK (Wojtaszewski et al., 2003). In addition, AMPK activity is higher following acute resistance training with aged skeletal muscle (Drummond et al., 2008). Elevated AMPK activation may cause an elevated pro-apoptotic response to resistance exercise in aged skeletal muscle. Therefore, the purpose of this study was to examine the response of cleaved (activated) caspase-3 to acute resistance exercise in the skeletal muscles of young versus old subjects. It was hypothesized that skeletal muscle cleaved caspase-3 content would increase in response to acute resistance exercise. It was further hypothesized that skeletal muscle cleaved caspase-3 content would be higher after acute resistance exercise in older versus younger individuals.
CHAPTER II
REVIEW OF LITERATURE

*Sarcopenia*

Aging is associated with notable alterations in body composition. Sarcopenia, which is the age-related loss in muscle mass, is a notorious part of these changes (Evans & Rosenburg, 1991). Sarcopenia is the result of decreases in both fiber number, size, and is specific with losses in fast twitch fibers (type II fibers) (Rogers & Evans, 1993; Welle, 2002). Some estimations state that anywhere from one-quarter to one-half of the population over the age of 65 have sarcopenia (Baumgartner et al., 1998). A continual decline in muscle mass is most often seen after the age of 45 years (Hudges et al., 2001). According to past investigations, sarcopenia has resulted in 14% of muscle loss and 34% muscle protein loss between the ages of 25 and 75. It has been determined that body fat can increase from 18 to 36% in women and 33 to 44% in men (Cohn et al., 1980). Sarcopenia is associated with age-related losses in metabolic rates, bone mineral density, strength, functional status, and daily caloric needs (Evans & Rosenburg, 1991).

Sarcopenia is highly correlated with physical disability within the elderly and can lead to multiple health concerns and healthcare expenditures (Janssen, Baumgartner, & Ross, 2004; Janssen et al., 2004). The combination of muscle loss and fat gain can lead to increases in many disorders: including hypercholesterolemia, atherosclerosis, hyperinsulinemia, insulin resistance, type 2-diabetes, and hypertension (Depres et al., 1990). Sarcopenia also plays a profound role in increased healthcare costs. In 2000, healthcare costs linked to diseases associated with sarcopenia were $18.5 billion (Janssen et al., 2004). This amount accounted for about 1.5% of
the United States total healthcare cost (about $1.3 trillion) for 2000. With such a high incidence of expenditure and age-related physical disability, it is vital to examine specific mechanism(s) underlying sarcopenia. Furthermore, it is necessary to examine potential compensatory methods, such as resistance training, to decrease the effects of increasing age on skeletal muscle mass and overall strength.

**Resistance Training**

The effects of sarcopenia are clearly evident, thus allowing compensatory methods such as resistance training to increase in popularity. Resistance training interventions have proven to be beneficial and can provide increases in both muscle strength and cross-sectional area of the muscle fiber (Kryger & Anderson, 2007; Rogers & Evans, 1993). However, fast-twitch fiber hypertrophy associated with resistance training is significantly reduced with age (Kosek et al., 2006; Trappe et al., 2001). Investigations have shown that even when fast-twitch hypertrophy is witnessed with resistance training, it is delayed when compared to slow-twitch fibers (Pyka et al., 1994). Others have shown that fast-twitch hypertrophy is still absent in the elderly even after one year of an appropriate intervention (Taaffe et al., 1996). Therefore, this impaired ability to hypertrophy in older individuals indicates that resistance-training interventions are not 100% effective (Welle, 1996). Previous investigations have also provided evidence suggesting that aged skeletal muscle from animal models have a weaker ability to respond to loading-induced muscle growth or regeneration from injury (Thomson & Gordon, 2005). Maintaining or even preventing muscle atrophy associated with aging is vital for all older individuals. The diminished effect of resistance training with the elderly can lead to an assortment of conditions,
including sarcopenia, type II diabetes, coronary artery disease, hypertension, and osteoporosis (Evans & Rosenberg 1991). Therefore, it is imperative to further investigate the possible mechanisms that may be associated with this diminished effect of resistance training among the elderly.

*Protein Synthesis and Degradation*

Protein synthesis is the formation of proteins, which leads to increases in muscle fiber size and mass of proteins within cells (Kryger & Andersen, 2007). Protein degradation is the continual breakdown of cells. Therefore, when degradation outweighs synthesis, there is a decrease in both muscle fiber size and mass (Viana et al., 2008). Aging alters the balance between synthesis and degradation in response to acute resistance exercise (Gordon et al., 2008). Investigations have shown that there is an increase in protein degradation at rest and after resistance exercise with the elderly (Kumar et al., 2009; Drummond et al., 2008). Additionally, there is a decrease in synthesis at rest in elderly when compared to young individuals. It may also be evident that protein synthesis within fast-twitch muscle is tapered with age (Welle et al., 1995 & 1996a). Mammalian target of rapamycin (mTOR) is an upstream signaling pathway that is associated with controlling protein synthesis and is decreased with age (Thomson & Gordon, 2005). Therefore, mTOR inhibition can play a definitive role in skeletal muscle atrophy (Bodine et al., 2001).
**Nuclear Apoptosis**

Cellular apoptosis is cell destruction that follows a formulated plan and design in order to fulfill specific intentions (Kerr, Wyllie, & Currie, 1972). Signaling pathways intervene specific signaling transduction leading to the execution and implementation of cellular apoptosis. These pathways include mitochondria-mediated, ligand-mediated, and endoplasmic reticulum stress-mediated (Danial & Korsmeyer, 2004). Most investigations have primarily dealt with mitotic single nucleated cell types, thus apoptotic components regarding postmitotic multinucleated cells, such as skeletal myofibers, are not entirely developed (Siu, 2009).

In single nucleated cells, apoptosis manages to eliminate nuclei, which leads to the destruction and eventual cell death. Multinucleated cells, such as skeletal muscle myofibers, can undergo losses of one or more nuclei, without complete destruction. Furthermore, there is a proposed physiological link of apoptosis in muscle adaptations and refers to the apoptosis-mediated down-regulation of the number of nuclei within the actual myofiber. This has become known as “nuclear apoptosis”. Therefore, not all myonuclei within the muscle fiber become apoptotic during muscle atrophy (Allen, Roy, & Edgerton, 1999). It has been hypothesized that the addition of new nuclei is necessary for muscle hypertrophy and the removal of myonuclei is required for muscle atrophy. It has been demonstrated that muscle hypertrophy can be promoted by the activation of myogenic satellite cells (Allen, Roy, & Edgerton, 1999; Charge & Rudnicki, 2004). Signaling pathways concerning muscle myofibers can fall into either an intrinsic or extrinsic pathway, which are affected by numerous apoptotic markers (proteins) and proteolytic enzyme activity.

Apoptotic markers are divided into two primary types of apoptotic proteins, which are
termed pro-apoptotic and anti-apoptotic, respectively. Present investigations have uncovered several apoptotic markers responsive to aging, including the pro-apoptotic markers Bax, AIF (apoptosis inducing factor), and Smac (second mitochondria-derived activator of caspases). In addition, some anti-apoptotic markers have also been investigated. These include Hsp70 (heat shock protein), XIAP (X-linked inhibitor of apoptosis protein), ARC (apoptosis repressor with caspase recruitment domain) and Bcl-2. Furthermore, a cascade of proteolytic enzymes known as caspases also play a vital role in nuclear apoptosis. Caspase-9, which is the most upstream member of the apoptotic protease cascade, mediates the mitochondria-associated apoptotic events; therefore, activating caspase cleavage. The activation of these caspases leads to the morphological changes that occur during apoptosis. More specifically, caspase-9 leads to the cleavage of procaspase-3 (32kD) to cleaved (active) caspase-3 (19kD) (Siu, 2009). As previously stated, most previous literature refers to the 32kD form of caspase-3 as caspase-3 or total caspase-3; however, for the purposes of this document, it will be referred to as procaspase-3. In addition, the 19kD or activated form of caspase-3 will be referred to as cleaved caspase-3. This cascade of downstream caspases carries out the final destructive processes of apoptosis (Li et al., 1998 & Quadrilatero et al., 2009). A number of the above specific markers that may be affected by acute exercise and age (Siu, 2009) (See Figure 2. Below)

Relation to Aging and Exercise Response

Apoptotic markers may play a definitive role in the development of sarcopenia. Aged skeletal muscle may also lead to increases in apoptotic events (Leeuwenburgh et al., 2005). According to Jejurikar at al., satellite cells from old rats have greater responses to pro-apoptotic proteins and caspases (Jejurikar et al., 2006). Bax, caspase-8, and caspase-9 levels are increased
in the gastrocnemius and plantaris of aged rats when compared to young rats (Alway et al., 2002 & 2003). It has been shown that 12 weeks of treadmill exercise training can alter apoptotic markers, such as increases in Bcl-2 and decreases in Bax in aged skeletal muscle (Song, Kwak,

Figure 2.1. Activation of caspase cascade leading to apoptosis

Mitochondria

Legend

ApaF-1 - apoptotic protease activating factor
Cyto C - cytochrome c
One investigation found no acute changes in caspase activity or apoptotic markers following a single bout of prolonged moderate-intensity aerobic exercise (Quadrilatero et al., 2009). Another investigation found that both pro and anti-apoptotic proteins are affected by age in rat fast-twitch skeletal muscle. Additionally, caspase activity and apoptotic index were also increased in aged rat fast-twitch skeletal muscle. (Pistilli, Jackson, & Always, 2006). Both caspase-9 and procaspase-3 have been shown to be elevated with age; therefore, these elevations are associated with the progression of sarcopenia (Baker & Hepple, 2006).

A study by Yang et al. showed that the Bax/Bcl-2 mRNA ratio increases in response to acute resistance exercise. This investigation showed increases in procaspase-3 mRNA in response to acute resistance exercise (Yang, Jemiolo, & Trappe, 2006). A study by Mahoney et al., showed Bcl-2 mRNA increases with aerobic exercise (Mahoney et al., 2005). An investigation by Kocturk et al. has shown that cleaved caspase-3 activity increases up to 3 hours post-aerobic exercise and that apoptotic responses are different when compared to different muscle types and oxidative metabolisms in young rats (Kocturk et al., 2007). Similar investigations have shown that age-related apoptosis has the potential to be reversed with effective exercise interventions. Procaspase-3 can be attenuated with exercise training (Marzetti et al., 2008).

*Upstream Signaling Pathways*

There are multiple upstream signaling pathways and protein kinases that stimulate nuclear apoptosis. One of the main contributors is Akt (protein kinase B), which acts as an apoptosis inhibitor and cell survival promoter. Akt has the ability to block multiple pro-
apoptotic markers; thus regulating cell survival. When Akt phosphorylation fails, the Akt pathway fails to regulate or maintain normal levels of anti-apoptotic proteins. This can lead to both signal interruption and multiple cellular system disruptions. These include disruption of protein synthesis and anti-apoptotic signals, which can lead to a higher expression of pro-apoptotic markers. These markers include Bax, Bim, and Puma, which are a result from FOXO3A (forkhead box O 3a) activation. FOXO3A is regulated (inhibited) by the P13K (phosphoinositide 3-kinase)/Akt pathway (Duronio, 2008). Furthermore, an investigation by Duronio found that Akt phosphorylates (deactivates) the pro-apoptotic protein Bad (Bcl-2/Bcl-XL-associated death domain protein) (Duronio, 2008). An investigation by Creer at al., found an increase in Akt phosphorylation following resistance exercise (Creer et al., 2005). Lastly, an investigation by Drummond et al., found that the Akt response (acute) to resistance exercise is diminished with age (Drummond et al., 2008).

**AMPK**

5′-adenosine monophosphate (AMP)-activated protein kinase is an energy sensor that attempts to maintain cellular energy stores and is activated in response to elevated AMP/ATP and Pi/PCr ratios. In muscle cells, AMPK activation leads to the stimulation of glycogen storage, mitochondrial biogenesis, and glucose/lipid catabolism. AMPK stimulation also increases with lower glycogen content (Wojazewski et al., 2003). Furthermore, AMPK activation inhibits energy-consuming processes, such as fatty acid and protein synthesis (Hardie, 2004). Research has shown that AMPK phosphorylation suppresses protein synthesis and activates protein degradation.

AMPK activation occurs 1-2 hours after acute resistance exercise in humans; therefore, it
may suppress early translational signaling leading to protein synthesis. In rat skeletal muscle AMPK has been shown to suppress Akt phosphorylation (Bolster et al., 2002). AMPK’s role with aged fast-twitch muscle may focus on the inhibition of mTOR and suppression of some specific downstream translational signaling intermediates (Thomson & Gordon, 2006). In terms of degradation, AMPK stimulates both atrophy-related FOXO (forkhead box) genes (especially the transcription factors FOXO1 and FOXO3A) and muscle-specific ubiquitin ligase genes, such as muscle atrophy F-box (MAFbx, or Atrogin-1) and muscle ring finger 1 (MuRF1) (Nakashima & Yakabe, 2007). It is believed that AMPK activation in aged skeletal muscle is elevated and that it plays an integrative role with atrophy and inhibition of overload-induced hypertrophy in fast-twitch fibers (Gordon et al., 2008). Additionally, AMPK phosphorylation has been shown to be higher in aged skeletal muscle in rats during rest and chronic overload (Thomson & Gordon, 2005), as well as in response to acute resistance exercise within elderly men (Drummond et al., 2008).

AMPK’s involvement with nuclear apoptosis is still being investigated. However, AMPK does seem to play an integrative role in pro-apoptotic signaling in mononucleated cells (Dyck & Lopaschuk, 2006; Niesler, Myburgh, & Moore, 2007). It has also been proposed that AMPK is pro-apoptotic in aged skeletal muscle and may be involved in the age-related increase in apoptotic nuclei (Dyck & Lopaschuk, 2006). Some investigations have shown that AMPK activation can initiate specific markers, such as p38 and MAPK (mitogen activated protein kinase), which can lead to a cascade of events leading to apoptosis. Therefore, AMPK activation may play a role in age-related apoptosis, by means of energy depletion (Dirks et al., 2006).

Recent investigations have shown that activation of AMPK (AICAR or other common activators) leads to increases in procaspase-3 activity (Sai et al., 2006; Riboulet-Chavey et al.,
Moreover the inhibition of AMPK activation can suppress both procaspase-3 and cleaved caspase-3 activity within non-muscle cells (Kim et al., 2009 & Khanal et al, 2011). A study by Riboulet-Chavey et al. found a fourfold increase in cleaved-caspase-3 after a period of incubation with an AMPK activator (Riboulet-Chavey et al., 2008). Therefore, these data provide evidence that AMPK activation alone may be a positive regulator of apoptosis, specifically elevating skeletal muscle cleaved caspase-3. Furthermore, we have preliminary data that show increases in skeletal muscle Bax and procaspase-3 contents when AMPK is stimulated for 1 week. Additionally, we also have data that show a decrease in Bcl-2 with 1 week of AMPK stimulation (Gordon Unpublished).

**AMPK, Glycogen, and Aging**

The diet of the elderly differs significantly from that of their younger counterparts. Elderly have a lower total caloric intake and consume lower amounts of both carbohydrates and fats (Wurtman, 1988). There is also an inverse relationship between dietary intake and age among the elderly (Sonn, Rothenberg, & Steen, 1998). This may account for the fact that there is a decrease in carbohydrate consumption which leads to decreases in muscle glycogen levels with aging (Cartee, 1994). When muscle glycogen levels decrease in young individuals, AMPK levels increase at both rest and after exercise. Investigations have shown that AMPK phosphorylation is higher in a glycogen-depleted state in comparison with a glycogen loaded state in young individuals (Wojtaszewski et al., 2003). Muscle glycogen content following acute resistance exercise needs further investigation when associated with aging. However, since aged skeletal muscle has lower glycogen levels (Cartee, 1994), as well as elevated AMPK
phosphorylation and activity than young skeletal muscle (Thomson & Gordon, 2005 & Drummond et al., 2008), additional decreases in glycogen following resistance exercise would further stimulate AMPK activation.

Specific Aim

Skeletal muscle atrophy, in association with aging, is specific to fast-twitch muscle fibers, and hypertrophy of these muscles is diminished with an overloading stimulus such as resistance training (Kosek et al., 2006; Thomson & Gordon, 2005; Rogers & Evans, 1993; Evans & Rosenberg 1991). These decreases in fiber size and strength may be partially due to increases in apoptotic markers that lead to nuclear apoptosis (Sui, 2009; Allen et al., 2003). Nuclear apoptosis is also elevated within aged skeletal muscle (Leeuwenburgh et al., 2005). AMPK phosphorylation is elevated in response to acute resistance exercise in aged human skeletal muscle (Drummond et al., 2008) and has been shown to lie upstream of caspase-3 cleavage (activation) and nuclear apoptosis in other cell types (Kim et al., 2009; Khanal et al, 2011; Riboulet-Chavey et al., 2008). Decreases in muscle glycogen associated with aging have been observed (Cartee, 1994), and depleted glycogen can activate AMPK (Wojtazewski et al., 2003), thus leading to the possibility of increasing specific apoptotic markers. Procasps-3 activity increases within the first 0-3 hours after acute running exercise in young rats (Kocturk et al., 2007); however, the response to resistance exercise is currently unknown, especially in young versus old human skeletal muscle. Therefore, the purpose of this study was to examine the response of cleaved (activated) caspase-3 to acute resistance exercise in the skeletal muscles of young versus old subjects. It was hypothesized that skeletal muscle cleaved caspase-3 content
would increase in response to acute resistance exercise. It was further hypothesized that skeletal muscle cleaved caspase-3 content would be higher after acute resistance exercise in older versus younger individuals.
CHAPTER III
METHODS

Subjects

There were a total of 18 subjects used for this study. There were 11 old subjects (5 male and 6 female), ranging from 55-85 years old and 7 young subjects (4 male and 3 female), ranging from 18-30 years old. Subjects were healthy men and women with no history of hypertension, cardiovascular disease, or diabetes. Subjects were non-smokers and were not on any medications that would alter glucose regulation. All potential subjects were screened prior to the study to determine, height, weight, age, exercise history, and medical history. Subjects were recruited through word of mouth, flyers, and East Carolina University mass emails.

Table 3.1. Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Young Adults (n=7)</th>
<th>Old Adults (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.7 ± 2.1</td>
<td>67.0 ± 8.6</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>23.5 ± 3.1</td>
<td>29.0 ± 3.2</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>21.9 ± 5.0</td>
<td>32.6 ± 4.0*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75 ± 14</td>
<td>85 ± 11*</td>
</tr>
</tbody>
</table>

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).
Experimental Design

The purpose of this study was to examine the response of cleaved (activated) caspase-3 to acute resistance exercise in the skeletal muscles of young versus old subjects. It was hypothesized that skeletal muscle cleaved caspase-3 content would increase in response to acute resistance exercise. It was further hypothesized that skeletal muscle cleaved caspase-3 content would be higher after acute resistance exercise in older versus younger individuals.

Table 3.2. Subject Visitations

<table>
<thead>
<tr>
<th>Initial Visit</th>
<th>Experimental Session (overnight fast)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Informed consent &amp; medical history</td>
<td>Exercise Session</td>
</tr>
<tr>
<td>2) Height and weight measurements</td>
<td>➢ Bilateral leg extension</td>
</tr>
<tr>
<td>3) 4-site skinfold measurements</td>
<td>➢ 3 warm-up sets (50, 70, &amp; 90% of 10RM)</td>
</tr>
<tr>
<td>4) 10-repetition maximum determination</td>
<td>➢ 3 sets to failure (100% of 10 RM)</td>
</tr>
<tr>
<td></td>
<td>Muscle Biopsy-alternating legs</td>
</tr>
<tr>
<td></td>
<td>➢ pre-exercise, immediately post (0 minutes), 60 minutes post, &amp; 120 minutes post</td>
</tr>
</tbody>
</table>

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

The initial screening took place at the Fitness Instruction, Testing, and Training (FITT) building at East Carolina University. The screening included baseline measurements, familiarization with the resistance exercise equipment, and determination of a 10-repetition maximum (10 RM) on the Cybex leg extension machine for each subject.

During the initial visit, participants completed all the necessary paper work. This included informed consent, the dietary log, and medical history. Height, weight, and skinfolds were also taken. A 4-site skinfold method (bicep, tricep, suprailiac, subclavical) was used. Each subject’s body density was estimated using the Durnin and Womersley skinfold assessment and body density formula ([495/(1.1714-.063 x LOG [sum of skinfolds] - 0.000406 x [age]) – 450]) (Durnin & Womersley, 1974).

Upon completion of the initial paper work, assessment, and measurements, the 10 RM was determined on the Cybex leg extension machine. Prior to the 10 RM testing, each subject became familiar with the machine in order to reduce the risk for possible cardiovascular events and injury. Additionally, each subject was asked to predict the maximum amount of weight they could lift 10 times. A warm-up period consisting of 50% of their 10 RM was also used. After completion of the warm-up, the weight was increased by 5-20 pounds, depending on individual performance. Each set included 10 repetitions, followed by a 1-2 minute rest period. Completion of testing occurred once subjects were unable to perform 10 repetitions with correct form. The prior 10-repetition amount was the subject’s 10 RM.

Prior to the end of the initial visit, subjects were given an information sheet that provided set guidelines for the 1-2 week period before the experimental session. For the 2 days prior to
the experimental section, subjects were told to consume 8 cups (64 oz) of water and refrain from alcohol consumption and moderate levels of caffeine intake. On the day before the experimental session, subjects underwent a 12-hour minimum fast. On the day of the experimental section, subjects were instructed to consume 2 cups (16 oz) of water before their laboratory visit. Furthermore, subjects were also told to refrain from blood donation and exercise throughout the duration of the study.

**Experimental Session**

The experimental session took place 1-2 weeks following the initial visit at the East Carolina Heart Institute. This waiting period allowed subjects to recover from any possible soreness that may have occurred after the 10 RM testing. The 10 RM testing was used for the resistance exercise bout in the experimental session in an effort to stimulate AMPK and the downstream signaling pathways. A muscle biopsy was acquired prior exercise for pre-exercise measurements, immediately post-exercise (after completion of the final resistance exercise set and completion of preparation for biopsy), 1 hour following the immediately post-exercise biopsy, and two hours after taking the immediately post-exercise biopsy. Muscle biopsy times are based upon the results of Drummond et al., which concluded that AMPK phosphorylation is greater in old versus young men by 1-2 hours post-exercise (Drummond et al., 2008) (See Figure Below for experimental timeline). Lastly, subjects were fasted for a minimum of 12 hours prior to this session.
Timeline of Experimental Session

Figure 3.1. Timeline of events for the experimental session for each subject. Pre-Ex is the period preceding 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 a Cybex Leg Extension machine following the pre-exercise biopsy. The subject began by performing a warm-up set consisting of 10 repetitions performed at 50% of their predetermined 10 RM. A 1-2 minute rest period was given between each set. The subject then performed an additional warm-up set consisting of 5-7 repetitions performed at 70% of their 10RM. The final warm-up set consisted of 3-5 repetitions performed at 90% of their predetermined 10 RM. The subject performed three working sets using their predetermined 10 RM. Each set was performed until failure. Once the subject reached failure, they completed 1-3 forced repetitions with the assistance of the investigator. This type of testing made an attempt to stimulate AMPK and downstream signaling pathways, such as apoptotic markers.
**Muscle Biopsies**

All biopsy procedures were performed under sterile conditions. First, a Povidone-Iodine swab stick was used to clean and disinfect the skin in the area where the biopsy took place. It was then wiped off and an ethyl chloride medium stream spray was administered in order to numb the area where approximately 5 mL of 1% Lidocaine will be administered. A small (about 1-2 cm) incision was made into the vastus lateralis of the left thigh with a No. 11 surgical blade. A 5-mm Bergstrom biopsy needle was inserted through the incision 2-3 centimeters into the actual muscle. A sterile procedure and proper suction was applied in order to minimize error and maximize biopsy size.

Immediately following each biopsy, direct pressure and ice was applied to specific area to alleviate bleeding. The wound was then dressed in an appropriate manner. A plain white strip was placed directly over the wound; along with an average size band-aid on top of that. A Tegaderm strip covered both of these. This total bandage wasn’t removed until 1-2 days later. It is semi-waterproof, but the subject was encouraged to cover with saran wrap when in the shower (Baths should be avoided). A thick piece of gauze was then placed on top of that and was held in place by a wrap that goes around the entire thigh. Athletic tap was wrapped on top of that (All the way around the thigh). The gauze and tape portion were removed after a few hours. This particular set-up allowed for proper healing and decreased the likelihood of scarring.

There were a total of four biopsies throughout the course of this study. Muscle biopsy times are based upon the results of Drummond et al., which concluded that AMPK phosphorylation is greater in old versus young men by 1-2 hours post-exercise (Drummond et al., 2008). The first occurred prior to the acute resistance exercise session. Subsequent biopsies
used alternating legs, with both the first and third biopsy being taken on the same side. Once the pre-exercise biopsy was complete, the subject performed the acute resistance exercise session. Immediately post-exercise, the subject was moved to a bed and the second biopsy was taken. Preparation for this biopsy was completed in association with the first. After 1 hour, the third biopsy was taken approximately 5 cm proximal to the first site. The final biopsy was taken 2 hours post-exercise approximately 5 cm proximal to the second biopsy site. Biopsy samples were placed into a cryovial and flash frozen in liquid nitrogen for future western blot analyses. Samples were stored at a constant temperature of -80 degrees Celsius.

**Sample Analysis**

*Analysis of Muscle Glycogen Content*

Muscle glycogen content was analyzed by using a method modified from a variety of methods derived from Roy and Tarnopolsky (1998), Passonneau and Lowry (1993), and Passonneau and Lauderdale (1974). An estimated amount of 5-15mg from each subject’s muscle sample was homogenized on ice in specified volumes of .1 M NaOH with a ground glass homogenizer. An aliquot of each homogenate was heated for an estimated amount of 20 minutes at 80° C. This attempted to destroy any free glucose. In addition, the pH of each sample was neutralized with acetic acid and vortexing. Furthermore, aliquots of each sample were placed in duplicate tubes of a sodium acetate buffer.

The following steps were performed in duplicate. In an attempt to obtain free glucosyl units, glyogenolysis was accomplished. Any remaining precipitate was centrifuged. A glucose assay that is commercially available (based upon hexokinase and glucose-6-phosphate
dehydrogenase reactions; Sigma-Aldrich # GAHK20) was performed to determine free glucosyl units within each sample. The glycogen content (µg/mg wet weight) of the 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 with the same approach as the muscle samples beginning with the NaOH step.

*Western Blotting Analysis for AMPK Phosphorylation, ACC, Procaspe-3, and Cleaved Caspase-3 Activity*

AMPK, ACC, protein contents, and phosphorylation, as well as total and cleaved caspase-3 protein contents were analyzed using the Western blot analysis method. The following antibodies are commercially available: anti-AMPK [Cell Signaling Technology (CST); Danvers, MA; Cat. # 2532], anti-phospho-AMPK (CST; Cat. # 2535), anti-acetyl CoA Carboxylase (streptavidin-HRP, GE Life Sciences, RPN1231), anti-phospho-Acetyl CoA Carboxylase (Millipore Corporation; Temecula, CA; Cat. # 07-303; Lot#: LV1506382), anti-caspase-3, and anti-cleaved caspase-3 (CST). Phospho-Acetyl CoA is an in vivo indicator of AMPK activity. A small portion of each frozen muscle biopsy sample was homogenized using a buffer that consists of 50 mM HEPES (pH 7.4), 0.1% Triton X-100, 4 mM EGTA, 10 mM EDTA, 15 mM Na₄P₂O₇•10H₂O, 100 mM β-glycerophosphate, 25 mM NaF, 50 µg/ml leupeptin, 50 µg/ml pepstatin, and 33 µg/ml aprotinin. All homogenizations were carried out in ice to prevent excessive heat build-up that can lead to protein denaturizing. Furthermore, a ground glass homogenizer using a variable speed motor executed all homogenizations.

Assessment of the homogenates for protein concentration was fulfilled in triplicate using alterations 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. Upon completion of staining, membranes were then dried and scanned into a digital image. This image permits measurement of the relative total protein loaded into each label through the gray scale integrated optical density of the full length of each specific lane. Membranes were then blocked for one hour at room temperature in blocking buffer. This buffer consisted of 5% nonfat dry milk in TBS-T (20 nM Tri-base, 150 mM NaCl, 0.1% Tween-20) pH 7.5. The membranes were then 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-procaspase-3: 1/1000; anti-cleaved caspase-3.

Membranes then underwent 4 washing periods with 5 minutes between each wash in TBS-T, incubated in secondary antibody in blocking buffer for an hour at room temperature. Another round of 4 x 5 minutes wash periods in TBS-T followed. Upon completion of 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 gauged on the image of the Ponceau stain that was formerly captured. The HRP-conjugated anti-rabbit secondary antibody was obtained from Amersham.

Statistics

An analysis of variance (ANOVA) with repeated measures was used to analyze the differences between and within groups over time for all western blot data. Fisher’s LSD post-hoc test for measurement of post-hoc differences was employed where necessary. All correlations were performed with Pearson-Product-moment Correlation Analyses. Subject characteristic data was 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 18 subjects completed both the initial and experimental session. Total and relative volume of exercise was used to measure exercise performance for both the old and young age groups. Total volume was determined by multiplying the total amount of weight lifted by the repetitions for each work set during the experimental session. Additionally, total volume excluded all warm-up sets. Relative volume was determined by taking the total volume divided by each individual’s fat-free mass (kg) (See Table 4.1 Below).

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

<table>
<thead>
<tr>
<th></th>
<th>Y (N=7)</th>
<th>O (N=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated 10 RM (kg)</td>
<td>48 ± 10</td>
<td>32 ± 14</td>
</tr>
<tr>
<td>Total volume (kg resistance x repetitions)</td>
<td>1644.6 ± 195</td>
<td>1131.8 ± 395</td>
</tr>
<tr>
<td>Relative volume (kg resistance/kg FFM)</td>
<td>28 ± 1.9</td>
<td>19 ± 4.9</td>
</tr>
</tbody>
</table>

No significant differences were found between groups for any measure. 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). 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.

Cleaved Caspase-3 Western Blotting

A significant main effect was observed for cleaved caspase-3 (elevated) and
cleaved/procaspase-3 ratio (elevated) for all post-exercise time points (0P, 1P, 2P) regardless of age (Figures 4.1 & 4.3). No significant differences were observed for procaspase-3 (Figure 4.2).

**AMPK Western Blotting**

A significant increase was found in AMPK phosphorylation at Thr$^{172}$ at 0P when compared to PRE in old subjects, but this effect was not observed within the young group (Figure 4.4). No difference was discovered in total AMPK between old and young subjects or at any time point (Figure 4.5). Furthermore, a main effect of the phospho/total AMPK ratio was seen at 0P when compared to PRE for old and young subjects combined (Figure 4.6).

**ACC Western Blotting**

Phospho-acetyl CoA (ACC) at Ser$^{79}$ displayed a significant main effect at time 0P and 1P (elevated) when compared to PRE in old and young groups combined (Figure 4.7). Similar main effect findings were consistent for total ACC (decreased at 0P and 1P) (Figure 4.8) and the phospho/total ACC ratio (increased at 0P and 1P) (Figure 4.9).

**Muscle Glycogen Content**

A significant difference was evident in glycogen content between old and young groups at the pre-exercise time point (PRE). In addition, there was a significant effect of time points immediately post-exercise (0P), 1-hour post-exercise (1P), and 2-hours post-exercise (2P) for both old and young groups. Furthermore, because there was a significant difference between age groups at PRE, but not post-exercise, it could be stated that the young group had a more pronounced drop in glycogen content following the acute resistance exercise in comparison to the old group (Figure 4.10). Glycogen percent change from baseline showed a main effect difference between age groups across all time points (Figure 4.11).
Figure 4.1. Mean ± SEM Cleaved Caspase-3 in young and old subjects and corresponding blots. PRE is the pre-exercise biopsy, 0P is immediately post-exercise muscle biopsy, 1P is the 1-hour post-exercise muscle biopsy, 2P is the 2-hour post-exercise biopsy. *Significant main effect of time points 0P, 1P, and 2P vs. PRE regardless of age.
Figure 4.2. Mean ± SEM Procaspase-3 in young and old subjects and corresponding blots. PRE is the pre-exercise biopsy, 0P is immediately post-exercise muscle biopsy, 1P is the 1-hour post-exercise muscle biopsy, 2P is the 2-hour post-exercise biopsy. No significant differences were seen in procaspase-3 between old and young groups, or across any time points.
Figure 4.3. Mean ± SEM in young and old subjects. PRE is the pre-exercise biopsy, 0P is immediately post-exercise muscle biopsy, 1P is the 1-hour post-exercise muscle biopsy, 2P is the 2-hour post-exercise biopsy. *Significant main effect of time points 0P, 1P, and 2P vs. PRE regardless of age.
Figure 4.4. Mean ± SEM phosphor-AMPK in young and old subjects and corresponding blots. PRE is the pre-exercise biopsy, 0P is immediately post-exercise muscle biopsy, 1P is the 1-hour post-exercise muscle biopsy, 2P is the 2-hour post-exercise biopsy. * Phospho-AMPK was significantly (p ≤ 0.05) elevated above PRE in old subjects at the 0P time point.
Figure 4.5. Mean ± SEM Total AMPK in young and old subjects and corresponding blots. PRE is the pre-exercise biopsy, 0P is immediately post-exercise muscle biopsy, 1P is the 1-hour post-exercise muscle biopsy, 2P is the 2-hour post-exercise biopsy. Total AMPK showed no significant differences between young and old groups, or across any time points.
Figure 4.6. Mean ± SEM phospho/Total AMPK in young and old subjects and corresponding blots. PRE is the pre-exercise biopsy, 0P is immediately post-exercise muscle biopsy, 1P is the 1-hour post-exercise muscle biopsy, 2P is the 2-hour post-exercise biopsy. * Phospho/total AMPK ratio showed a significant main effect of time point, for young and old groups combined at 0P vs. PRE.
Figure 4.7. Mean ± SEM phospho-ACC in young and old subjects and corresponding blots. PRE is the pre-exercise biopsy, 0P is immediately post-exercise muscle biopsy, 1P is the 1-hour post-exercise muscle biopsy, 2P is the 2-hour post-exercise biopsy. * Significant (p < 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 4.8. Mean ± SEM Total ACC in young and old subjects and corresponding blots. PRE is the pre-exercise biopsy, 0P is immediately post-exercise muscle biopsy, 1P is the 1-hour post-exercise muscle biopsy, 2P is the 2-hour post-exercise biopsy. *Significant (p ≤ 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 4.9. Mean ± SEM phospho/Total ACC in young and old subjects and corresponding blots. PRE is the pre-exercise biopsy, 0P is immediately post-exercise muscle biopsy, 1P is the 1-hour post-exercise muscle biopsy, 2P is the 2-hour post-exercise biopsy. *Significant (p < 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.
Figure 4.10. Mean ± SEM glycogen content in young and old subjects. PRE is the pre-exercise biopsy, 0P is immediately post-exercise muscle biopsy, 1P is the 1-hour post-exercise muscle biopsy, 2P is the 2-hour post-exercise 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.
Figure 4.11. Mean ± SEM glycogen content in young and old subjects. 0P is immediately post-exercise muscle biopsy, 1P is the 1-hour post-exercise muscle biopsy, 2P is the 2-hour post-exercise biopsy. *Main effect difference between age groups versus PRE across all time points.
**Association of AMPK Activity and Cleaved Caspase-3 with Glycogen Content**

We found a significant, or close to significant, relationship between glycogen content and the phospho/total ACC ratio at time points PRE, 0P, 1P, but not 2P, regardless of age (Figs 4.12-4.15). In addition, we chose to further examine the relationship of glycogen content with ACC phosphorylation status as opposed to AMPK phosphorylation because it is a better indicator of AMPK activity (Park et al., 2002). Increased muscle glycogen content was also associated with a lower phospho/total ACC ratio. Furthermore, no significant correlation between AMPK activity and cleaved caspase-3 was observed.
Figure 4.12. Relationship between glycogen content and the phospho/total ACC ratio (top) and between AMPK activity and cleaved caspase-3 (bottom) at the pre-exercise time point. Significance is set at $p \leq 0.05$. 

The top graph shows a scatter plot with a linear regression line. The correlation coefficient $r = -0.43$ and the p-value $p = 0.076$. The bottom graph also shows a scatter plot with a linear regression line. The correlation coefficient $r = 0.03$ and is not significant (N.S.).
Immediately (0 hrs) Post-Exercise

![Graph showing the relationship between glycogen content and the phospho/total ACC ratio](image)

Glycogen Content (µg / mg wet weight)

AMPK Activity as Assessed by Phospho (Ser79) / Total ACC (IOD / IOD, Arbitrary Units)

\[ r = -0.45 \]
\[ p = 0.06 \]

Immediately (0 hrs) Post-Exercise

![Graph showing the relationship between AMPK activity and cleaved caspase-3](image)

AMPK Activity as Assessed by Phospho (Ser79) / Total ACC (IOD / IOD, Arbitrary Units)

Cleaved Caspase-3 (IOD, Arbitrary Units)

\[ r = 0.35 \]
\[ \text{(N.S.)} \]

Figure 4.13. Relationship between glycogen content and the phospho/total ACC ratio (top) and between AMPK activity and cleaved caspase-3 (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 AMPK activity and cleaved caspase-3 (bottom) at the one-hour post-exercise time point. Significance is set at $p \leq 0.05$. 

$1$ Hour Post-Exercise

![Graph showing linear relationship between glycogen content and AMPK activity](image)

- **r** = -0.69
- **p** = 0.002

$1$ Hour Post-Exercise

![Graph showing linear relationship between AMPK activity and cleaved caspase-3](image)

- **r** = -0.18
- **(N.S.)**
Figure 4.15. Relationship between glycogen content and the phospho/total ACC ratio (top) and between AMPK activity and cleaved caspase-3 (bottom) at the two-hour post-exercise time point. Significance is set at $p \leq 0.05$. 
CHAPTER V: DISCUSSION

The purpose of this study was to examine the response of cleaved (activated) caspase-3 to acute resistance exercise in the skeletal muscles of young versus old subjects. It was hypothesized that skeletal muscle cleaved caspase-3 content would increase in response to acute resistance exercise. It was further hypothesized that skeletal muscle cleaved caspase-3 content would be higher after acute resistance exercise in older versus younger individuals.

Cleaved caspase-3 and cleaved/procaspase-3 ratio showed a significant main effect (elevated) at all post exercise time points (0P, 1P, & 2P) in response to acute resistance exercise regardless of age. Similar results were observed in an investigation by Yang, Jemiolo, and Trappe, which showed an acute increase in procaspase-3 mRNA expression following resistance exercise (Yang, Jemiolo, & Trappe, 2006). Again, most previous literature refers to the 32kD form of caspase-3 as caspase-3 or total caspase-3; however, for the purposes of this document, it is referred to as procaspase-3. In addition, the 19kD or activated form of caspase-3 is referred to as cleaved caspase-3. An investigation by Kocturk et al. found significant increases in cleaved caspase-3 activity in response to aerobic exercise in young rats. More specifically, increases in cleaved caspase-3 were observed at all post-exercise time points (Kocturk et al., 2007). Kocturk et al. found that cleaved caspase-3 activity at 3-hours post-exercise was the most elevated when compared to control (Kocturk et al., 2007). Therefore, cleaved caspase-3 responses to acute resistance exercise may be more prevalent at later time points than were examined within this investigation. Moreover, there was more cleaved caspase-3 activity within the soleus muscle in comparison to the gastrocnemius (Kocturk et al., 2007). Within the present investigation, fast-twitch fibers were mixed via homogenization of muscle biopsy samples with slow-twitch fibers. Sarcopenia and diminished muscle hypertrophy occur in fast-twitch muscle fibers; however, this
is unseen among slow-twitch fibers (Rogers & Evans, 1993). On the contrary, an investigation by Quadrilatero et al., found that a prolonged moderate intensity aerobic exercise does not alter procaspase-3 activity (Quadrilatero et al., 2009). The current investigation also displayed an increase in cleaved caspase-3 at both PRE and 0P in the old when compared to the young group; however, these values failed to reach significance. To our knowledge this is the first study investigating cleaved caspase-3 acute response to resistance exercise. Procaspase-3 showed no significant difference in response to resistance exercise or between old and young groups across time points in this present investigation.

In agreement with the hypothesis, AMPK phosphorylation at Thr$^{172}$ was elevated in old subjects immediately post-resistance exercise (0P). This response was not present among the young. In addition, there was a significant main effect in the phospho/total AMPK ratio immediately post-resistance exercise in both old and young groups; however, no differences between age groups alone were evident. No differences between old and young groups at any time point in phospho-acetyl CoA carboxylase (ACC) were observed. ACC is a downstream marker of AMPK activity (Hawley et al., 1996). In contrast to phospho-AMPK, there was a significant increase (main effect) in phospho-ACC and phospho/total ACC at 0P and 1P (1-hour post-exercise) for both old and young groups compared to pre-exercise levels (PRE). No significant effect of age groups for phospho/total ACC ratio were observed at any time point.

Current results concerning AMPK phosphorylation partially agree with previously published literature. Research conducted by Drummond et al. displayed a significant increase from baseline in AMPK phosphorylation in older subjects, but not in younger, at both 1 and 3 hours post-resistance exercise (Drummond et al., 2008). The results concluded by this investigation illustrated a significant elevation of AMPK phosphorylation in old subjects 0P;
however, the phosphorylation of AMPK fell to levels comparable to young subjects by 1P. More specifically, phosphorylation of AMPK approached baseline in old and returned to baseline in young by 1P. On the contrary, Drummond et al. found phospo-AMPK levels to remain elevated for up to 3-hours post-exercise. Factors contributing to these dissimilarities could include the fact that Drummond et al. used 8 sets of 10 repetitions at 70% of a 1 repetition maximum, while this investigation used only 3 working sets at 100% of a 10-repetition maximum. Even though the present investigation went until failure, the extra working sets utilized within the Drummond et al. investigation may have contributed to higher and prolonged phosphorylation of AMPK in response to leg extension exercise (Drummond et al., 2008). An investigation by Thomson and Gordon concurred with this likelihood. Thomson and Gordon found that phosphorylation of AMPK is higher in old versus young muscle both a rest and after chronic overload in rats (Thomson & Gordon, 2005). Furthermore, the Drummond investigation required subjects to ingest an amino acid and carbohydrate supplement (leucine-enriched EAA) in addition to the resistance exercise. The use of non-fasted subjects by Drummond et al. may have had an impact of phosphorylation of AMPK, since AMPK plays a role in internal energy stores and creating homeostasis. The current investigation was conducted with the use of fasted subjects.

The present investigation found an age-related difference in the phosphorylation of AMPK in response to resistance exercise; however, ACC phosphorylation, an indicator of AMPK activity, was similar between both the old and young groups. More specifically, ACC phosphorylation was elevated equally in both old and young groups and in a more sustained manner when compared to AMPK phosphorylation. ACC phosphorylation and phospo/total
ACC ratio displayed a significant main effect at 0P and 1P in comparison to the PRE values in old and young age groups combined. These data further conclude that AMPK activation may not have differed between the groups.

Many factors could have been contributors to the lack of differences observed in the phospho-ACC between the old and young groups within the present investigation. One such factor to why significant differences may not have been observed could be due to the possibility that the older adults were healthier than the average older adult. Therefore, the group of old subjects within this study may not have been truly representative of the general older adult population. The invasiveness of the present investigation may have also had an effect on the willingness to participant. Therefore, an N of only 18 was used for the duration of the study. The delimitations of this study omitted all individuals that participated in regular physical activity. Consequently, the young group may not abide by the same lifestyle factors as the older subjects; therefore, the older group may simply be healthier than the young group. Another aspect to consider is the fact that the differences seen in phospho-ACC between the old and young group may have only occurred in the fast-twitch fibers. These fibers were mixed via homogenization of muscle biopsy samples with slow-twitch fibers in the current study. According to Thomson and Gordon, the utmost AMPK and ACC results occur only in fast-twitch muscle fibers and not slow-twitch (Thomson & Gordon, 2008). Homogenization processes leading to the mixing of fiber types could also be a contributor to the dearth of noticeable differences between phospho-ACC among the age groups.

Our results failed to show a definitive relationship between AMPK and cleaved caspase-3 activation before or after acute resistance exercise within young and old skeletal muscle; however, other investigations have shown that activation of AMPK (by AICAR or other
common activator) leads to increases in caspase-3 activity within other cell types (Sai et al., 2006; Riboulet-Chavey et al., 2008; Jung et al., 2010). Investigations have shown that inhibition of AMPK activation can suppress both total and cleaved caspase-3 activity in cancer cells (Kim et al., 2009; Mukherjee et al., 2008; Khanal et al., 2011). A study by Riboulet-Chavey et al. found a fourfold increase in cleaved-caspase-3 after a period of incubation with an AMPK activator within pancreatic β-cells (Riboulet-Chavey et al., 2008). In contrast, investigations by Konishi et al. & Yeh et al. found that AMPK was anti-apoptotic and was associated with cardiovascular protection in cardiac myocytes (Konishi et al., 2011 & Yeh et al., 2010). More specifically, the AMPK inhibitor dorsomorphin induced apoptosis via increased caspase-3 activity (Konishi et al., 2011). An investigation by Nakashima and Yakabe found that AICAR-induced activation of AMPK failed to induce mRNA expression of caspase-3 within C2C12 myotubes (Nakashima & Yakabe, 2007). AMPK may provide increased protection against apoptosis in multinucleated myotubes in comparison to single-nucleated myoblasts (Niesler, Myburgh, & Moore, 2007). As previously stated, fast-twitch fibers were concealed via homogenization of muscle biopsy samples with slow-twitch fibers. Moreover, sarcopenia predominately affects these fast-twitch muscle fibers; (Rogers & Evans, 1993). These investigations are in contrast to our pilot data, which found increased procaspase-3 protein expression in response to 1 week of AMPK activation in whole muscle of rats. Regardless of whether AMPK induces procaspase-3 protein or mRNA expression in skeletal muscle, the results of the current investigation indicate that AMPK activation may not lead to increased cleaved caspase-3 in response to resistance exercise within aged human skeletal muscle.

Investigations have shown that AMPK also has the ability to sense cellular energy reserves in the form of glycogen. Therefore, glycogen content is known to affect AMPK activity
by increased phosphorylation. Glycogen content may also affect AMPK activity independently (McBride et al., 2009). It is to our knowledge that glycogen content before and after acute resistance exercise in old versus young subjects has not been formerly investigated. The current investigation observed a significant difference in glycogen content between both age groups at PRE. This illustration is supported by previous data, which displayed a decrease in resting muscle glycogen levels with increasing age (Cartee, 1994). The present study exhibited a significant post-exercise decrease for both age groups. This data is also consistent with previous literature. Tesch, Colliander, & Kaiser showed a significant decrease in muscle glycogen content following a variety of resistance exercises in young subjects (Tesch, Colliander, & Kaiser, 1986).

Within the present investigation, glycogen content declined more in young than in old subjects following acute resistance exercise and was statistically equal at 0P, 1P, and 2P. One potential explanation for these variances in glycogen depletion may be a result of the different skeletal muscle fiber types found in old and young adults. Sarcopenia is specific to losses in fast-twitch muscle fibers as opposed to slow-twitch (Rogers & Evans, 1993). Therefore, the elderly have a lower percentage of fast-twitch fibers when compared to younger individuals (Anderson, 2003). Fast-twitch muscle fibers are required for quick and powerful movements; therefore, they store and use more glycogen. In addition, this utilization of more glycogen may be associated to a more intense depletion in overall glycogen content from PRE to 0P in young subjects. The analogous glycogen content observed in the present investigation may have had a significant main effect on ACC phosphorylation and thus AMPK activity. This in turn may have generated an analogous ACC phosphorylation in both old and young groups. There was a significant negative correlation between glycogen content and ACC phosphorylation status at 1P. There was also a negative correlation between glycogen content and ACC phosphorylation at
both 0P, and 1P that approached significance. No significant correlation between AMPK activity and cleaved caspase-3 was observed.

The control of procaspase-3 activity is a multifaceted process and encompasses several interconnected signaling pathways and cascades. Investigations have shown that procaspase-3 is activated by the initiator caspase-9 and is responsible for fulfilling the final executional processes of apoptosis (Siu et al., 2009). In addition, AMPK’s involvement in nuclear apoptosis within skeletal muscle still needs further investigation. In contrast, the present investigation does provide relevance for future research efforts. There were no significant differences between age groups displayed in ACC, cleaved caspase-3, and procaspase-3. However, although no significant differences were seen between age groups, there was slightly higher cleaved caspase-3 content in the skeletal muscle of old at time points 0P and 1P. Furthermore, it may be advantageous to separate the muscle sample by fiber type (fast-twitch versus slow-twitch) to analyze nuclear apoptosis changes on the specific fiber types of old and young groups.

In summary, the current investigation examined cleaved caspase-3 response to acute resistance exercise and its relation to muscle glycogen content and AMPK activation in old versus young subjects. AMPK phosphorylation was increased among the old subjects, but not in young, immediately following resistance exercise. Additionally, glycogen content was significantly lower in old subjects in comparison to young at time point PRE. In both age groups, glycogen content decreased and remained in a depleted state for time points 0P, 1P, and 2P. Cleaved caspase-3 was elevated at all post-exercise time points (0P, 1P, 2P) regardless of age. Therefore, these data suggest a robust response of cleaved caspase-3 (e.g. caspase-3 activity) in response to acute resistance exercise in both young and old subjects. However, exercise-induced AMPK activation may not be the mechanism by which this occurs. Regardless,
future research is necessary in order to fully conclude whether older adults with an increased progression of sarcopenia show dissimilar results pertaining to the relationship between glycogen content and AMPK phosphorylation, ACC, and other markers of apoptosis.


Pyka, G., Lindenberger, E., Charette, S., Marcus, R. Muscle strength and fiber adaptations to


Taaffe, D.R., Pruitt, L., Pyka, G., Guido, D., Marcus, R. Comparative effects of high and low-


APPENDIX A: ECU INSTITUTIONAL REVIEW BOARD APPROVAL DOCUMENT

UMCIRB #: 08-0706

UNIVERSITY AND MEDICAL CENTER INSTITUTIONAL REVIEW BOARD
REVISION FORM

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 Labs

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):

<table>
<thead>
<tr>
<th>Fund</th>
<th>Organization</th>
<th>Account</th>
<th>Program</th>
<th>Activity (optional)</th>
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<tr>
<td>73050</td>
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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:
- [x] East Carolina University
- [ ] Beaufort County Hospital
- [x] Pitt County Memorial Hospital, Inc
- [ ] Carteret General Hospital
- [x] Heritage Hospital
- [ ] Bolce-Willis Clinic
- [ ] Other

The following items are being submitted for review and approval:
- [x] Protocol: version or date: 1/20/10
- [x] Consent: version or date: 1/20/10
- [ ] Additional material: version or date

Complete the following:
1. Level of IRB review required by sponsor:
   - [ ] full
   - [x] expedited

2. Revision effects on risk analysis:
   - [x] increased
   - [ ] no change
   - [ ] decreased

3. Provide an explanation if there has been a greater than 60 day delay in the submission of this revision to the
UMCIRB.

4. Does this revision add any procedures, tests or medications?
   - [ ] yes  
   - [x] no If yes, describe the additional

5. Have participants been locally enrolled in this research study?
   - [x] yes  
   - [ ] no

6. Will the revision require previously enrolled participants to sign a new consent document?
   - [x] yes  
   - [ ] no

Briefly describe and provide a rationale for this revision:

1. One of the testing locations has been changed from Brody School of Medicine Room 35-08 to East Carolina Heart
   Institute Room 2379. All appropriate equipment and supplies will be moved to the new location, and all procedures
   and medical support will remain exactly the same. There is no increased risk. The forms have been changed to reflect
   the new location.

2. I would like to add LaDonna Lynn Maddy to the research team (IRB training completion date approximately 1/12/10).

Principal Investigator Signature: [Signature]
Print: Scott E. Gordon
Date: 1/20/10

Box for Office Use Only

The above revision has been reviewed by:
- [ ] Full committee review on 1/12/10
- [x] Expedited review on 1/12/10

The following action has been taken:
- [x] Approval for period of 1/12/10 to 1/12/10
- [x] Approval by expedited review according to category
- [ ] See separate correspondence for further required action.

Signature: [Signature]
Print: [Print]
Date: [Date]

UMCIRB Version 2/21/08
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 an 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.

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<tr>
<th>Participant’s Name (print)</th>
<th>Signature</th>
<th>Date</th>
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<tbody>
<tr>
<td>Authorized Representative Name (print)</td>
<td>Relationship</td>
<td>Signature</td>
</tr>
<tr>
<td>Person Obtaining Authorization</td>
<td>Signature</td>
<td>Date</td>
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</table>

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
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)

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Principal Investigator’s Name (Print)

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<tr>
<th>Signature of Principal Investigator</th>
<th>Date</th>
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APPENDIX C: PERSONAL HISTORY FORM

PERSONAL HISTORY FORM

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) ....
   ____________________________ __________________________
   ____________________________ __________________________
   ____________________________ __________________________
   **Any major illnesses in the past?** (if yes, explain) ..... **(date)** ______ yes no
   ____________________________ __________________________
   ____________________________ __________________________
   ____________________________ __________________________
   **Any hospitalization or surgery?** (if yes, explain) ...... **(date)** ______ yes no
   ____________________________ __________________________
   ____________________________ __________________________
   ____________________________ __________________________
   **Have you ever had an EKG (electrocardiogram)?** ...... **(date)** ______ yes no
   ____________________________ __________________________
   ____________________________ __________________________
   ____________________________ __________________________
   **Are you diabetic?** ....If yes, at what age did you develop diabetes: ______ yes no
   ____________________________ __________________________
   ____________________________ __________________________
   ____________________________ __________________________
   **Are you currently taking any medications?** ............................ yes no
   ____________________________ __________________________
   ____________________________ __________________________
   ____________________________ __________________________

Medication  Dosage  Reason  Times taken per day

   _____________________________________________________________

5. **Family History**
<table>
<thead>
<tr>
<th>Relationship</th>
<th>Age of occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Age of occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>High blood pressure</td>
<td></td>
</tr>
<tr>
<td>Heart attack</td>
<td></td>
</tr>
<tr>
<td>By-pass surgery</td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
</tr>
<tr>
<td>Gout</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Cigarette history</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Quit months/years</td>
<td>1-10 daily</td>
</tr>
<tr>
<td>Cigarette</td>
<td>11-20 “</td>
</tr>
<tr>
<td>Snuff</td>
<td>21-30 “</td>
</tr>
<tr>
<td>Chewing tobacco</td>
<td>31-40 “</td>
</tr>
<tr>
<td>Pipe</td>
<td>more than 40</td>
</tr>
</tbody>
</table>

Total years of tobacco use? ______

<table>
<thead>
<tr>
<th>Snuff history</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.5 cans daily</td>
<td></td>
</tr>
<tr>
<td>0.5-2.5 cans “</td>
<td></td>
</tr>
<tr>
<td>&gt; 2.5 cans “</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chewing history</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.5 pouches daily</td>
<td></td>
</tr>
<tr>
<td>0.5-2.5 pouches “</td>
<td></td>
</tr>
<tr>
<td>&gt; 2.5 pouches “</td>
<td></td>
</tr>
</tbody>
</table>

7. **Weight History**

What do you consider a good weight for you? ______

Weight at age 21? ______

Weight since age 21? ______

Weight one year ago? ______

Weight now? ______

8. **Cardio-Respiratory History**

Any heart disease now?...yes no

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

Heart murmur?...yes no

Occasional chest pains?...yes no
Chest pains on exertion?.................................................................................................................. yes no
Fainting?........................................................................................................................................ yes no
Daily coughing?.......................................................................................................................... yes no
Cough that produces sputum?........................................................................................................ yes no
High blood pressure?.................................................................................................................. yes no

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

9. **Muscular History**

   Any muscle injuries or illnesses now?......................................................................................... yes no
   Any muscle injuries in the past?.................................................................................................. yes no
   Muscle pain at rest?.................................................................................................................... yes no
   Muscle pain on exertion?............................................................................................................. yes no

10. **Bone-Joint History**

    Any bone or joint (including spinal) injuries or illnesses now?................................................ yes no
    Any bone or joint (including spinal) injuries or illnesses in the past?................................. yes no
    Ever had painful joints?............................................................................................................ yes no
    Ever had swollen joints?........................................................................................................... yes no
    Flat feet?..................................................................................................................................... yes no

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

    Are you post-menopausal (e.g., not had menstrual flow for at least one year)? ...................... yes no
    Have you had a hysterectomy? ................................................................................................... yes no
    If you have had a hysterectomy, were the ovaries removed? ................................................. yes no
If pre-menopausal: On what date did your last period start (beginning of flow)? ____________
Are your periods regular? .......................................................... yes no
Approximately how many days apart are your periods? _____________
Are you on any hormonal supplements, such as a birth control pill or estrogen replacement therapy? .......................................................... yes no
If so, what? ________________________________

12. **Nutritional Survey**

How many times do you usually eat per day? ______

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

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

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

How many servings per week do you usually consume?

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

13. **Physical Activity Survey**

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

- ___ much less
- ___ somewhat less
- ___ about the same
- ___ somewhat more
- ___ much more

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

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

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

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

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

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

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

h. What aerobic activity or activities would you prefer in a regular exercise program for
   yourself?
   ___ Walking and/or running    ___ Tennis    ___ Bicycling
   ___ Racquetball    ___ Swimming    ___ Basketball
   ___ Aerobic dance    ___ Stationary cycling    ___ Soccer
   ___ Stair climbing    ___ Rowing    ___ Other

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

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

16. **Education**
Please indicate the highest level of education completed.
___ Grade School    ___ Junior High    ___ High School
___ College    ___ Graduate    ___ Postgraduate
Please indicate degree earned (i.e. B.A., M.S., Ph.D.) _____________________________

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

18. **Family Physician**
Name: _____________________________
Address: _____________________________
Phone: _____________________________
Should it be necessary, may we send a copy of your results to your physician?
19. **Insurance** I, _____________________ understand that this evaluation is not reimbursable under Medicare and the cost of the evaluation must be paid by me.

Signature: ________________________________
Date: ___________________________________
# APPENDIX D: DIETARY LOG FOR A TYPICAL DAY

**Date** _________________  **Subject ID #** _______________

**Dietary Log for a Typical Day**

<table>
<thead>
<tr>
<th>Meal</th>
<th>Time of day</th>
<th>Serving Size</th>
<th>Food Item</th>
<th>Prepared by:</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

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

___________________________________________________________________

___________________________________________________________________
APPENDIX E: EXPERIMENTAL SESSION INSTRUCTIONS

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

Principal Investigator: Scott E. Gordon, Ph.D.
Telephone #: (252) 737-2879
Sub Investigators: Hope Tharrington/Jen Macesich/Eric Choplin
Telephone #: (252) 883-2001/(919) 606-2853/(919) 671-1482

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/Eric Choplin.