The Effects of Acute Aerobic and Resistance Exercise on mTOR Signaling and Autophagy in Human Skeletal Muscle

by

Corey Mazo

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Graduate Supervisory Committee:

Jared M. Dickinson, Chair
Chad C. Carroll
Siddhartha S. Angadi

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ABSTRACT

Exercise serves as a powerful stimulus to induce skeletal muscle adaptation. For instance, it is well understood that aerobic exercise (AE) elicits an adaptive response ultimately leading to increased fatigue resistance and capillarization, whereas resistance exercise (RE) is known to elicit an adaptive response leading to increased muscle strength and size. However, the precise molecular mechanisms mediating these unique adaptations to different forms of exercise remain to be completely resolved. The purpose of this study was to investigate the adaptive cellular response of skeletal muscle following acute AE and RE. Specifically, this study focused on two molecular processes: 1) mammalian/mechanistic target of rapamycin (mTOR) signaling pathway, a regulator of muscle protein synthesis, and 2) autophagy, a process through which proteins and organelles are broken down in the muscle fiber. In a counterbalanced, crossover design, six healthy, recreationally active young men (27±3 yr) completed acute AE (40 min of cycling ~70% maximal HR) and acute RE [8 sets, 10 reps, ~65% 1-repetition maximum (1RM)] separated by ~1wk. Muscle biopsies (*vastus lateralis*) were obtained before, at 1 and 4h post exercise and western blot analyses were used to examine the phosphorylation of mTOR signaling proteins and various markers of autophagy. Phosphorylation of mTOR^{Ser2448} increased only following RE at 4h (P < 0.05). However, phosphorylation of p70S6K1^{Thr389}, a downstream marker of mTOR, increased following both AE and RE at 4h (P < 0.05). However, p70S6K1^{Thr389} was phosphorylated to a greater extent at 1h following RE compared to AE (P < 0.05). LC3BII was decreased at 1h and 4h postexercise in response to both AE and RE (P < 0.05). These data indicate that both acute AE and RE stimulate, to some degree, mTOR signaling in skeletal muscle, a pathway associated with increased muscle protein synthesis. Further, based on markers examined in the current study, both acute AE and RE similarly stimulate autophagy, which is associated with muscle protein breakdown. These data indicate that, at least in the immediate hours post exercise, the unique adaptations to AE and RE exercise may be mediated through cellular pathways other than mTOR and autophagy.

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CHAPTER ONE

INTRODUCION

Skeletal muscle is unique in its ability to adapt to various stimuli. In particular, exercise serves as a powerful stimulus for skeletal muscle adaptation, or the change in function of skeletal muscle. However, different forms of exercise can elicit very unique adaptations in skeletal muscle. For instance, when performed chronically, aerobic exercise (i.e., cycling, running) generally results in increased mitochondrial density, through mitochondrial biogenesis (76, 78), leading to increased fatigue resistance of the skeletal muscle. Ultimately when coupled with increased capillary concentration, this skeletal muscle adaptation allows for exercise to be maintained for longer periods of time and often at an increased intensity (61). Conversely, chronic resistance exercise training (e.g., weight lifting) results in a muscle with a greater ability to produce force as a result of accumulation of contractile proteins (24, 94). This increase in contractile protein is often associated with muscle hypertrophy (55). It is well understood that the adaptation of skeletal muscle is ultimately facilitated through specific cellular processes. To that end, we have previously shown in untrained individuals that several genes are uniquely responsive to acute cycling and weight lifting exercise in the immediate hours following exercise (27) highlighting that these different forms of exercise stimulate a unique transcriptional response in skeletal muscle. However, what remains to be determined are the specific cellular pathways/processes that may be governing the unique adaptation of skeletal muscle to different forms of exercise (i.e., aerobic vs. resistance exercise).

Adaptations of skeletal muscle are, in part, regulated through the activation of specific molecular signaling pathways (48, 84). In general, these molecular pathways are acutely simulated in response to each bout of exercise, leading to increased gene expression (transcription) (27) and/or increased mRNA translation (131). One such pathway, the mammalian/mechanistic target of rapamycin (mTOR) signaling pathway, is one of the most well-known regulators of cell growth, as this pathway controls, among other cellular processes, the ability to stimulate translational rates (i.e., muscle protein synthesis) (28, 31). In regards to a role for mTOR signaling as it relates to regulating the specific adaptation of muscle to different forms of exercise, Coffey et al. has

demonstrated that mTOR^{Ser2448} phosphorylation is increased in response to acute aerobic and resistance exercise bouts when compared to rest in previously trained subjects (20). Conversely, Atherton et al. utilized high and low frequency electrical stimulation to mimic resistance exercise and aerobic exercise, respectively in isolated rat muscles and found mTOR phosphorylation at Ser^{2448} was increased only in response to the resistance exercise frequency, demonstrating ambiguity of the mTOR signaling response (3). In addition, Coffey et al. demonstrated the downstream target of mTOR, S6K1, was significantly increased in endurance trained athletes, but not in resistance trained athletes performing a an acute bout resistance exercise (22). Similarly, S6K1 significantly increased in response to resistance exercise in subjects who perform concurrent training (i.e., both aerobic and resistance exercise training) (19). While these results indicate that activation of the mTOR pathway may differ between modes of exercise, these studies were employed in trained participants, which may have impacted the findings given the muscle is already adapted to the exercise stimulus. To what extent mTOR signaling is stimulated in response to acute aerobic exercise or resistance exercise has yet to be thoroughly investigated in an untrained population.

Skeletal muscle adaptation is governed by a relationship between protein synthesis and protein breakdown. While mTOR signaling is well characterized for its role in regulating protein synthesis following exercise, it is also well understood that acute exercise elicits changes in muscle protein breakdown (49, 85). In particular, autophagy is a process that regulates clearance of damaged cells and proteins to maintain homeostasis (125). Specifically, autophagy removes and recycles long lived contractile structures (i.e., actin and myosin) as well as organelles (i.e., mitochondria), and acute exercise has routinely been shown to stimulate autophagy in mouse and human skeletal muscle (69, 71, 72, 126). With respect to different forms of exercise, relative to resistance exercise, acute aerobic exercise appears to elicit a more robust and longer time course for proteolysis in skeletal muscle, at least when examining components of the ubiquitin proteasome systems (27, 86). It is interesting to speculate that an increase in breakdown following acute exercise is to maintain skeletal muscle homeostasis and stimulate mitochondrial turnover (108). However, markers of autophagy have yet to be investigated in untrained subjects performing

different forms of acute exercise. This level of investigation is important given the role of autophagy is regulating mitochondrial turnover and the targeted mitochondrial adaptations that are unique to aerobic exercise.

Whereas many studies have examined the cellular adaptive response of skeletal muscle following acute exercise, most of these studies have investigated only one form of exercise (e.g., either acute aerobic or acute resistance exercise) (24, 49, 55, 57, 69, 71, 78, 103, 126, 131). Further, those studies that have compared different forms of exercise have either examined the response of trained athletes (19, 22, 24, 41, 69, 84) or examined different muscles (107). Collectively, comparisons with respect to the molecular mechanisms that may be regulating the unique adaptation of skeletal muscle to different forms of exercise are difficult in the context of the existing literature. This thesis will take a unique approach to identifying those mechanisms that may be regulating the unique adaptation of skeletal muscle, by employing a cross-over design in which the same untrained individuals will perform both acute aerobic and acute resistance exercise. Importantly, both forms of exercise will focus on activation of the quadriceps muscle to allow for examining the acute response of the same muscle to each exercise mode. Investigation into the response of cellular signaling pathways to acute divergent exercise allows a greater understanding of the specific adaptations of the muscle to exercise. A better understanding of the acute response of skeletal muscle to different forms of exercise could provide a basis for prescription exercise to elicit specific responses or adaptations in persons with skeletal muscle dysfunction or diseased populations.

SPECIFIC AIMS AND HYPOTHESES

The purpose of this study was to investigate the effects of acute aerobic and resistance exercise on mTOR signaling and autophagy in human skeletal muscle. This study will use a unique crossover design in which untrained subjects will perform acute aerobic or resistance exercise during two separate experimental trials. In conjunction, muscle biopsies will be taken at rest, and at 1 and 4 hours after each acute exercise trial. In particular, we will examine key targets in the mTOR signaling pathway as well as several proteins associated with autophagy to analyze the effects of acute exercise on these molecular processes.

Specific Aim #1: To determine the extent to which acute aerobic and acute resistance exercise stimulate the mTOR signaling pathway in human skeletal muscle.

Hypothesis #1: Acute resistance exercise will stimulate mTOR signaling more than acute aerobic exercise in skeletal muscle.

Specific Aim #2: To determine the extent to which acute aerobic and acute resistance exercise impact markers of autophagy in human skeletal muscle.

Hypothesis #2: Acute aerobic exercise stimulate autophagy more than acute resistance exercise in skeletal muscle.

All experimental trials outlined below have previously been conducted (27). The work outlined herein consisted of new analyses on the skeletal muscle samples collected during these experimental trials. Specifically, the new objectives outlined in this Thesis was to perform analyses to examine how acute aerobic and resistance exercise impact 1) the mTOR signaling pathway, which is important for the regulation of muscle growth, and 2) markers associated with autophagy, which is a process through which protein and organelles (i.e., mitochondria) or degraded in the muscle fiber. These objectives do not overlap with those data previously reported from this cohort of participants (27) but rather provides new and additional insight.

CHAPTER TWO

REVIEW OF LITERATURE

I. INTRODUCTION TO REVIEW OF LITERATURE

The contents of this review of literature are largely focused on skeletal muscle physiology. Attention is given to the composition, structure and function of skeletal muscle, with an emphasis on skeletal muscle adaptation. Discussion includes aspects related to the function of the whole skeletal muscle and to the individual skeletal muscle fibers. With regards to skeletal muscle adaptation, attention is given to the specific adaptations elicited by different types of exercise and to the cellular events that regulate skeletal muscle adaptation. The literature cited largely includes human-subjects research, however, data derived from animal models are also included.

II. SKELETAL MUSCLE STRUCTURE, FUNCTION AND ADAPTATION

Skeletal muscle is a very complex and dynamic tissue that constitutes nearly 40% of one's body weight. Although skeletal muscle is responsible for a variety of bodily functions, arguably its main function is to convert chemical energy into mechanical energy to allow for muscle contraction and movement (42). This section will focus on the structure and function of skeletal muscle, followed by a general discussion of skeletal muscle adaptation to exercise.

A. Whole Skeletal Muscle

1. General Structure

Aside from water, skeletal muscle is comprised primarily of proteins. Moreover, roughly 75% of proteins in skeletal muscle constitute contractile proteins (i.e., actin and myosin) (42) that form the basis of muscle contraction and allowing for voluntary movement. In addition, the skeletal muscle also includes connective tissue, blood vessels and nerves. The whole muscle is surrounded by a layer of connective tissue called the epimysium. Within the whole muscle are groups of fascicles, which are bundles of muscle fibers (cells), that is surrounded by the perimysium. Within the muscle fascicles, the endomysium surrounds each individual muscle fiber. These layers of

connective tissues ultimately attach to the bone and form the tendon, allowing for the transmission of force from the sarcomere to the bone to create joint movement (46).

2. Whole Muscle Function

The force generated within the skeletal muscle is dependent upon nervous system activation, muscle size and muscle architecture. When the skeletal muscle is signaled to contract, an action potential travels down the motor neuron to the neuromuscular junction. Each motor neuron innervates several hundred to several thousand muscle fibers, termed a motor unit, and upon activation of the motor neuron, all muscle fibers innervated contracted maximally. Skeletal muscles contain anywhere from 200-400 motor units in young adults and 100-200 in older adults, thus muscle force can be regulated, in part, based upon the number of motor units activated (30).

The structure of the skeletal muscle also plays a role in force generating capabilities. For instance, a muscle with a larger cross-sectional area generally produces greater force due to having more fibers and sarcomeres (see below for description of a sarcomere) in parallel. Along with cross-sectional area, pennation angle contributes to force production. Pennation angle is defined as the angle at which muscle fibers intersect with the skeletal muscle midline. For example, the greater the intersection angle is, the greater force the muscle is able to produce. Muscle cross sectional area and pennation angle contribute to physiological cross sectional area, which predicts the maximum capability of muscle force generation (13).

B. Skeletal Muscle Fiber

1. Skeletal Muscle Fiber Structure

As discussed above, skeletal muscle is ultimately comprised of a bundle of muscle fibers. For example, Klein et al. found an average of 253,000 fibers in the *biceps brachii* (74), whereas Lexell found anywhere from 500,000 to 900,000 fibers in the *vastus lateralis*, with fiber number decreasing with advancing age (83). Below each endomysium, the connective tissue surrounding each muscle fiber, is another protective tissue layer called the basal lamina (110). The collective

membrane that surrounds the muscle fiber is called the sarcolemma. The sarcolemma is comprised of networks that allow the diffusion of metabolites involved in initiating contraction.

Each individual muscle fiber is comprised of a bundle of myofibrils, which can be further divided into sarcomeres (Figure 1), the contractile unit of the skeletal muscle. These sarcomeres are arranged in a series. The Z-disks mark the start and end of the individual sarcomeres. Within the sarcomere are thick and thin filaments, giving the muscle its striated appearance (35). The thick filament contains myosin, which is a tail-like structure with two heads. The myosin molecule is comprised of two myosin heavy chains (MHC) and four myosin light chain components, however, MHC is responsible for the force-velocity characteristics (7). Moreover, different isoforms of MHC exist (in adult human muscle there are three isoforms: Type I, Type IIa, Type IIx) that contain different ATPase activity, which provides the basis of differences in maximum velocity for shortening (112) and fiber typing (see below).

Thin filaments are composed of actin, troponin and tropomyosin. The thin filaments are attached to the Z-disk creating the I-band. The interaction between thick and thin filaments provides the basis for contraction and force production. Troponin and tropomyosin regulate the ability for the thick and thin filaments to interact.

2. Skeletal Muscle Fiber Contraction

The process of muscle contraction begins with a nerve impulse arriving at the neuromuscular junction. This action potential causes the release of acetylcholine from the motor neuron, which binds to receptors on the membrane of the muscle fiber leading to depolarization of the muscle cell. Ultimately, calcium is released into the muscle cell from the sarcoplasmic reticulum, which then binds to troponin-C. When calcium is bound to troponin-C, tropomyosin is shifted exposing a myosin binding site on the thin filament. For contraction to occur, ATP must bind to the myosin head, causing detachment from the actin binding site. ATP is then hydrolyzed to ADP and an inorganic phosphate, causing a conformational change in the myosin head. The inorganic phosphate is then released, causing the myosin head to move closer to the thin filament. Finally, the ADP is released and the myosin head returns to its original conformation (106) sliding the thin

filament over the thick filament. The Sliding Filament Theory, first described in 1954 by two independent laboratories, explains a model of muscle contraction (66, 67). This cyclic process will continue as long as calcium is present within the muscle.

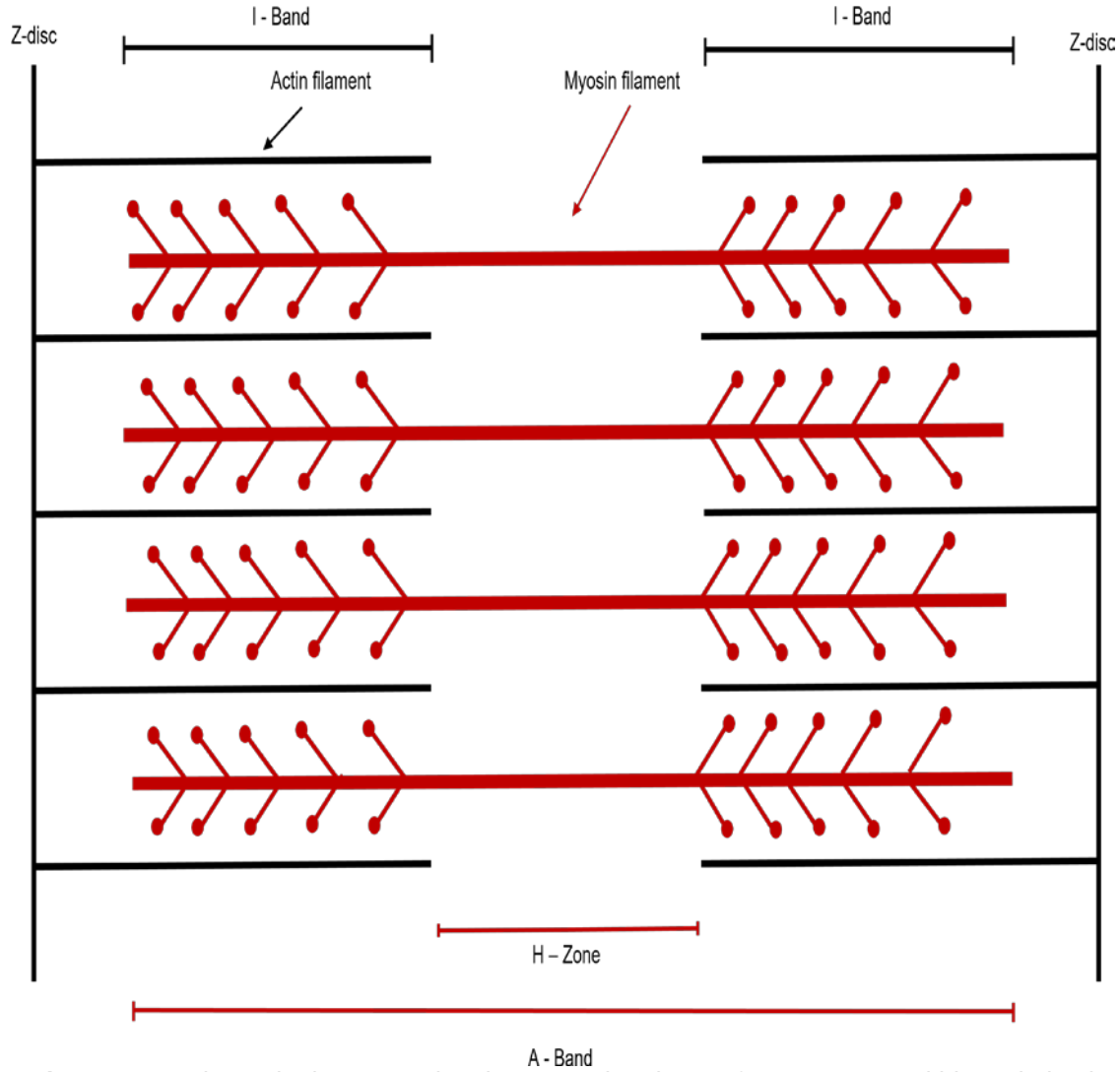

Figure 2-1. Schematic demonstrating the general makeup of a sarcomere within a skeletal muscle fiber.

3. Skeletal Muscle Fiber Types

Human skeletal muscle is comprised of multiple fiber types. The type of muscle fiber is determined by the presence of one or more of the three MHC isoforms, at least for adult human skeletal muscle. The three main isoforms include type I, IIa and IIx (12). However, about 15-20%

of muscle fibers can have a combination of MHC isoforms, giving a unique fiber profile termed a hybrid fiber (124). Fibers containing Type I MHC are referred to as slow twitch fibers and fibers containing type IIa or IIx MHC are referred to as fast twitch fibers. The isoforms display their own set of properties, respectively. Generally, type IIa and IIx fibers generate a higher velocity and power when compared to type I as a result of higher rates of cross bridge cycling. Conversely, type I fibers tend to be generally more fatigue resistant, as a result of more mitochondrial and oxidative enzymes (12).

C. Relationship Among Structure, Fiber Type, and Function

In addition to differences in the structural characteristics of skeletal muscle, the fiber type profile of a given muscle can also influence the contractile characteristic of a muscle. As summarized above, characteristics of skeletal muscle are governed by structure and fiber type profile. Skeletal muscle force generation is dictated by the physiological cross sectional area and pennation angle of fibers. Meanwhile, velocity and power are dictated by the MHC isoform(s) within the muscle fiber. Alterations in these components of skeletal muscle will change the contractile properties of the muscle. Similarly, in a very general sense, adaptation of skeletal muscle is facilitated through the accumulation of specific proteins over a given time.

III. SKELETAL MUSCLE ADAPTATION

Skeletal muscle is very adaptive, and in particular, exercise serves as a powerful stimulus for adaptation. This section is devoted to discussion of the molecular events regulating adaptation. Specifically, this section will review molecular processes such as cell signaling, transcription and translation. More so, how these processes occur and the resulting adaptations are highlighted.

A. Overview

Adaptation is defined as a change in the function (e.g., contractile, metabolic) of a skeletal muscle. Muscle adaptation is ultimately facilitated through a series of molecular events that when routinely stimulated mediate an accumulation of specific proteins. Exercise, is a powerful stimulus

to promote muscle adaptation when performed chronically. Specifically, each acute bout of exercise acutely elicits changes in several molecular processes and it is the accumulation of these acute changes in response to each exercise bout that promotes eventual adaptation. In brief, cellular signaling pathways stimulated by exercise upregulate gene expression, ultimately leading to increased concentration of mRNA in the muscle fiber (18, 54). Increased mRNA concentration is coupled with an increase in translation rate, or muscle protein synthesis (34), ultimately producing more protein. Rates of translation are also regulated through specific signaling pathways. The specific events leading to muscle adaptation are discussed below.

B. Cell Signaling

Cellular signaling pathways are responsible for converting a stimulus into the events that alter the phenotype of skeletal muscle. These pathways are imperative to the highly plastic nature of skeletal muscle (63). Cellular signaling pathways are comprised mostly of molecules (namely proteins) that interact with one another through phosphorylation, and many of these pathways control the transcription of specific genes and the rates of translation. For example, signaling pathways can be stimulated by specific cues, including, but not limited to, reactive oxygen species, exercise or circulating nutrients (33). Consequently, activation or inhibition of specific pathways can regulate the unique adaptation of skeletal muscle and thus, signaling events represent the initial regulator of transcriptional and translational activity during muscle adaptation. This section serves as a general overview of signaling, please see section IV (specific signaling pathways) for discussion of specific signaling pathways.

C. Transcription

Transcription is the synthesis of RNA from the specific base sequence within the genes contained in DNA. For skeletal muscle to adapt, new functional proteins must be created. With regards to forming new proteins, transcription is stimulated by cellular signaling pathways to increase specific mRNA in the cell that can be translated to proteins. This process is regulated by transcription factors that bind to the specific sequence of DNA signifying initiation (12). Upon

initiation, RNA-dependent polymerase moves along a specific sequence of DNA to create messenger RNA (mRNA), signifying elongation (140). Transcription also produced ribosomal RNA (rRNA) as well as transfer RNA (tRNA), however, it is the mRNA molecule produced that contains a complementary nucleotide sequence that will ultimately be translated to a protein. Once the DNA-dependent polymerase is finished transcribing the gene (i.e., termination), the mRNA strand is then transported out of the nucleus to the cytosol where it undergoes modification and interacts with the ribosome. The quantity of mRNA produced signifies gene expression. Moreover, transcription provides the specificity of adaptation by producing specific mRNA.

D. Translation

Translation is the formation of a specific chain of amino acids that uses the base sequence of the mRNA strand as the template. This processes is also often referred to as protein synthesis. Along with transcription, translation can also be stimulated by cellular signaling pathways. Translation initiation is regulated by eukaryotic initiation factors (eIFs) (114), which regulation the formation of the pre-initiation complex. Once the mRNA strand moves to the cytoplasm, two ribosomal subunits are bound to the mRNA molecules where translation occurs (12). mRNA molecules are read in three letter combinations of nucleotides called codons. As the subunits travel down the mRNA molecule, transfer RNA (tRNA), carry specific amino acids based on the sequence of nucleotides in the codon. Once translation is terminated, the end result is a polypeptide/protein. The final product then undergoes post-translational modifications to ensure proper function. Translational activity can be upregulated for 12-36 hours after exercise (92).

E. Summary

Adaptation is defined as the accumulation of specific proteins over a given period of time. In particular, muscle adaptation is largely governed by repeatedly stimulating signaling pathways, transcription and translation. The acute activation of these cellular processes lead to the accumulation of specific proteins and the adaptation of skeletal muscle.

IV. SPECIFIC SIGNALING PATHWAYS

The molecular mechanisms of skeletal muscle adaptation involve multiple signaling pathways. As briefly discussed above, cell signaling pathways are regulatory pathways that control cellular growth and degradation through feedback mechanisms (119). These pathways contain several molecules, mostly proteins that interact with one another through phosphorylation. However, signaling pathways respond to different degrees based on the stimuli. For example, resistance exercise, known as low-frequency, high intensity contractions, is thought to stimulate signaling pathways promoting contractile protein synthesis. Contrary, aerobic exercise is thought to stimulate pathways to increase mitochondrial content (105). This section will elaborate on how different signaling pathways are stimulated by exercise, including the downstream events regulated by specific signaling pathways. The first part of this section will discuss pathways largely related to anabolic processes while the latter part of this section will discuss pathways largely related to catabolic processes. Moreover, it is important to note that several other "pathways" are involved in the regulation of skeletal muscle adaptation, however, those pathways directly examined in this Thesis will be discussed. For detailed discussion on other pathways see (120, 127).

A. Anabolic / Growth Related Pathways

1. Mammalian/Mechanistic Target of Rapamycin (mTOR)

The mTOR (mammalian/mechanistic target of rapamycin) pathway is composed of a variety of proteins and its activity largely regulates cell growth and muscle protein synthesis. More so, mTOR can be activated by exercise, nutrition and growth factors (10, 129, 139). Although the mTOR signaling pathway contains many downstream targets, mTOR itself is seen as the central molecule determining the activation of this cascade (129). mTOR is formed by two distinct complexes, mTOR complex 1 (mTORC1) and mTORC2 (73). However, mTORC1 is primarily activated by anabolic stimuli (82). The activation of the mTOR signaling cascade can be measured by phosphorylation of downstream proteins, namely S6 kinase 1 (p70S6K1), 4E-binding protein 1 (4E-BP1), and ribosomal protein S6 (rps6) (81, 88). For muscle hypertrophy to occur, protein synthesis rates must be greater than protein breakdown rates over time. Moreover, mTOR is

known to downregulate markers of muscle breakdown (73). In particular, mTOR phosphorylates Unc-51 like autophagy activating kinase (ULK1), ultimately preventing a key step in stimulating autophagy (73), which is associated with muscle breakdown and is discussed below.

It is well documented that acute resistance exercise enhances mTOR signaling (14, 26, 65). However, the response of mTOR varies with the time course of muscle biopsies and exercise protocol. For example, Fujita et al. (44) examined subjects performing 10 sets of 10 repetitions of leg extension at 70% 1 repetition max. Muscle biopsies were taken 1 hour pre-exercise, immediately before exercise, immediately after exercise, and at one and two hours post exercise. mTOR signaling along with downstream markers were all upregulated, or activated, in response to the acute exercise bout. Similarly, Terzis et al. (122) recruited subjects to perform either 1, 3, or 5 sets of 6 repetition max, in a random order, on 3 separate occasions. Muscle biopsies were taken immediately before and 30 minutes after exercise. Although the mTOR signaling cascade was enhanced by all set variations, it was most drastically enhanced by the 3 and 5 set scenarios. In a another study, Koopman et al. (79) had subjects perform 8 sets of 10 repetitions at 75% 1 repetition max on both leg extension and leg press. Muscle biopsies were taken before, immediately after, 30 minutes and 120 minutes post exercise. In this study, downstream markers of mTOR signaling were upregulated starting 30 minutes after termination of acute resistance exercise.

The above mentioned studies contain a common theme, in that resistance training increasing mTOR signaling activity. However, the exercise protocol and time of muscle biopsies greatly vary. Likewise, mTOR signaling has also been shown to be increased by acute aerobic exercise. Carraro et al. (15) recruited subjects to perform 4 hours of cycling at 40% VO_{2max}. Tracers were used to assess changes in muscle protein synthesis rate during and post exercise. It is to be noted that the synthesis rate was increased in response to the acute exercise bout. Although this study does not investigate activation of signaling pathways, it demonstrates the acute effect of aerobic exercise on muscle protein synthesis rates, which is largely regulated my mTOR signaling (10). More directly, Mascher et al. (90) conducted a study where 6 subjects performed cycling for 1 hour at 75% VO_{2max}. Muscle biopsies were taken at rest, immediately after, 30 min and 2 hours

after exercise. mTOR along with downstream markers (S6K1 and eEF2) were phosphorylated at all biopsy times post exercise.

Collectively, these studies demonstrate how acute aerobic and resistance exercise both increase the activation of the mTOR pathway. This leads to the belief that both acute aerobic and resistance exercise leads to increased mTOR signaling, as both lead to adaptation, or accumulation of specific proteins. However, there has been no direct comparison as to the level of activation of mTOR between aerobic and resistance exercise, particularly within the same subjects (see **Divergent Exercise** below**)**, and the timing of biopsies are also greatly varied.

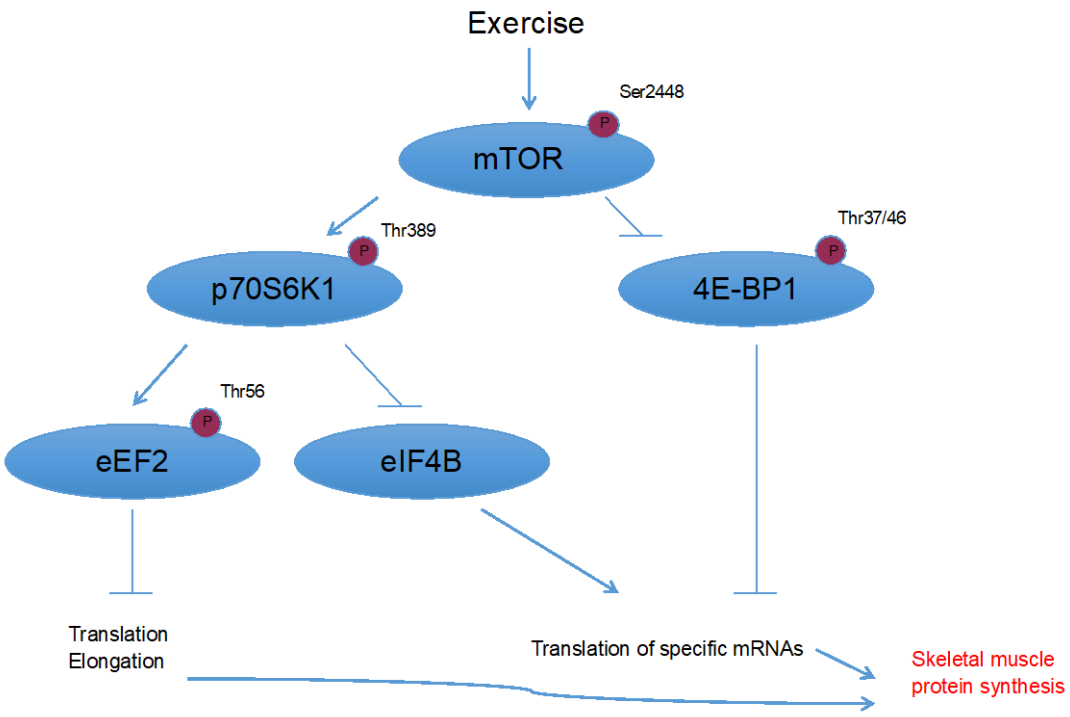

Figure 2-2. Conceptual schematic of mTOR signaling cascade, ultimately leading to skeletal muscle protein synthesis. As exercise serves as a stimulus, mTOR becomes phosphorylated. In turn, phosphorylating 4E-BP1 and p70S6K1. P70S6K1 then phosphorylates eEF2 and eIF4B. These events ultimately lead to elongation and translation of specific mRNAs.

2. AMP-activated protein kinase (AMPK)

The AMP-activated protein kinase (AMPK) pathways is a key regulator of energy balance. AMPK is activated by an increase in cellular AMP concentration (53). AMPK is known to inhibit mTOR signaling pathway and also known to increase the rate of catabolic pathways (95, 136). However, the direct effect that AMPK has on skeletal muscle is more ambiguous. AMPK is regulated by the ratio of AMP:ATP, and based on that ratio, AMPK attempts to return to energy homeostasis (52). The changes in AMP:ATP can be the result of exercise. However, there is a great amount of overlap between AMPK and other signaling pathways. In recent studies in rats, AMPK activation is suggested to induce mitochondrial biogenesis (134). To that end, in humans, AMPK signaling elicits with increased peroxisome proliferator-activated receptor-gamma (PCG-1α) expression increased after performing aerobic exercise. In particular, PGC-1α, is a transcriptional cofactor regulating transcription of mitochondrial specific genes and promoting adaptation of

skeletal muscle to endurance training. Most notably, this response increases mitochondrial content thus inferring AMPK signaling is associated with increased mitochondrial biogenesis (113, 115).

3. Akt / Protein Kinase B

Akt, also known as protein kinase B, plays a crucial role in the balance between anabolic and catabolic signaling. Akt is activated by stimuli such as growth factors and muscle contraction (38). Akt is phosphorylated by insulin-like growth factor 1 (IGF-1) receptor, which is a receptor activated by insulin. When the IGF-1 receptor is stimulated, phosphatidylinositol-3-kinase is phosphorylates phosphoinositide-dependent kinase 1 (PDK1). PDK1 then activates Akt, which represses the transcription factor FoxO (see also below) and stimulates mTOR signaling, promoting growth (111). Specifically, in response to insulin, Akt becomes phosphorylated at threonine 308. Currently, Akt serves as a biomarker for cell growth, and can be targeted for tumor growth (128).

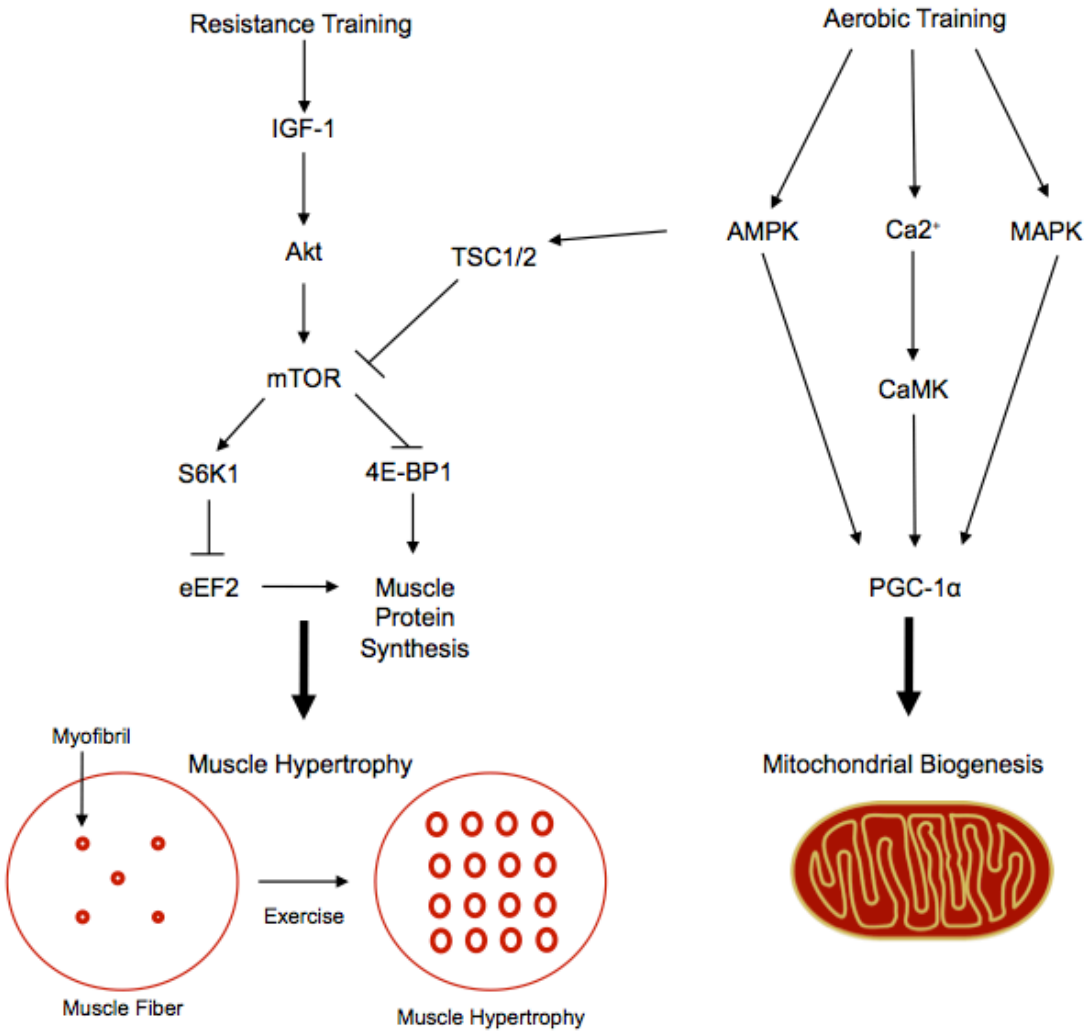

Figure 2-3. Simplified conceptual schematic of mTOR signaling cascade and the interaction with AMPK and MAPK signaling, ultimately leading to muscle hypertrophy and mitochondrial biogenesis, respectively.

B. Catabolic Processes / Pathways

1. Autophagy

Autophagy is regulatory mechanism that recycles cells of dysfunctional or damaged molecules and organelles (37). There are three types of autophagy, however, macroautophagy is the most prevalent (138). Macroautophagy itself is a process in which autophagosomes are formed from accumulation of autophagic vacuoles, partially regulated by Autophagy-related genes (Atgs). However, for the autophagosome formation, microtubule-associate protein 1 light chain 3 (LC3)

must be produced. For this to occur, Atg4 is activated to produce cytosolic LC3B-I. LC3B-I is then transferred to Atg3 where it is modified to active LC3B-II, targeting conjunction of autophagosome membranes (121). The autophagosome then fuses with lysosomes to form a vesicle called the autophagosome (36). More so, autophagy is also regulated by many signaling pathways, a central pathway being mTOR. Specifically, mTOR interferes with autophagosome formation through hyperphoshphorylaion of Atg13 (17). Also, mTOR interferes with ULK phosphorylation, ultimately inhibiting phosphorylation of Atg13 (73). The final product results in degradation and reutilization of damaged cellular components to maintain homeostasis.

Several markers related to autophagy are changed in response to exercise. Performing resistance exercise elicits increased rates of muscle protein synthesis and muscle protein breakdown up to 48 and 24 hours post exercise, respectively (103). Many other studies also demonstrate acute resistance exercise leads to increase muscle protein breakdown (29, 43). Lira et al (85) investigated mice that completed treadmill running for four days. Western blot analysis found not only markers of mitochondrial biogenesis to be upregulated, but also markers of autophagy. Comparably, acute endurance exercise has been shown to increase markers of autophagy (68, 69).

As mentioned above, one of the main markers of autophagy are LC3BI and LC3BII. However, simply looking at concentrations of these markers can be difficult to interpret. Decreased LC3BII concentration can indicate decreased autophagy, for instance less conversion of LC3BI to LC3BII. Conversely, decreased LC3BII can also indicate increased autophagy, since LC3BII is degraded in the lysosome during autophagy (29). To navigate this, p62 is often analyzed as it is in part responsible for recruitment of autophagic membranes. As a result, p62 is decreased during the formation of autophagosomal structures (9). Nonetheless, interpretation of autophagy based on assessment of these markers is used to examine the changes in autophagic flux in response to acute exercise bouts.

2. Ubiquitin-Proteasome System

The ubiquitin-proteasome system (UPS) regulates several processes including cellular quality control, DNA repair and cell stress response. The main function of this system is to tag proteins for proteasome degradation. While a detailed description of this pathway is beyond the scope of this literature review, research indicates that aerobic exercise may actually stimulate the UPS pathway more than RE, in particular with respect to the time course of expression (23). However, as mentioned earlier, expression of ubiquitin-proteasome system genes are regulated by Akt. Specifically, FoxO3a is a transcription factors that increases expression of UPS genes, namely muscle-specific RING finger-1 (MuRF1) muscle atrophy F-box (atrogin-1) (109). When Akt is activated, Akt phosphorylates FoxO3a, which moves FoxO3a to the cytosol, attenuating its transcriptional activity (29). Consequently, an increase in the expression of MuRF1 or atrogin-1 often serves as a marker of activity of the UPS. Notably, FoxO3a is also responsible for regulating the expression of several genes associated with autophagy (130). Consequently, examining of the cellular location of Foxo3a, for instance in the cytosol vs. in the nucleus, can serve as an indicator of the catabolic activity of the muscle.

V. SKELETAL MUSCLE ADAPTATION TO EXERCISE

Skeletal muscle adaptation occurs as a result of accumulated molecular response to acute exercise bouts, resulting in chronic adaptations. However, exercise can be performed in a variety of ways. Performing both aerobic and resistance in the same bout is considered concurrent exercise, while divergent exercise refers to a single bout of exercise. This section will review whole and cellular adaptations to aerobic, resistance, concurrent and divergent exercise.

A. Skeletal Muscle Adaptation to Resistance Exercise

1. Whole Muscle

Resistance training ultimately leads to increased muscle size, strength and power output. However, the initial adaptations to occur that attribute to increases in strength are mainly neural

adaptations (96). These neurological adaptations include increased rate of discharge rate of motor units (32). Moreover, previous research has shown that subjects completing isokinetic training for two weeks had no hypertrophic adaptations, but the training resulted in increased muscle strength (2, 97). Studies involving longer timelines demonstrate more prominent hypertrophy (4, 118). Hypertrophy, defined as increases in muscle size, is a result of accumulated contractile proteins in the muscle. As a result of hypertrophy, muscle strength increases as discussed above (see section II. Skeletal muscle structure, function and adaptation). For instance, Bechshoft et al. (5) found a 3.4% increase in quadriceps cross-sectional area after 12-weeks of heavy resistance exercise training in an elderly population. Furthermore, resistance exercise training increased their peak torque and power by 91%, compared to baseline measures. In addition, Haun et al. (56) found an average hypertrophy change of +10.7% after six weeks of high volume resistance training. Using amino acid tracers, Chesley et al (16) showed increased rate of muscle protein synthesis after resistance exercise. However, resistance training also stimulates muscle protein breakdown (8, 103). Thus, muscle protein synthesis must exceed the rate of muscle protein breakdown for hypertrophy to occur (14). When performed chronically, resistance training increases muscle protein synthesis rates more than breakdown, therefore promoting hypertrophy over time (99, 103).

2. Cellular level

One of the most important adaptations to resistance training is to increase force production. For this to occur, new contractile proteins must be synthesized. As discussed above, the addition of contractile proteins such as actin, myosin, etc., is dependent on the relation between muscle protein synthesis and muscle protein breakdown (93). Muscle contractions lead to increased quantity of mRNA as a result of gene transcription. mRNA's are then translated into functional contractile proteins, which assist in enlargement of myofibers (6). In addition, resistance training is known to elicit shift toward type II muscle fibers. For instance, eight weeks of lower extremity heavy resistance exercise training significantly increased maximum dynamic strength in exercises performed in both men and women, and while there were no significant changes in fiber cross sectional area, there was a significant decrease in type IIx fibers (116). Additionally, 19 weeks of

heavy resistance training has been shown to elicit a significant decrease in percentage of type IIx fibers along with a significant increase in percentage of type IIa fibers (1). On the other hand, obese, insulin resistant men who performed 16 weeks of progressive exercise training demonstrated a shift towards type IIx fibers (118). Several other studies also demonstrate shift towards type II muscle fibers, specifically from hybrid fibers (40, 102, 133). Fiber shift can result from increased myokinase activity, which assists with the rapid utilization of ATP (123). Resistance training is also shown to increase type II fiber cross sectional area (62).

Muscle protein synthesis is mediated by signaling pathways such as the Akt-mTOR cascade, which regulates transcription and translation, as previously stated in the review (22). As discussed in section *Mammalian/Mechanistic Target of Rapamycin (mTOR)*, resistance exercise is a powerful stimulus to increase the activation of the mTOR pathway and enhance muscle protein synthesis rate. In fact, Drummond et al. (31) demonstrated that activation of the mTOR pathway is an integral contributor to increased muscle protein synthesis following resistance exercise. Subjects were randomized to a control group or a rapamycin treatment group that was administered rapamycin prior to performing resistance exercise. Specifically, rapamycin is a known inhibitor of the mTOR protein. Muscle protein synthesis in response to acute resistance exercise was increased in the control group, along with the activation of the mTOR signaling cascade. However, this response was attenuated in the subjects that were administered rapamycin. This study demonstrates that the increase in muscle protein synthesis following resistance exercise is regulated by mTOR signaling.

B. Skeletal Muscle Adaptation to Aerobic Exercise

1. Whole Muscle

Aerobic exercise is defined as a high quantity of contractions at a small resistance for an extended period of time (57). Classically, aerobic training adaptations raise the ability to perform at the highest average power output or speed for a given period of time (62). Aerobic training adaptations include the ability to increase energy production from oxidative systems and decrease fatigue of working muscles (57). Ultimately, the increased performance is a result of increased

mitochondrial density (89). Mitochondria resynthesize ATP for sustained muscle contraction. Mitochondria contain enzymes that allow for the potential energy within carbohydrates, fats and proteins to be captured as ATP, largely through electron transport. Therefore, as mitochondrial density increases, so does the ability to resynthesize ATP through oxidative phosphorylation (61). Along with increased mitochondrial density, increased capillary density is also a notable adaptation to endurance exercise training. Increased capillary supply allows more oxygen to be delivered to mitochondria (64). However, it is to be noted that aerobic exercise can increase muscle fiber size. Harber et al. (50) investigated recreationally active runners and highly trained, varsity collegiate, distance runners before and after the competitive season. The recreational runners ran no more than 25 km/week. Muscle fiber analysis showed the highly trained runners had about 20% larger diameter of MHC type I (50). Similarly, older women were investigated that underwent 12 weeks of progressive cycle ergometer training at about 60-80% max heart rate. The results showed an average of 16% increase in MHC I fiber size following training (51).

2. Cellular Level

Aerobic exercise elicits a unique response within the skeletal muscle. Ultimately, there will be a shift in fiber type towards type I fibers (51, 77). As type I fibers are more oxidative, they allow the skeletal muscle to perform more work all while resynthesizing ATP in the mitochondria. As discussed above, endurance training also increases the mitochondria content within the muscle. (47). Early work by Hollozy demonstrated adaptations to aerobic exercise training, specifically a 2-fold increase in mitochondrial ATPase enzymes in exercising mice. Increased content of mitochondrial enzyme activities allow for greater rates of ATP resynthesis. This can be a result of increased slow twitch fiber size and greater concentration of muscle glycogen. (47, 100). Furthermore, increased mitochondrial content allows a higher amount of ATP generation as a result of increased beta oxidation of long chain fatty acids (60). It is to be speculated that these adaptations are a result of AMPK, PGC-1α upregulation, as discussed above.

At the onset of exercise, ATP is converted to ADP and an inorganic phosphate to develop tension within the muscle fibers. To keep up with ATP demand, mitochondria utilize oxidative phosphorylation as a form of ATP production. As mitochondrial content increases, so does the ability to consume oxygen molecules necessary to continue to resynthesize ATP (57) as oxygen serves as the final electron accepter in the electron transport chain. For example, if mitochondria content is doubled, the rate of ATP production through oxidative phosphorylation is also doubled, requiring more oxygen to accept electrons. Endurance exercise adaptations also include increased utilization of fat as a fuel as opposed to carbohydrates (59). This is a result of increased capillary density, leading to increased free fatty acid transport to the active muscle coupled with increased mitochondrial content.

C. Concurrent Exercise

Concurrent exercise is defined as performing both aerobic and resistance exercise during a specified training program (45). Although performing both resistance and aerobic exercise may seem beneficial, there is evidence suggesting molecular mechanisms are attenuating the potential beneficial adaptations. As discussed previously, resistance exercise training is known to elicit fiber hypertrophy. Contrarily, endurance training increases the oxidative capacity and mitochondrial density, leading to increased aerobic capacity (57). Hickson (58) examined how individuals adapted to a combination of resistance and endurance training when compared to individuals performing only strength or endurance training for 10 weeks. The resistance along with the resistance and endurance group both showed increases in strength gains during the first 7 weeks of training, but the concurrent group leveled off in the latter weeks. However, both endurance and concurrent groups developed similar increases in VO_2max. Hickson pioneered the idea of interference of adaptation during concurrent training. Further studies confirmed the results of Hickson, that concurrent training attenuates strength but not endurance adaptations (80). However, recent papers have called into question the attenuation of hypertrophic adaptations (87, 98), which may be the result of different designs around the concurrent training programs.

The molecular processes effected by concurrent training have not been fully investigated, however, there are studies looking into theories of this phenomenon. The mTOR signaling pathway is one of the most well-known regulators of transcription and translation. Research has suggested that AMPK activation, which is stimulate by increased cellular AMP, negatively regulates mTOR (11). AMPK activation also upregulates FOXO and MuRF-1 which promote muscle protein breakdown through the ubiquitin proteasome system (101). The upregulation of breakdown factors along with decreased mTOR signaling activation may be responsible for the attenuation of hypertrophic adaptations to concurrent exercise training. Other speculations as to why there is a decrease in hypertrophic adaptations include fatigue, timing and volume of training, satellite cell proliferation and nutrition (45, 104).

D. Divergent Exercise Review

Both aerobic and resistance exercise promote distinct, yet unique adaptations within the skeletal muscle. “Concurrent” exercise as discussed previously in this review, is defined as an individual performing both aerobic and resistance exercise as part of the same training program. Conversely, “divergent” exercise, as discussed in this Thesis, is comparing the effects of different forms of exercise, i.e., aerobic vs. resistance exercise. For instance, what are the unique molecular processes through which aerobic and resistance exercise elicit their specific muscle adaptations. As discussed above, the early signaling events that are elicited from each individual exercise bout lead to adaptations (22). These studies are summarized in Table 1.

Atherton (3) investigated the response of isolated rat skeletal muscle to high or low frequency stimuli, simulating resistance and endurance exercise, respectively. High frequency stimulus were isometric contractions, involving 10 sets of six repetitions at 50 V and 100 Hz, whereas, low frequency stimulus were isometric contractions at 50 V at 10 Hz with a 90 ms delay for three hours. Interestingly, AMPK-PGC-1a was stimulated by low frequency, whereas mTOR was stimulated specifically by high frequency stimuli. It is hypothesized that the high frequency stimulations were not long enough to decrease phosphocreatine (PCr) levels. Low frequency stimuli were able to decrease PCr levels, therefore increasing AMP levels, activating AMPK signaling

cascade. High frequency stimuli significantly increased downstream targets of mTOR, while low frequency stimuli did not elicit this response. This study demonstrates that the signaling response of muscle is dependent on type of muscle stimulation. In particular, high frequency stimulus, mimicking resistance exercise, activated markers of the mTOR signaling cascade, while low frequency stimulus, mimicking aerobic exercise, activated AMPK phosphorylation.

Raue (107) investigated the response of the TWEAK-Fn14 pathway to both endurance and resistance exercise in 12 healthy, nonobese and physically active volunteers. Participants completed either a resistance exercise (RE) or a submaximal running (RUN) protocol. The subjects in the RE group had been performing resistance exercise about two times per week, while RUN participants had been running three to five times per week. Subjects in RE performed three sets of 10 reps at 70% 1-RM. RUN subjects performed 30 min of treadmill running at 75% VO_2max. Muscle biopsies were taken pre exercise, immediately post, and 1, 2, 4, 8, 12, and 24 hours after exercise. Notably, the *vastus lateralis* was biopsied following RE whereas the gastrocnemius was biopsies following RUN. TWEAK-Fn14 pathway is an inflammation pathway sensitive to muscle atrophy, regeneration and mitochondrial function. Prolonged activation of TWEAK also upregulates NF-kB, a known marker of autophagy, and also increased NF-kB signaling. The RE protocol increased Fn14 stimulation substantially more than the RUN protocol. NF-kB signaling was also stimulated by RE protocol, signified by activation of downstream markers such as NIK (NF-kB inducible kinase) and p100-p52. In contrast, NF-kB signaling was not stimulated by RUN protocol. These data show the response of muscle to TWEAK-Fn14 and downstream markers are differentially stimulated by exercise type. Specifically, resistance exercise serves as a greater stimulus to TWEAK signaling pathways.

Coffey et al. (21) investigated chronically trained endurance and chronically training strength trained athletes in a crossover design in which subjects performed familiar and unfamiliar training modes. The subjects had been participating previously in aerobic or resistance training for at least 8 years. The acute resistance exercise consisted of 8 sets of 5 repetitions at maximal effort, while the acute aerobic exercise consisted of 1 hour of cycling at 70% peak O_2 uptake. Muscle biopsies were taken at rest, immediately post and 3 hours post exercise. Real time PCR was used

to quantify gene expression. After acute endurance exercise, both groups experienced a significant increase in PGC-1a mRNA, signifying a response including mitochondrial biogenesis. Myogenic genes such as MyoD, Myogenin, and Myostatin responsible for muscle cell proliferation were increased after endurance exercise in endurance trained subjects but not in strength trained subjects. There were also significant increases in markers of muscle breakdown, such as MAFbx, in both groups after endurance exercise. Similarly, only endurance trained subjects showed increased PDK4 mRNA abundance in response to endurance exercise. It is to be hypothesized that the increases of myogenic genes are an adaptive response to greater force production. Interestingly, it seems the endurance trained subjects had greater increases in myogenic mRNA abundance in response to acute exercise bouts. Coffey hypothesizes that prior training history can affect the acute gene response to exercise.

In a separate study, Coffey et al. (22) also examined signaling response in the same subject pool, with the same study design, as mentioned in the previous paragraph. Western blot analyses were used to quantify protein response, as this study investigated mTOR-Akt, a marker of increased muscle protein synthesis, and AMPK-PGC-1α signaling, a marker of endurance training adaptations. Interestingly, AMPK was elevated in both groups when performing unfamiliar exercise bouts (endurance subjects performing RE, or strength subjects performing AE). S6K1, a downstream marker of mTOR activation, was simulated in endurance trained subjects performing resistance exercise, but was unresponsive in both groups in response to endurance exercise. As a result, this study identified a term coined “response plasticity”, meaning prior training attenuates the signaling responses of acute exercise bouts. Similar to the study mentioned previously, a higher intensity of exercise may be required for to stimulate an adaptive signaling response in subjects familiar with the particular exercise bout.

Dickinson et al. (27) conducted a crossover designed study in which untrained subjects performed aerobic exercise (70% maximal heart rate for 40 minutes) or resistance exercise (8 sets of 10 repetitions at 60-65% 1-repetition max). Muscle biopsies were taken at baseline, 1 hour and 4 hours post exercise. This study utilized whole-transcriptome RNA sequencing to examine whole-

genome expression in response to the acute exercise bouts. There were 348 genes uniquely responsive to resistance exercise while 48 genes were uniquely responsive to aerobic exercise. Specific to the aerobic exercise bout, there was an increase in Estrogen Related Receptor Gamma (ESRRG), which is associated with increased oxidative capacity, increased vascularization and a shift to MHC I fibers. However, resistance exercise demonstrated a more robust genetic response. Notably, there was an increase in Fn14, which has been shown to be correlated with increase size and strength of the muscle, helping confirm the findings from Raue (107). More so, resistance exercise resulted in stimulation of NF-kB signaling and activation of heat shock proteins, which are responsible for response to cellular stress and returning the muscle to homeostasis. The results demonstrate that different forms of exercise, when performed acutely, stimulate unique transcriptional activity in skeletal muscle.

Yang et al. (137) investigated the metabolic (CD36, CPT1,HKII and PDK4) and myogenic (MRF4, Myf5, MyoD and myogenin) gene expression in response to acute bouts of resistance (3 sets of 10 repetitions at 70% 1-repetition max) and aerobic exercise (30 minutes of treadmill running at 75% VO_2 max). Muscle biopsies were taken at rest, 12 and 24 hours post exercise. Real time PCR assays were used to determine gene expression. Each subject was untrained and randomly assigned to either the resistance or aerobic protocols. Acute resistance exercise increased MRF4, myogenin, MyoD and HKII expression. Similarly, acute aerobic exercise increased MyoD, HKII and PDK4. However, there was no change in gene expressions 24 hours post exercise. This study further confirms the unique genetic response to acute aerobic and resistance exercise.

Finally, Louis et al. (86) examined the time course of proteolytic genes in response in trained subjects to either resistance exercise (3 sets of 10 repetitions at 70% 1-repetition max) or running protocol (30 min of treadmill running at 75% VO_2 max). Muscle biopsies were taken from *the vastus lateralis* before, immediately after, 1, 2, 4, 8, 12, and 24 h post exercise. Specifically, this study aimed to investigate genes involved with the ubiquitin proteasome pathway, mainly muscle ring finger-1 (MuRF-1), atrogin-1, which are both regulated by forkhead box 3A (Foxo3a). After both exercise protocols Atrogin-1, MuRF-1 and Foxo3a were all significantly upregulated.

However, beyond the 8 h time point, only run protocol showed elevated levels of the aforementioned genes. Although this study investigates mRNA expression and not functional protein concentration, it provides insight to the differing adaptive responses of skeletal muscle to aerobic and resistance exercise. Furthermore, this study demonstrates that aerobic exercise, at least in the form of running, is perhaps a greater stimulus to proteolytic processes in skeletal muscle compared to resistance exercise.

E. Divergent Exercise Summary

As shown in the previous paragraphs, exercise elicits a unique response in skeletal muscle. In the immediate hours postexercise, both acute aerobic and resistance are well-known to elicit responses in gene expression and stimulation of cellular signaling pathways in the skeletal muscle. However, aerobic and resistance exercise have unique effects on the immediate molecular response of the muscle. For example, aerobic exercise acts as a more powerful stimulator genes regulating muscle protein breakdown than resistance exercise (107). Similarly, an increase in myogenic gene expression was demonstrated in response to resistance exercise, ultimately leading to muscle protein synthesis (21). Additionally, Coffey et al. (22) demonstrates a blunted mTOR signaling response when the subject is previously trained. More so, the aforementioned studies investigate the response of skeletal muscle in a variety of populations such as untrained (27), trained (21, 22, 86, 107) and rodent models (3). In particular, the study that investigated untrained subjects (27) specifically looked at the genetic response. However, there is no literature on the cellular signaling response of untrained subjects performing aerobic and resistance exercise, as cellular signaling is largely responsible for muscle adaptation. Further identification of the signaling response in untrained subjects will show how muscle adaptation is regulated.

IV. SUMMARY AND CONCLUSIONS

This review of literature outlines the processes and various stimuli that lead to muscle adaptation. The aforementioned studies demonstrate that skeletal muscle is adaptive in nature. Both acute aerobic and acute resistance exercise elicit adaptive responses, however, the responses are both very unique. In immediate hour post exercise, aerobic exercise prompts an increase in expression of genes promoting mitochondrial biogenesis, while resistance exercise prompts expression of genes promoting contractile protein accumulation. Prior training also can attenuate the response of signaling pathways to a familiar exercise stimulus. However, the majority of these studies do not involve subjects performing both aerobic and resistance exercise. Moreover, none of these studies (3, 21, 22, 27, 86, 107, 137) investigate the response of intracellular signaling in response to divergent bouts of exercise. More so, these studies compare subjects performing both aerobic and resistance exercise, but on different occasions. In addition, many of these studies included either athletes who are previously trained or animal models. With inclusion criterion of untrained subjects, this allows exploration of a baseline unique response to unfamiliar exercise. Being able to identify specific markers of muscle synthesis and breakdown, will then allow more Intel as to using exercise as a therapeutic measure. Specifically, what exercise stimulus can be used to elicit the adaptions desired in the target population.

Table 2-1. Summary of divergent exercise studies including study protocol and outcomes.

Author, Year, Reference	N	Inclusion Criteria	Exercise Mode	Exercise Duration	Time of Biopsy	Outcome
Atherton et al. Feb, 2005 (3)	-	Mice	Electrical Stimulation	Aerobic – Low Frequency Stimulus 50V, 10Hz. 10ms contractions for 3h Resistance – High frequency stimulus 50V 100Hz. 3s contraction w/ 10s recovery	Soleus/EDL - Muscle stimulation began 30 min after sacrifice	HFS stimulates increased AMPK-PGC-1α signaling LFS stimulates increased PKB-TSC2-mTOR signaling
Raue et al. March 2015 (107)	12	Healthy, nonsmoking, physically active	Aerobic – Treadmill Resistance – Knee extension	Aerobic – 30 min run at 75% VO_2 max Resistance – 3 sets of 10 repetitions at 70% 1-RM, 2 min rest between sets	Gastrocnemius - Rest, immediately post, 1, 2, 4, 8, 12 and 24 h post	RE stimulates Fn14 and Nf-KB signaling Although all exercise stimulates TWEAK-Fn14, RE is greater stimulus than AE
Coffey et al. May 2006 (22)	13	All male and highly trained, 6 cyclists and 7 power lifters	Aerobic – Cycle ergometer Resistance – leg extension	Aerobic – 60 min at 70% VO_2 max Resistance – 8 sets of 5 maximal repetitions with 3 min rest between sets	Vastus Lateralis - Rest, immediately after and 3 h post exercise	AMPK-PGC-1α signaling increased in resistance trained group Akt-TCS2-mTOR signaling increased in aerobic trained group Response plasticity based on previous training
Coffey et al. May 2006 (21)	13	All male and highly trained, 6 cyclists and 7 power lifters	Aerobic – Cycle ergometer Resistance – leg extension	Aerobic – 60 min at 70% VO_2 max Resistance – 8 sets of 5 maximal repetitions with 3 min rest between sets	Vastus Lateralis - Rest, immediately after and 3 h post exercise	Prior training history altered gene expression

Table 2-1. Summary of divergent exercise studies including study protocol and outcomes.

Dickinson et al. June 2018 (27)	6	Untrained	Aerobic - Cycle ergometer, leg extension Resistance - Subjects performed both bouts	Resistance - 8 sets of 10 reps at 65% 1-RM Aerobic – 40 minutes at 70% max HR	Vastus Lateralis - Rest, 1 h and 4 h post exercise	Aerobic -Increased Estrogen-related receptor-gamma – correlated with improved mitochondrial function, greater oxidative capacity Resistance – increased TNFRS12A and TWEAK-Fn4 –Nf-kB, which is highly correlated with muscle fiber growth
Yang et al. October 2004 (137)	12	Nonsmoking, nonobese and physically active	Aerobic – treadmill Resistance – leg extension	Aerobic – 30 min running at 75% VO_2 max Resistance – 3 sets of 10 reps at 70% 1RM	Vastus Lateralis - Rest, immediately after, 1, 2, 4, 8, 12 and 24 h	Aerobic exercise increased mRNA of MyoD, HKII, PDK4. Resistance exercise increased mRNA of MRF4, MyoD, myogenin HKII and PDK4. Both exercises increased metabolic and myogenic gene expression
Louis et al. August 2007 (86)	12	Previous aerobic or resistance training. Had been performing the exercise 2-5 times per week	Aerobic – treadmill Resistance – leg extension	Aerobic – 30 min of treadmill running at 75% VO_2 max Resistance – 3 sets of 10 reps at 70% 1RM	Vastus Lateralis – Rest, immediately after, 1, 2, 4, 8, 12 and 24 h	Both RE and RUN protocol elicit increases in Atrogin-1, MuRF-1 and Foxo3a mRNA expression 4 h post exercise. After 4 h only RUN shows increased expression

Table 2-1. Summary of divergent exercise studies including study protocol and outcomes.

Wilkinson et al. July 2008 (132)	10	Had not been performing aerobic or resistance exercise 8 months prior to the study	Aerobic – cycle ergometer Resistance – leg extension	Aerobic – 30 min of single leg cycling at 75% VO_2 max Resistance – 3 sets of 10-12 reps at 80% 1RM 10 weeks of training	Vastus Lateralis – Pre exercise, rest, immediately after, and 4 h	Prior to training, RE stimulated both myofibrillar and mitochondrial protein synthesis, while AE only stimulated mitochondrial protein synthesis. After training, only myofibrillar protein synthesis was stimulated by RE, and mitochondrial by AE

CHAPTER THREE

MATERIALS AND METHODS

Participants

Six healthy men volunteered to participate in the original study. All participants were recruited from the greater Phoenix area. All of these participants were untrained and not participating in regular physical activity (<2 days/week). All subjects completed a medical activity and physical activity questionnaire along with a blood draw for blood coagulation profile. All procedures were approved by the Institutional Review Board of Midwestern University (in compliance with the Declaration of Helsinki, as revised in 1983).

Preceding the experimental trials, subjects completed baseline testing of unilateral leg extension 1-repetition maximum (1RM) along with maximal heart rate and peak workrate (W) on a cycle ergometer. 1RM was taken on two separate visits using a knee extension device (Cybex VR3, Medway MA), first during the initial screening and the second test completed ~1 week before the commencement of the experimental trials. The heaviest weight lifted between the two visits was considered the subjects 1RM. Maximal exercise heart rate and peak workload was determined on a stationary cycle (model 828E; Monark Exercise, Vansboro, Sweden) using a ramp protocol. Subjects completed a warm-up phase at 50 W, followed by a progressively increasing workload by 15 W every minute until volitional fatigue. During the test, subjects HR was measured by telemetry, and maximal heart rate and peak W were recorded. VO_{2Peak} was also calculated using peak cycling workload and standard metabolic equations for leg cycling: VO_2 ($ml \bullet kg^{-1} \bullet min^{-1}$) = 1.8 X (work rate in $kg \bullet m^{-1} \bullet min^{-1}$)/(body mass in kg) + 3.5 $ml \bullet kg^{-1} \bullet min^{-1}$ + 3.5 $ml \bullet kg^{-1} \bullet min^{-1}$.

Study Design

Each participant completed two separate experimental trials (Fig. 3-1). These trials were performed utilizing a randomized, counterbalanced crossover design with a washout period of ~1 week between each trial. The exercise trials were identical except for the mode of exercise

performed (aerobic or resistance). Subjects were instructed to abstain from alcohol consumption for 72 h and caffeine for 24 h before each experimental trial.

Experimental Trials

On the morning of each experimental trial (Fig. 3-1), subjects arrived to the laboratory (~0700) following a 12 hour overnight fast, and remained fasted until completion of the trial. Participants were instructed to record their last meal the night before the first exercise trial and to replicate for the second experimental trial. Prior to the start of exercise, a basal muscle biopsy was taken following 30 minutes of supine rest. The basal biopsy was used for both exercise trials to minimize invasive procedures, therefore no basal biopsy prior to the second exercise trial. After the basal biopsy (1st trial) or 45 min supine rest (2nd trial, to imitate the basal biopsy), subjects performed a 5 minute warmup at a low workload on a cycle ergometer (model 828E; Monark Exercise) and then performed either acute aerobic (AE) or resistance exercise (RE). After completion of exercise, subjects rested supine in a clinical bed, engaging in minimal physical activity. Muscle biopsies were taken at 1 and 4 hours, relative to the end of the exercise trial. After the first exercise trial, a 1-week washout phase was observed, before the subjects perform the second trial. Six participants completed the experimental trials, three performed AE during their first trial and three performed RE during their first trial. The dominant leg for the muscle biopsies at basal and post exercise was also counterbalanced. The counterbalance design limits the influence of factors unrelated to exercise particularly to find unique responses in muscle signaling.

Acute exercise bouts

Exercise sessions consisted of either acute bouts of aerobic or resistance exercise. Aerobic exercise consisted of 40 minutes of cycling on a stationary bike (model 828E; Monark Exercise, Vansboro, Sweden) at a prescribed workload to elicit ~70% maximal heart rate. Heart rate was continuously monitored (Polar Electro, Lake Success, NY) throughout the exercise bout. Along with heart rate, W and rating of perceived exertion were measured every two minutes during the exercise bout. Resistance exercise consisted of isotonic unilateral leg extensions (Cybex VR3),

where each subjects performed eight sets of ten repetitions at 60-65% 1RM. Both legs were exercised to simulate the aerobic exercise bout, but unilateral exercise is chosen to ensure the leg chosen for the biopsy received equal work (3 of the participants had dominant leg biopsied and 3 had non dominant leg biopsied post RE). Participants alternated legs for each set first, and were given 3 minutes of rest between each set. Total exercise time for the RE was ~40 min.

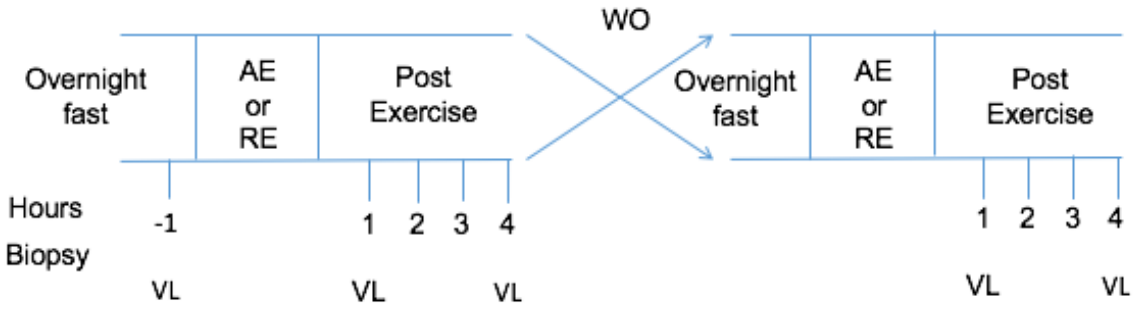

Figure 3-1. Experimental trial schematic. Subjects arrived to the lab after an overnight fast and a resting muscle biopsy from the *vastus lateralis* is taken. The subject then performed either acute aerobic or resistance exercise. After the cessation of exercise, muscle biopsies were taken at 1 h and 4 h respective to the end of exercise. After about a week long washout period subjects completed the second experimental trial, which consisted of the other exercise mode. Muscle biopsies are taken 1 and 4 h post exercise during the second experimental trial.

Muscle Biopsies

Muscle biopsy procedures were conducted under strict sterile conditions. All muscle biopsies were obtained from the lateral portion of the *vastus lateralis* following local anesthesia (1% lidocaine without epinephrine) using a 5-mm Bergström needle with suction. The nondominant leg was selected for basal muscle biopsies during the first experimental trial, and was used as a basal value for both exercise trials. During both experimental trials, the 4 hour biopsy was obtained from the same incision as the 1 hour biopsy; however, the biopsy needle was inclined at a different angle such that the 4 hour postexercise biopsy will be taken ~5cm proximal to the 1 hour postexercise biopsy, as previously performed (27). All muscle samples were obtained from a single pass of a needle for each time point. The 1 and 4 hour biopsies during the first experimental trial were obtained from the opposite leg as the basal biopsy, however, the 1 and 4 hour biopsies during the second trial were obtained from the same leg used for the basal biopsy during the first experimental

trial. Muscle tissue was immediately blotted, dissected of connective tissue and adipose tissue and frozen in liquid nitrogen. Muscle tissue was stored at -80°C until analysis.

Analytical Techniques

Cytosolic and Nuclear extraction. Frozen muscle tissue was weighted, placed in buffer, homogenized (1:9 wt/vol), and centrifuged at 3,400 g for 10 min at 4°C, followed by removal of supernatant, which was used for Western blotting of cytosolic proteins (p/t mTOR, p/t p70S6K1, p/t 4E-BP1, p/t e-EF2, p/t rps6, p/t Akt, p62, FOXO3a, LC3B, GAPDH). The resulting pellet was suspended in isolation buffer (1 M sucrose, 1 M Tris-MCl, 1 M KCl, 0.5 M EDTA, pH 7.4) containing protease and phosphatase inhibitors and centrifuged for 10 min at 4°C and 700 g. After three series of PBS buffer suspensions and centrifugations at 15,000 g for 5 min at 4°C, the pellet was resuspended and agitated on ice for 2 X 20 min and in a 4°C sonication bath in high-salt buffer (1:4 weight/volume). The slurry was then centrifuged at 15,000 g for 10 min at 4°C, the supernatant was taken as the nuclear fraction and used for Western Blotting of nuclear fractions (FOXO3a etc.). Please note, nuclear protein contents are not presented as part of this Thesis.

Western Blot Analysis. Total protein concentrations were determined in cytosolic and nuclear fractions using the Bradford assay (Smartspec Plus, Bio-Rad, Hercules, CA). Samples were diluted (1:1) in a 2X sample buffer mixture containing 1255 mM Tris, pH 6.8, 25% glycerol, 2.5% SDS, 2.5% ß-mercaptoethanol, and 0.002% bromophenol blue and then boiled at 3 min at 100°C. Equal amounts of total protein (cytosolic, 40 µg; nuclear, 20 µg) were loaded into each lane and the samples were separated by electrophoresis (150 V for 60 or 55 min) on a poly acrylamide gel (Criterion, Bio-Rad) based on a size of the target protein (7.5% or 12%). Each sample was loaded in duplicate and each gel contained an internal loading control and molecular weight ladder (Precision Plus, Bio-Rad). Following electrophoresis, protein was transferred to a midi-size™ polyvinylidene difluoride membrane (Bio-Rad) using the Transblot® Turbo™ Transfer System according to manufacturer's instructions. Blots were then blocked for 1 hour in 5% nonfat dry milk and incubated with primary antibody dissolved in nonfat dry milk or bovine serum albumin overnight at 4°C. Cytosolic proteins of interest included in this Thesis are: total mTOR (1:1000), mTOR^{ser2448}

(1:500), total p70S6K1 (1:500), p70S6K1^{thr389} (1:500), total EEF2 (1:2000). EEF2^{thr56} (1:5000), total 4E-BP1 (1:1000), 4E-BP1$^{thr37/46}$ (1:1000), total Akt (1:1000), Akt^{thr308} (1:500), total rpS6 (1:1000), rpS6$^{ser240/244}$ (1:250), LC3B (1:1000), p62 (1:1000), FOXO3a (1:500), GAPDH (1:1000) served as a loading control). After the overnight incubation, blots were incubated with anti-rabbit HRP linked antibody for 1 hour at room temperature. Blots were then washed in TBST (TBS + Tween20) for 15 min followed by three 5 min washes. Blots were then incubated in a chemiluminescent solution (ECL plus, Amersham Biosciences, Piscataway NJ) for 5 min and optical density measurements were made with a phosphoimager (ChemiDoc, Bio-Rad) and densitometric analysis was performed by using Quantity One 4.5.2 software (Bio-Rad). Phosphorylation and total density values were normalized to the internal control, and the phospho/total protein ratios were determined. Western blot data are expressed as phosphorylation divided by total protein or total protein and adjusted to represent fold change in relation to basal. The ratio of LC3B II to LC3B I was determined by optical density measurements. All antibodies will be purchased from Cell Signaling Technologies (Beverly, MA).

Statistical Analysis

All data were tested for normality through skewness and kurtosis and visual inspection of the normality plots using SPSS. Data with a non-normal distribution were transformed prior to statistical analyses. A one-way repeated measures analysis of variance (ANOVA) was used to test for differences in GAPDH across time. A two way repeated measures ANOVA was used to test group by time differences. Pairwise comparisons using the Tukey posthoc test were used to determine differences within ANOVA. Data analyses were performed using SigmaStat version 12.5 (Systat Software). Significance will set at $P \leq 0.05$. Data are presented as mean ± SE unless otherwise noted.

Table 3-1. Targets investigated along with the role of each target.

Target	Signaling Mechanism	Role
mTOR (Ser2448)	Hypertrophy	Regulates cell growth, proliferation and protein synthesis
S6K1 (Thr308)	Hypertrophy	Regulates cell growth and cycle progression
eEF2(Thr56)	Hypertrophy	Regulates Elongation
4E-BP1(Thr37/46)	Hypertrophy	Repress Translation
p62	Autophagy	Recruitment of lysosome
FOXO	Autophagy	Regulates autophagy and ubiquitin proteasome system
LC3B	Autophagy	Recruitment of Autophagosome membrane
GAPDH	Control	Control

CHAPTER FOUR

RESULTS

Physiological Exercise Data

Maximal heart rate and peak workrate (means ± SD) achieved during the stationary cycling ramp protocol were 184 ± 14 beats/min (bpm) and 225 ± 41 W, respectively. The participants' unilateral knee extension 1RM was 65 ± 17 kg. Descriptive exercise data during the AE and RE experimental trials are presented in Table 4-1. Mean power output during the AE bout (minutes 6–40) was 51% peak power (115 W). The mean %1RM for all sets during the RE bout was 61%.

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No changes in the density of GAPDH were observed across time ($P > 0.05$). Phosphorylation of mTOR (Ser^{2448}) was increased above baseline at 4 h after resistance exercise ($P = 0.004$), whereas there was a trend for increased phosphorylation of mTOR above baseline at 4 h after aerobic exercise ($P = 0.066$), as shown in Figure 4-2A. Further, phosphorylation of mTOR was higher after resistance vs. aerobic exercise at 4 h post exercise ($P = 0.026$). Phosphorylation of S6K1 (Thr^{389}) was increased above baseline in both aerobic ($P < 0.001$) and resistance ($P < 0.001$) at 4 h after exercise. However, phosphorylation of S6K1 was increased above baseline 1h post exercise only after resistance exercise ($P < 0.001$). Phosphorylation of S6K1 was also higher in resistance when compared to aerobic at 1 h post exercise ($P = 0.004$) as shown in Figure 4-2B. Phosphorylation of 4E-BP1($Thr^{37/46}$) was unchanged at all-time points when compared to baseline in both trials, however there is a trend for higher phosphorylation following resistance vs. aerobic exercise at 1h ($P = 0.062$) and at 4h ($P = 0.073$) as shown in Fig 4-2C. eEF2 (Thr^{56}) phosphorylation was also unchanged compared to baseline at all-time points in both trials. However, there was a trend for increased phosphorylation of eEF2 at 4 h ($P = 0.06$) following the aerobic exercise vs. resistance exercise, as shown in Figure 4-2D. There were no significant changes in LC3BI protein content across all time points compared to baseline in both trials ($P = 0.434$). Moreover, there were also no differences between groups at 1 and 4 h post exercise, as shown in Figure 4-3A. However,

LC3BII protein content decreased significantly ($P < 0.05$) below baseline at 1 h and 4h following both aerobic and resistance exercise, as shown in Figure 4-3B. There were no changes in LC3BII/I ratio from baseline or between groups, as shown in Figure 4-3C.

Table 4-1. Physiological data from the acute aerobic and resistance exercise bouts.

Variable	Average During Exercise	Peak Value
	Aerobic Exercise	
Heart rate, beats/min	147 ± 12	163 ± 15
%HR_{max}	79 ± 4	89 ± 6
Work (W)	115 ± 128	130 ± 26
%Peak Work	51 ± 5	58 ± 4
RPE	15 ± 1	
	Resistance Exercise	
Absolute weight lifted, kg	38 ± 12	44 ± 11
%1RM	61 ± 6	68 ± 7

Data are means ± SD. Presented data are from minutes 6 to 40 of exercise to account of stead state exercise. Table is derived from Dickinson et al. 2018 (27).

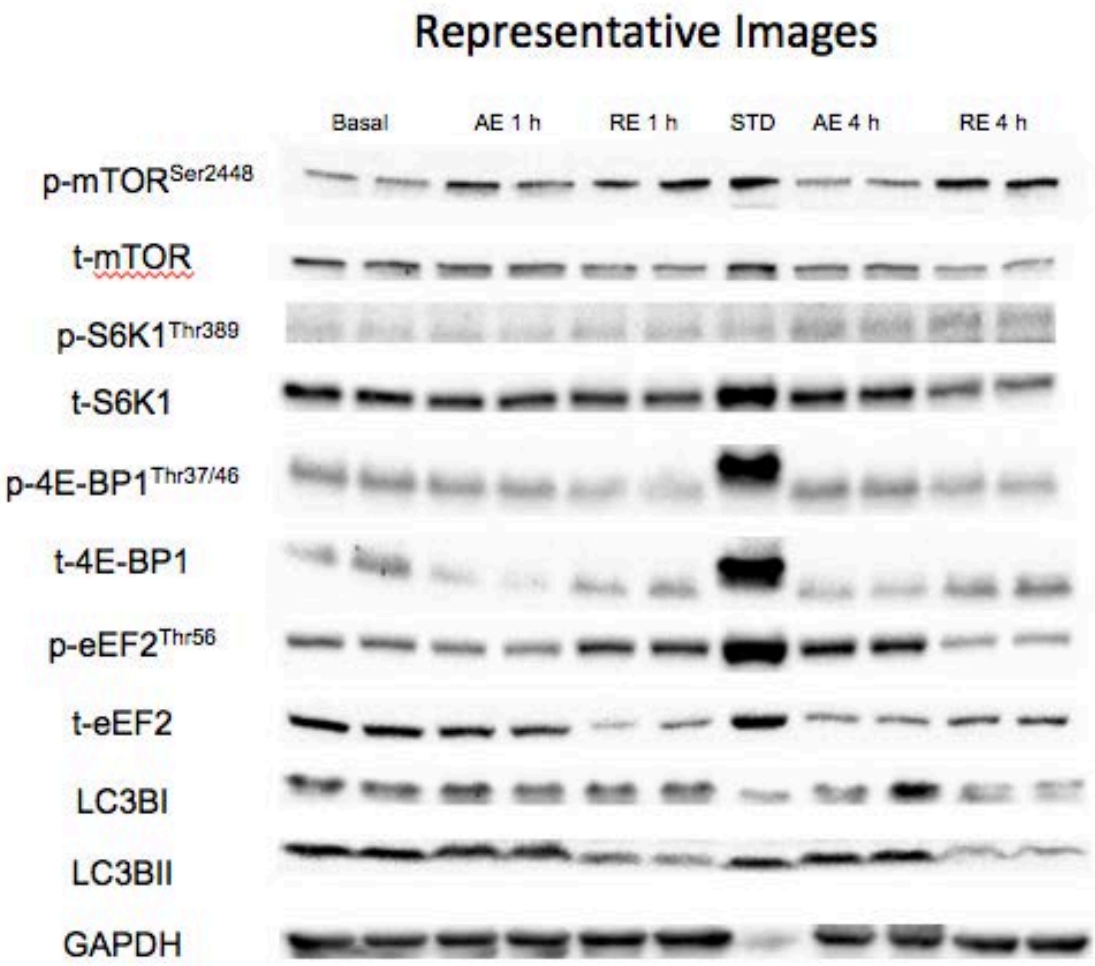

Figure 4-1. Representative western blot images for basal, 1h and 4 h following either aerobic or resistance exercise. All samples were loaded in duplicate.

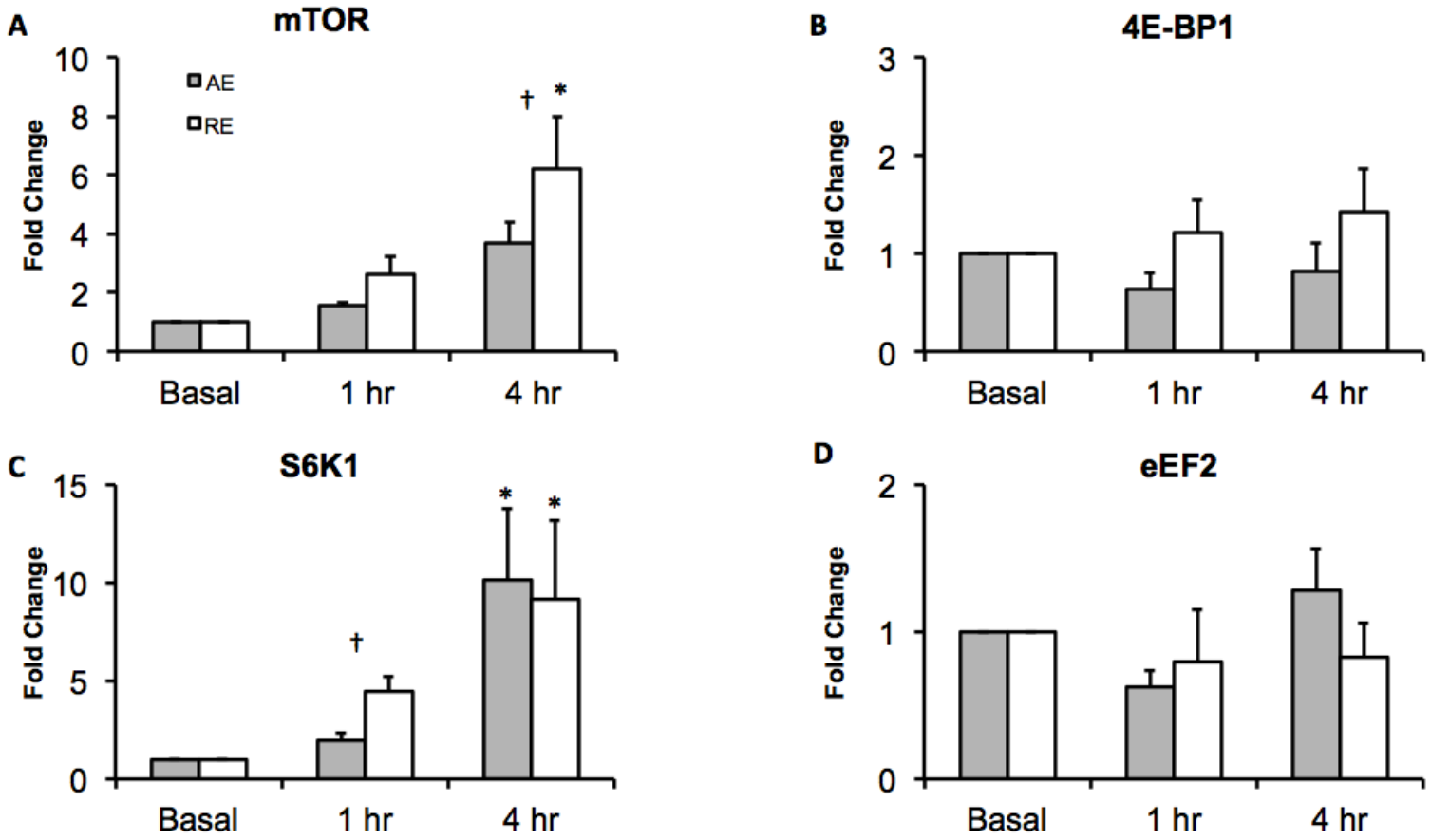

Figure 4-2. Phosphorylation of mTOR (A), S6K1 (B), 4E-BP1 (C), eEF2 (D). Subjects completed either 40 min of cycling at 70% HR max (AE, aerobic exercise) or 8 sets of 10 repetitions at 65% 1-RM (RE, resistance exercise). Data are mean ± SE and are presented as fold change relative to basal levels. Significant difference ($P < 0.05$) vs. basal *, difference between trials † ($P < 0.05$).

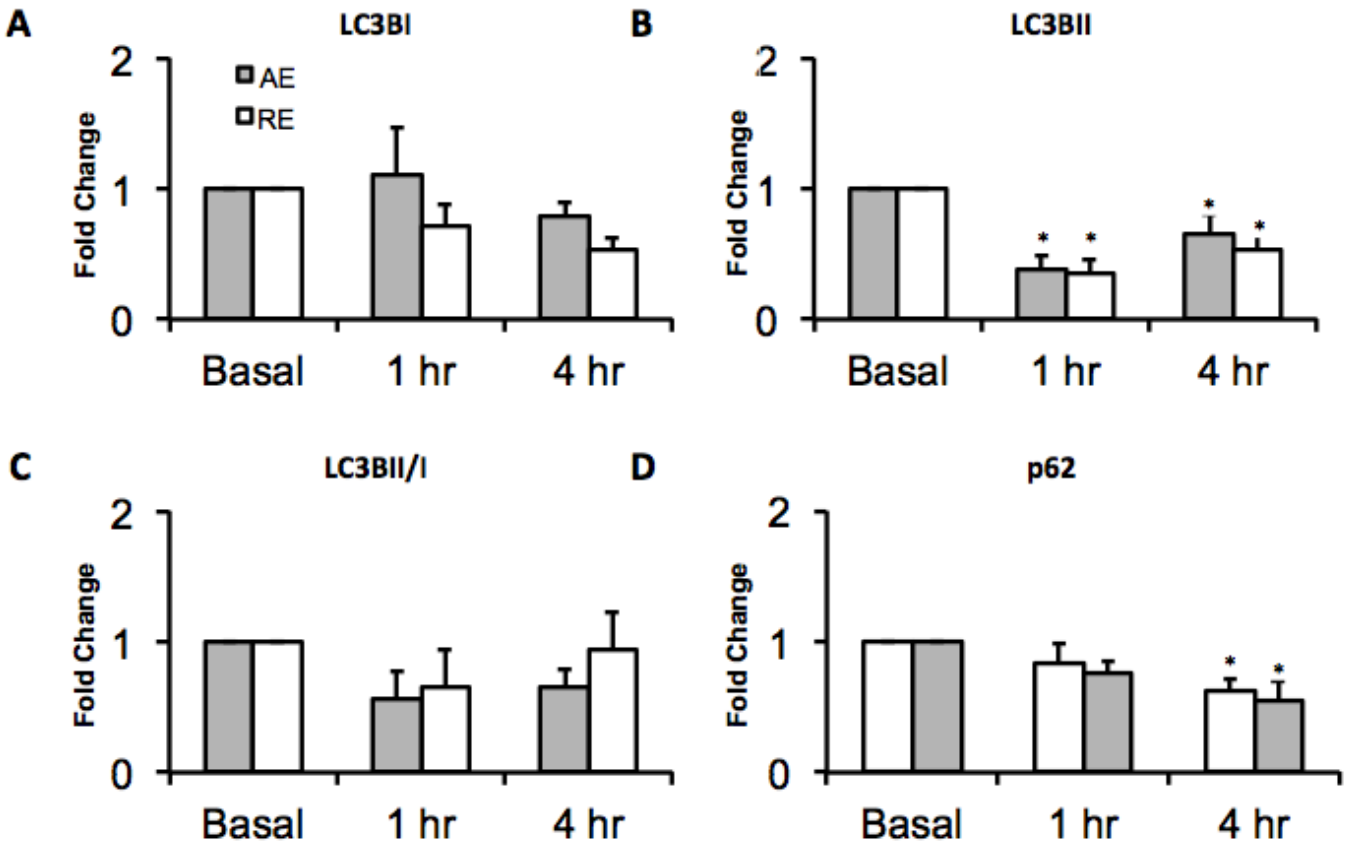

Figure 4-2. Protein content of LC3BI (A), LC3BII (B), LC3B II/I (C), p62 (D), Subjects completed either 40 min of cycling at 70% HR max (AE, aerobic exercise) or 8 sets of 10 repetitions at 65% 1-RM (RE, resistance exercise). Data are mean ± SE and are presented as fold change relative to basal levels. Significant difference ($P < 0.05$) vs. basal *, difference between trials † ($P < 0.05$).

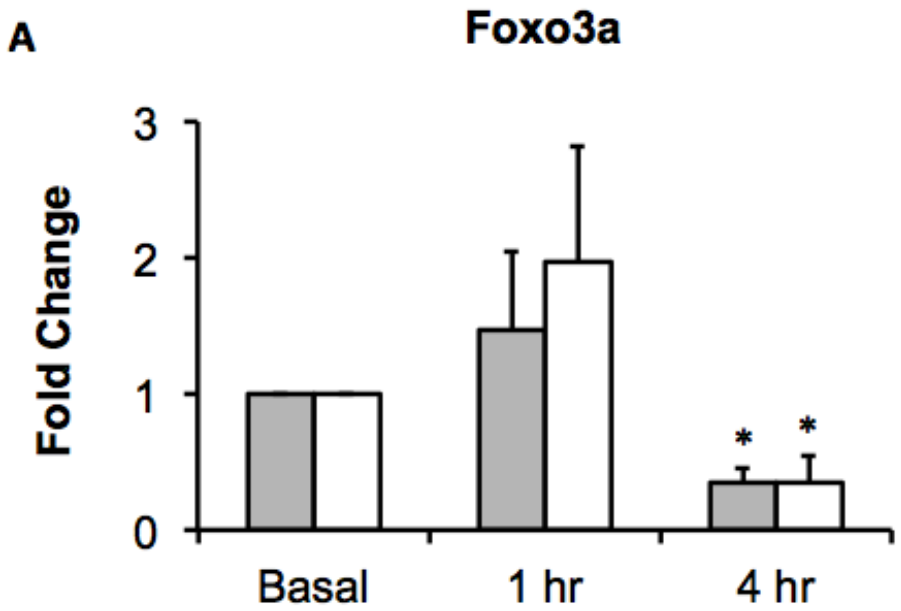

Figure 4-4. Protein content of Foxo3a (A). Subjects completed either 40 min of cycling at 70% HR max (AE, aerobic exercise) or 8 sets of 10 repetitions at 65% 1-RM (RE, resistance exercise). Data are mean ± SE and are presented as fold change relative to basal levels. Significant difference ($P < 0.05$) vs. basal *, difference between trials † ($P < 0.05$).

Table 4-2. Raw values of protein concentration.

Target	1 h AE	1 h RE	4 h AE	4 h RE
mTOR^{Ser2448}	1.5604 ± 0.1095	2.6462 ± 0.5933	3.6966 ± 0.6782	6.2062 ± 1.7842*
S6K1^{Thr389}	1.9303 ± 0.4151	4.4526 ± 0.7767	10.1831 ± 3.5824*	9.2022 ± 4.0062*
4E-BP1$^{Thr37/46}$	0.6408 ± 0.1572	1.2151 ± 0.3374	0.8128 ± 0.2924	1.4292 ± 0.4357
eEF2^{Thr56}	0.6277 ± 0.1095	0.8026 ± 0.597	1.2790 ± 0.2853	0.8266 ± 0.2335
LC3BI	1.1072 ± 0.3611	0.7114 ± 0.1637	0.7863 ± 0.1084	0.5387 ± 0.0796
LC3BII	0.3771± 0.1147*	0.3546 ± 0.0976*	0.6489 ± 0.1517*	0.5302 ± 0.1220*
LC3BII/I	0.5607 ± 0.2181	0.6551 ± 0.2814	0.6482 ± 0.1451	0.9398 ± 0.2786
p62	0.8336 ± 0.1557	0.7515 ± 0.0987	0.6185 ± 0.1002	0.5515 ± 0.1472
GAPDH	0.9496 ± 0.32	0.9717 ± 0.138	0.7019 ± 0.0540	0.8915 ± 0.1309

Data represents mean ± standard error. Values represent fold change of phosphorylated protein content / total protein content. * Significant difference between time point and baseline ($P < 0.05$).

CHAPTER FIVE

DISCUSSION

The goal of this study was to examine the early molecular response of skeletal muscle to divergent acute exercise. Specifically, we investigated the response of the mTOR pathway along with markers associated with autophagy in skeletal muscle in the immediate hours following acute aerobic and resistance exercise. We chose these cellular processes largely because it is well known that aerobic and resistance exercise each elicit unique skeletal muscle adaptations. Specifically, resistance exercise promotes muscle growth (1, 39, 116), a processes shown to be regulated through the mTOR pathway (129, 139) , whereas aerobic exercise promotes proteolytic activity (86) and mitochondrial adaptations (89), which are processes in which autophagy has a role (73). In this study, we found that mTOR was phosphorylated four hours postexercise only after resistance exercise, while S6K1 was phosphorylated four hours postexercise by both exercise bouts. Moreover, both exercise bouts also resulted in reduced LC3BII, p62 and cytosolic FOXO protein in the immediate hours post exercise. Collectively, while subtle differences were observed, these findings suggest that the mTOR signaling pathway and autophagy appear to be stimulated in the immediate hours following both resistance and aerobic exercise in untrained subjects.

The mTOR signaling pathways is a well-known regulator of muscle growth (31, 79). Stimulation of mTOR leads to the activation of downstream markers such as S6K1, 4E-BP1 and eEF2, and ultimately, enhanced transcription and translation (81, 88). Specifically, we examined $mTOR^{Ser2448}$ and $S6K1^{Thr389}$, both positive regulators of translation, $4E\text{-}BP1^{Thr37/46}$, which is a negative regulator of translation, and $eEF2^{Thr56}$ which is a positive regulators of elongation. The current study found that while resistance exercise resulted in a greater phosphorylation of mTOR at 4h postexercise and S6K1 at 1h postexercise, both exercises stimulated S6K1 phosphorylation at 4 hr postexercise. Despite these subtle differences, it appears that both acute aerobic and resistance exercise stimulate some level of mTOR signaling in untrained individuals. Specifically, activation of S6K1 is an important step to promote the formation of the translation preinitiation

complex and to stimulate an increase in translation (i.e., muscle protein synthesis) (79). Thus, given protein synthesis has been shown to be stimulated by both aerobic and resistance exercise (15, 90), particularly in the untrained state, is not surprising that S6K1 was phosphorylated by each exercise mode. Similarly, Wilkinson et al. (132) found a similar increase in mTOR signaling response to both aerobic and resistance exercise, showing that exercise elicits a response in protein synthesis. Specifically, aerobic exercise stimulates mitochondrial protein synthesis, whereas resistance exercise increases contractile protein synthesis. Importantly, S6K1 can be phosphorylated by other growth-promoting pathways, such as the MAPK pathway (120). However, to what extent these subtle differences in mTOR phosphorylation are related to the unique adaptation facilitated by each exercise mode, and to what additional pathways may be regulating S6K1 activation following each exercise stimulus, require further investigation.

Previous work in isolated rat muscle has demonstrated that electrical stimulation to mimic resistance exercise (high frequency) resulted in activation of the mTOR signaling cascade, whereas electrical stimulation to mimic aerobic exercise (low frequency) did not result in mTOR signaling activation (3). Interestingly, this “exercise mode” specific response observed in this previous study occurred in both the isolated extensor digitorum longus and soleus muscles, which differ considerably in fiber type profile and fiber size (117). While in the current study we did observe that mTOR was phosphorylated only following resistance exercise (4 h post), both aerobic and resistance exercise stimulated an increase in S6K1 phosphorylation at 4 h post exercise. Thus, our results appear to contrast those of Atherton et al., which may be explained by the *in vitro* design used by Atherton et al (3). On the other hand, Coffey et al. (22) has shown that strength trained subjects have an attenuated mTOR signaling response following acute resistance exercise, whereas aerobically trained subjects showed a significant increase in mTOR, S6K1 and 4E-BP1 phosphorylation when following acute resistance exercise. Coffey coined “response plasticity”, meaning subjects accustomed to their respective exercise bouts did not elicit a signaling response. Consequently, the increased phosphorylation of S6K1 observed in the current study following both RE and AE is likely the result of the untrained status of our subjects and the unaccustomed nature

of each form of exercise. Studying the response of mTOR signaling in untrained individuals may provide insight as to how muscle adaptation to exercise is regulated.

Autophagy is a regulatory mechanism that helps recycle dysfunctional or damaged proteins, to prevent muscle dysfunction (37). Autophagy is a crucial process to ensure healthy and proper muscle function (135), and relevant to this study, autophagy appears to have a role in regulating proteolytic activity and the turnover of mitochondria (68, 69, 85). Thus, in addition to the examination of mTOR signaling, we also examined the response of various markers associated with autophagy given prior research has demonstrated that aerobic exercise may stimulate proteolytic processes more than resistance exercise (29, 86). In the current study, we observed that changes in autophagy were similar following both aerobic exercise and resistance exercise protocols. Specifically, both acute aerobic and resistance resulted in reduced LC3BII, p62 and cytosolic FOXO postexercise. While assessment of autophagy remains difficult due to the array of molecules involved, we specifically assessed changes in LCBI, LC3BII, the ratio of LC3BII/I, and p62, as they have previously been shown to change with acute exercise (75). Moreover, our finding of reduced LC3BII protein content following acute exercise is consistent with previous acute exercise studies (29, 43), both of which interpreted the reduced LC3BII to signify an increase in autophagy. Specifically, although LC3BII concentrations can increase during autophagosome formation, a decrease in LC3BII may also signify increased autophagy activity as LC3BII can be degraded in the autophagosomes (75). As further support that the reduced LC3BII was associated with an increase in autophagy, we also observed a decrease in p62 concentration, a marker of autophagosome degradation (9, 25), which was similar after both exercise bout. Collectively, autophagy appears to similarly increase in the immediate hours following both aerobic and resistance exercise, at least in untrained individuals.

While it is well known that proteolytic markers are upregulated in response to acute exercise, the time course for this response may differ between exercise modes. For instance, Louis et al. (86) has demonstrated that mRNA expression for proteolytic markers such as atrogin-1, MuRF-1 and Foxo3a are upregulated in response to both acute resistance and treadmill running protocols up to 4 hours postexercise. However, beyond that point, mRNA expression of MuRF-1

and Foxo3a only remained elevated after RUN. While the study of Louis et al. (86) focused mainly on markers of the ubiquitin proteasome system (UPS), there is interplay between UPS and autophagy as both processes are transcriptionally regulated by the transcription factor Foxo3a (70). Foxo3a regulates transcription via moving in and out of the nucleus, and we observed that the cytosolic fraction of Foxo3a was reduced following both resistance and aerobic exercise. Moreover, while the time course of autophagy post exercise is less well defined, markers of autophagy have been shown to be upregulated up to 24 h postexercise (29, 103). Collectively, the time course of the present study may not have extended far enough beyond exercise to identify exercise mode specific differences in autophagy. Thus, future studies should extend the time course following acute exercise to more comprehensively examine the time course of autophagy following resistance and aerobic exercise. Such insight would provide insight as to what mediates the unique adaptations of skeletal muscle to exercise.

We recognize that our study does include some limitations. For instance, our study included a relatively small sample of participants, all of whom were college-aged males. However, the results of this study provide a foundation for performing larger studies, perhaps including diseased or aged populations. Similarly, as discussed above our time course only extended to 4 h postexercise. A longer post exercise time course may provide a more comprehensive strategy to compare the acute molecular response between aerobic and resistance exercise. However, we chose to perform this study utilizing a crossover design, which minimized the number of biopsies that could be performed and thus limited the post exercise time points. We also recognize that stationary cycling and leg extension exercise do not represent the entire spectrum of aerobic and resistance exercises, and consequently, changing the type of exercise (i.e., treadmill running or squats) may elicit a different signaling response. However we chose to study these forms of exercise as they both activate the *vastus lateralis*, which was the muscle sampled in the current studied. Lastly, we also recognize that other pathways are involved in the regulation of muscle adaptation, such as Nf-KB, AMPK and Akt (91). Consequently, to what extent these pathway may be mediating exercise-mode specific adaptation requires further investigations.

In conclusion, both acute resistance and aerobic exercise increased S6K1 phosphorylation and resulted in a similar response in markers of autophagy in skeletal muscle, namely a reduction in LC3BII and p62, in the immediate hours post exercise. These findings indicate that, at least in the immediate hours post exercise, the unique adaptations to resistance and aerobic exercise may be mediated through cellular pathways other than mTOR and autophagy, or occur at later post exercise timepoints. The rather similar response of these cellular processes in skeletal muscle following acute resistance and aerobic exercise observed in the current study may be the result of the unaccustomed nature of each form of exercise as all subject in this study were considered untrained (22). However, we did identify subtle differences in the response of mTOR signaling in skeletal muscle to acute AE and RE in untrained subjects. To what extent these subtle differences in mTOR phosphorylation are related to the unique adaptation facilitated by each exercise mode requires further investigation. In particular future studies should include a longer time course following exercise and perhaps examine the molecular response of skeletal muscle to acute AE and RE in both the untrained and trained state. Further, determining the specific molecular response to each bout of exercise will provide further insight to the regulatory mechanisms controlling specific skeletal muscle adaptations, which could have important implications for health and performance. In particular, such insight would provide a foundation for developing targeted exercise therapies aimed to improve muscle function in those suffering from conditions of muscle wasting and dysfunction.

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APPENDIX A

WESTERN BLOT PROTOCOL

Before day 1

1. Stock Chemicals / Reagents
    a. 10X TBS – 96.8 g Tris base, 320g NaCl,, pH to 7.6 with 12 N HCl, add dd H_2O final volume of 4L
    b. TBST (1X) – 400 ml of 10X TBS + 3600 ml ddH_2O + 4 ml Tween 20
    c. 10X Electrode buffer – 120 g Tris Base, 576 g Glycine, 40 g SDS, final volume 4L
    d. Electrode buffer (1X) – 400 ml of 10X concentrated electrode buffer + 3600 ml ddH_2O
    e. Nonfat Dry Milk (NFDM) - 5% solution: 0.5 grams NFDM per 10 ml TBST
    f. Bovine Serum (BSA) – 5% solution: 0.5 grams NFDM per 10 ml TBST
2. Fill out western gel template (see back page)
    a. Determine loading volume of each sample (ul)
    b. Calculate volumes based on loading 50 ug of each sample and standard
    c. 8 ug of ladder

Day 1

1. Turn Fisher Scientific Isotemp to high
    a. Heat must reach 100 °C
2. Prepare Gel
    a. Select appropriate gel
        i. 7.5% gel: targets proteins between 60-200 kDa
        ii. 12/15% gel: targets proteins 10-60 kDa
    b. Remove comb and **tape from bottom of cassette**
    c. Place gel in cassette holder, with tall plate facing outward
    d. Label gel based on position in cassette holder
    e. Place cassettes into electrophoriesis tank (BioRad Mini-Protean Tetra System)
    f. Fill cassette holder with electrode buffer until overflow (check for leak in cassette holder)
3. Retrieve desired samples
    a. Thaw samples on ice
    b. Heat samples for 3 min on Isotemp
    c. Cool samples for 1 min

Loading Samples

Items needed: Western data sheet, micropipettes, micropipette loading tips, electrode buffer solution

1. Use western loading sheet to determine order of loading
2. When loading a sample into a well
    a. Vortex each sample, angle tip towards body in straight line
        i. Prevents sample from going into adjacent wells
3. Gradually lift pipette tip out of well as sample is loading
    a. Prevents sample bubble formation
4. Add more electrode buffer to fill line on electrophoresis tank
5. Return samples to freezer

Running the Western

1. Power settings – BioRad Power Pac Basic
    a. 7.5% Gels: 150 V for 50 min
    b. 12/15% Gels: 150 V for 60 min
2. Make sure wires are in correct colored shots

During last 10 min of run

Items needed: sharpened pencil, 1 transfer membrane, scissors

Prep membrane

1. Take membrane from in between two blue sheets (BioRad)
2. Label membrane
3. **Do not directly touch membrane**
4. Place membrane in transfer buffer solution

After Run

Items needed: wide flat spatula, transfer buffer solution

1. Turn off power unit, unplug wire from power unit
2. Use tools to extract gel from case
    a. Start with key shaped tool, open top corners followed by lower corners
    b. Run key along sides to separate front and back plates
    c. Cut wells off top of gels
    d. Place gels in transfer buffer solution (MAKE SURE GELS ARE PROPERLY LABELED IN THE LOWER RIGHT CORNER)

Preparing for Transfer

Items needed: transfer buffer solution (Cat #10026938) 100% methanol, 2 reservoir stacks, 1 membrane, tweezers and mini roller

1. One set of reservoir stacks for every 2 gels
2. Submerge both stacks in transfer buffer solution
3. Bathe membrane in 100% methanol for 1 min
    a. Place membrane in transfer buffer solution
4. Transfer cassettes to RioRad Transblot Turbo Transfer system
5. SEE DIAGRAM BELOW

Setting up the BioRad Transblot Turbo Transfer System

1. Turn system on
2. Select LIST
3. Select Bio-Rad
4. Determine molecular weight category for desired protein
5. Select settings
    a. Make sure setting is for mini gels; includes 2.5 A and up to 2%V
    b. Proteins < 30 kDa is low molecular weight
        i. 5 min
    c. 5-150 kDa is mixed molecular weight
        i. 7 min
    d. > 150 kDa is high molecular weight
        i. 10 min
    e. To RUN, press button that corresponds to loaded cassette
        i. A is top, B is bottom
    f. Transfer automatically starts
    g. After transfer, time will go off
    h. Disassemble gel sandwich and place in transfer buffer

After Transfer Run

Items needed: tweezers, blocking solution, 1 plastic container

1. Disassemble transfer cassettes
2. Transfer membrane to plastic container, using tweezers
3. Add blocking solution (NDFM or BSA) and place container on rocker
4. Let membrane rock for 60 minutes

Primary Antibody

Items needed: primary antibody, one 15 ml centrifuge tube, NFDM or BSA

1. Determine which primary antibody will be used
2. Determine the dilution of the primary solution
3. Incubate overnight in 4°C fridge

Day 2

1. Remove ECL (Pierce ECL Western Blot Substrate, Thermo Fisher) from fridge and allow to equilibrate to room temp
    a. Prepare:
        i. Plastic tray and conical tube labeled ECL only
        ii. 1-5mL pipette set to 1.5 mL
        iii. 2 1-5 mL pipette tips
        iv. Transparent sheet
2. Removing primary antibody
    a. Pour primary into previous conical tube and store in 4°C fridge
3. Wash membrane 3x in TBST to remove residual antibody
4. Preparing secondary antibody
    a. Determine required volume for 10mL solution
    b. Make BSA or NFDM diluent
    c. Remove secondary antibody from -20°C fridge, vortex and spin
    d. Add secondary diluent at a concentration of 1:10,000
5. Membrane will incubate for 60 min
6. Discard secondary into waste, perform 2-3 quick TBST washes
7. Rock membrane in fresh TBST for 15 min
8. Perform 3, 5 min washes in fresh TBST
9. Aliquot 1.5 of ECL solution 1 and 2 into ECL labeled conical tube, swirl to mix
10. Incubate in ECL for 6 min
    a. Transfer membrane to labeled ECL container
    b. Pour on solution, start timer for 6 min
    c. Use mini transfer pipette to redistribute ECL
11. Transfer membrane to transparent sheet
    a. Using tweezers dab bottom left corner of membrane on sides of plastic container
    b. Drag corner of membrane along paper towel to remove excess ECL
    c. Place on transparent sheet
    d. Discard the used ECL

Imaging

1. Open image lab 5.0
    a. Select protocol titled “ECL mini gel”
2. Select position gel and center membrane on blue box on screen
3. Select “run protocol”
    a. 10 images across 500 sec exposure
4. Right click on 500 sec image and select “save all”
    a. Select appropriate folder to save images to
    b. Save ECL images as “YEAR-MONTH-DAY STUDY_GELNAME_SUBJECT#_PROREIN TARGET_Exposure_xsec”
5. Click select “image an continue”
6. Under “Gel Imaging”, click “Select”, “Blots” and select “colormetric”
7. Select “Run protocol”

8. Click “Select image and continue”
9. Return membrane to TBST to rock until storage

Identifying Band of Interest

1. With the ECL image in “channel 1” and colormetric image open, go to “File” and select “Create Multichannel image”
    a. Place ECL image in “channel 1” and colormetric image in “channel 2”, click “OK”
    b. Ladder will appear RED and ECL bands will appear GREEN
2. Very band is present at expected molecular weight and visible in all wells
3. If multiple bands are present on membrane, identify band of interest on ECL image
    a. Select “annotation tools”
    b. Select “arrow” and draw next to bands of interest
4. Savel colormetric image as “YEAR-MONTH-DAY”

Western Blots: Mini-Protean Gel

Study _____ **Date** _____

Purpose _____

Block _____

Primary Antibody _____

Gel _____ **Gel %** _____

Lane	1	2	3	4	5	6	7	8	9	10	11	12
Sample												LAD
µl Loaded												8
µg loaded	50	50	50	50	50	50	50	50	50	50	50	50

Gel _____ **Gel %** _____

Lane	1	2	3	4	5	6	7	8	9	10	11	12
Sample												LAD
µl Loaded												8
µg loaded	50	50	50	50	50	50	50	50	50	50	50	50

APPENDIX B

WESTERN BLOT IMAGE ANALYSIS

1. Open Image Lab
2. Open image for desired analysis
3. Select volume tools
    a. Select rectangle
    b. Start with standard band
    c. Draw rectangles that capture the entire density of desired band
        i. Use shift to rotate rectangle
        ii. Do this for every band
    d. Double click on each drawn rectangle
        i. Add custom label
        ii. Label based on sample ran (i.e. BASAL, AE 1h)
    e. Draw two rectangles on background of the image
        i. Label BKGD 1 and BKGD 2
4. Open analysis table
    a. Export to excel
    b. Copy and paste the analysis table to desired location