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Examination of Anabolic Signaling and Muscle Growth with Caffeine Treatment in Overloaded Hindlimb Muscle and Electrically Stimulated Muscle Lacking Liver Kinase B1

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Examination of Anabolic Signaling and Muscle Growth with Caffeine
Treatment in Overloaded Hindlimb Muscle and Electrically
Stimulated Muscle Lacking Liver Kinase B1

Timothy Michael Moore

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of
Master of Science

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ABSTRACT

Examination of Anabolic Signaling and Muscle Growth with Caffeine Treatment in Overloaded Hindlimb Muscle and Electrically Stimulated Muscle Lacking Liver Kinase B1

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Master of Science

Skeletal muscle has the ability to increase in size (hypertrophy) after resistance is placed upon it. This hypertrophy is marked by significant upregulation of the mammalian target of rapamycin (mTOR) and its downstream targets. The upstream kinases, protein kinase B (also known as Akt) and AMP-activated protein kinase (AMPK), are two of the many regulators of the mTOR pathway. Recent studies suggest that the widely consumed neuroactive compound caffeine could potentially inhibit mTOR by acting through Akt and/or AMPK. The purpose of this thesis was to: 1) determine if caffeine can inhibit the mTOR pathway and ultimately attenuate skeletal muscle hypertrophy and 2) determine if this inhibition is through LKB1, an upstream regulator of AMPK.

First, 3 month old male rats underwent unilateral tenotomy of the gastrocnemius, resulting in overloading (OVL) of the synergistic plantaris muscle. The contralateral limb was sham-operated (SHAM) on. Rats were given *ad libitum* access to tap water or tap water + caffeine (1 g/L). The OVL procedure resulted in significant hypertrophy of the plantaris which was attenuated after 1 wk of caffeine treatment. However, after two wks this effect was not observed. mTOR targets were examined in both the SHAM and OVL plantaris muscle which showed significant upregulation with OVL but no impact with caffeine treatment. Akt and AMPK was also assessed in the plantaris muscle which showed diminished Akt phosphorylation in 1 wk treated rats while the phosphorylation of AMPK remained relatively unaffected. Notably, caffeine caused decreased atrophy of the tenotomized gastrocnemius after 1 wk along with decreased body weight gains, food consumption, and retroperitoneal fat pad weight in both 1 and 2 wk treated rats.

Sec, to elucidate how caffeine could be impacting the mTOR pathway and how LKB1/AMPK might be involved, skeletal muscle specific LKB1 knockout (skmLKB1-KO) mice were subjected to high-frequency electrical stimulation (HFES) of the sciatic nerve resulting in contraction of the tibialis anterior (TA) and extensor digitorum longus (EDL) muscles against the larger gastrocnemius. All mice were given an intraperitoneal injection of saline or saline + caffeine (20 mg/kg BW at 1 g/L). HFES resulted in marked upregulation of mTOR targets in the TA/EDL of mice 0, 3, and 8 h post HFES. mTOR targets remained relatively unchanged with caffeine treatment. We also observed that these markers were consistently upregulated in our skmLKB1-KO mice with or without HFES. Our findings indicate that caffeine, at physiological concentrations, does not impact anabolic signaling. Furthermore, diminished LKB1 levels resulted in increased levels and activation of markers of protein synthesis.

Key Words: mTOR, hypertrophy, caffeine, AMPK, LKB1

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CHAPTER 1: Literature Review

Skeletal Muscle: A Brief Overview

Skeletal muscle is one of the three major muscle types in mammalian organisms. This mesoderm derived striated tissue accounts for 30-40% of total body mass and permits the contractile activity necessary to sustain movement [Janssen, 2000]. A muscle comprises all tissue ensheathed by the outer most layer of connective tissue called the epimysium. This includes all blood vessels, muscle tissue, connective tissue, lymphatics, and resident immune cells naming only the more abundant types. Skeletal muscle tissue is further divided into long strands of fibers encompassed by a sheath of connective tissue called the perimysium. Several myocytes, also known as myofibers, fill the perimysium running parallel to one another and are one of the few cell types that are multinucleated within mammalian organisms. Such cells form from the fusion of several myoblasts, a process that occurs during both embryonic development and adult life. Each myofiber is further ensheathed by an additional layer referred to as the basal lamina. The striated nature of myofibers is due to the repetition of sarcomeres [Scott, 2001]. Sarcomeres are considered the functional unit of skeletal muscle and are composed of overlapping myosin and actin filaments that interact in such a way as to allow for contraction and subsequent movement. See Figure 1.1 for a simplified cross section of a skeletal representation.

Skeletal Muscle Hypertrophy

Skeletal muscle cells have the ability to grow (hypertrophy) after increased loading. Not only is skeletal muscle able to hypertrophy (change its mass/volume ratio), it can also alter the particular myosin isoform expressed. This transforms the muscle fiber into an adapted fiber type better able to meet its demands. Hypertrophy in response to training is marked by an overall increase in the size of the skeletal muscle fibers instead of amplification of fiber number [Glass,

2010]. For fibers to undergo hypertrophy, the rate of protein synthesis must be greater than the rate of protein degradation. Thus, there is an essential role for protein synthesis pathways and related pathways that either promote the formation of proteins or impede their degradation.

Protein synthesis is pivotally dependent upon a number of key pathways resulting in myofibrillar protein accumulation and subsequent skeletal muscle hypertrophy. The mammalian target of rapamycin (mTOR) pathway has been shown to be a regulator of the increase in muscle size that results from mechanical stimulation (increased loading upon the muscle) [Bodine, 2001]. Work in *S. cerevisiae* has shown that the timing and location of growth are both dependent upon this pathway [Wullschleger, 2006]. Furthermore, in the rat, blocking mTOR prevents muscle growth during development and under overload (OVL) conditions [Bodine, 2001; Bodine, 2006; Reynolds, 2002].

Upstream of mTOR is Akt, also known as protein kinase B. Work utilizing Akt knockout mice results in a myriad of conditions ranging from diabetes, neural tube defects, and growth deficiency showing the versatility this molecule has within various cell types [Yang, 2004]. Phosphorylation of Akt through interaction with phosphatidylinositol 3 kinase (PI3K) results in downstream activation of mTOR [Krakstad, 2010]. Refer to Figure 1.2 for a simplified representation of the mTOR pathway.

Activation of the PI3K/Akt/mTOR pathway seems to be able to happen in immediate and later fashions after muscle stimulation. The later activation can happen through a number of factors, either autocrine or paracrine in nature, including various nutrients such as amino acids or muscle growth factor (MGFs) such as insulin-like growth factor-1 (IGF-1) [Rennie, 2003; Hornberger, 2006]. The immediate response is believed to originate from the mechanical stimuli [Reynolds, 2002]. This contraction based activation results in phosphorylation of a different

serine residue, Ser2035 than the traditional IGF-1 based activation site on mTOR, Ser2448. Nevertheless, hypertrophy is still a result and activation of a number of the same effector pathways occurs [Reynolds, 2002].

Activation of mTOR increases protein synthesis by subsequent phosphorylation of the serine/threonine kinase p70S6 (S6k) which further targets and phosphorylates the S6 ribosomal protein (S6) [Baar, 1999]. mTOR has additional targets including eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) that also aides translation [Kubica, 2005]. mTOR and its effectors are essential for a hypertrophic response as a result of increased muscle loading [Reynolds, 2002].

The robust activation of the mTOR pathway through a mechanical stimulus can be accomplished experimentally in rodents by a number of different methods. Each method typically falls into one of the following categories: exercise training, electrical stimulation, or compensatory overload.

Exercise training can be either aerobic or anaerobic. Both protocols can induce skeletal muscle hypertrophy although aerobic training protocols have difficulty inducing substantial muscle hypertrophy. Anaerobic training protocols in rodent have been used for decades and resemble weight bearing exercises in humans. However, rodents do not train well often needing an external stimulus such as food or an electrical shock. Such stimuli can be seen as forced exercise inducing a high amount of stress upon the animal. An additional drawback is the amount of time needed to gain significant results. Some studies report 10-30% increases in muscle cross sectional area (CSA) requiring anywhere from 8 – 36 wks of training [Ho, 1980; Fluckey, 1995; Lowe, 2002]. Increasing CSA size over 30% is strenuous on the animals and

burdensome on researchers due to time constraints with more realistic growth rates ranging from 10-15% over a few months.

High frequency electrical stimulation of the sciatic nerve (HFES) has been shown to activate mTOR targets, namely S6K and 4EBP1, [Thomson, 2008] and result in skeletal muscle hypertrophy of the tibialis anterior (TA) and extensor digitorum longus (EDL) muscles [Baar 1999]. In addition, this model allows direct comparisons between resting and stimulated muscles within the same animal. Other advantages includes: independence from the animal's motivation, stimulation of any muscle desired, and large control over the type of stimulation including duration, frequency, or intensity. Such control can allow for a wide range of studies to be employed. Nevertheless, surgery, however minor or careful, is needed and results in some amount of injury and inflammation to the animal. Furthermore, these animals must repeatedly undergo anesthesia and care to prevent further injury to the wound if they are to undergo repeated bouts.

Compensatory overload (OVLD) of a muscle occurs when its synergists are rendered unable to function. OVLD experiments have repeatedly been used to induce skeletal muscle hypertrophy [Adams, 1996; Armstrong, 1979]. One of three different protocols can be used: tenotomy of synergist, synergist ablation, or synergist denervation. A tenotomy refers to severing the tendon of a muscle rendering it unable to function. This procedure can result in a 40 and 20% increase in the mass of the soleus and plantaris muscles respectively when performed on the tendon of the gastrocnemius muscle after 14 d [Goldberg, 1967]. Synergist ablation involves at least partial removal of a muscle along with the tendon and also induces significant hypertrophy. In female rats whose gastrocnemius and soleus muscles had been partially excised, the plantaris grew 65% over a 9-wk period [Baldwin, 1982]. The final OVLD experiment

involves synergistic denervation where nerve branches to specific muscles are severed. One study showed that when the gastrocnemius and soleus muscles of rodents were denervated, the plantaris muscle grew 30-40% in 3 wks [Degens, 1995]. Taken together, the main advantage of these techniques is the large scale hypertrophy that occurs. Drawbacks include the higher levels of initial inflammation within the first few days immediately following the surgery and the direct applicability to a human condition.

AMPK and its link to mTOR

5' adenosine monophosphate-activated protein kinase (AMPK) is a highly conserved metabolic sensor expressed in a wide range of eukaryotes including yeast and humans as well as a variety of tissues including hepatocytes, myocytes, and neurons [Steinberg, 2009]. AMPK forms a heterotrimeric complex combining various isoforms of alpha, beta, and gamma subunits. The beta and gamma subunits are regulatory in function while the alpha acts as the catalytic domain [Winder, 1999]. AMPK becomes activated by interaction with liver kinase B1 (LKB1) complexed with STE-related adaptor alpha (STRAD α) and mouse protein 25 alpha (MO25 α) [Hawley, 2003; Suter, 2006; Smith, 2011]. LKB1 is typically nuclear bound and must complex with STRAD α and MO25 α before it is exported to the cytosol [Dorfman, 2008]. As metabolic activity increases within a cell and the ratio of AMP:ATP begins to increase, AMPK becomes activated. Two AMPs bind to the gamma subunit causing a conformational change that exposes Threonine 172 within the alpha subunit [Adams, 2004]. The complex of LKB1:STRAD α :MO25 α is then able to phosphorylate AMPK at Thr 172 allowing it to activate catabolic, ATP producing, processes and to inhibit anabolic, ATP consuming, processes [Shackelford, 2009].

AMPKs inhibition of anabolic processes extends to the inhibition of the mTOR pathway in skeletal muscle [Bolster 2002; Thomson, 2008]. This inhibition is believed to attenuate protein synthesis and ultimately diminish muscle growth [Mounier, 2011]. AMPK can deter the mTOR pathway in a few ways such as: phosphorylation of tuberous sclerosis complex 2 (TSC2) an upstream regulator of mTOR [Inoki, 2003; Corradetti, 2004], or phosphorylation of raptor, a member of the mTOR complex that is essential for mTOR activity [Gwinn, 2008].

As mentioned previously, the alpha subunit of the heterotrimeric complex that comprises AMPK is responsible for the catalytic activity [Winder, 1999]. Two distinct isoforms of the alpha subunit exist, namely alpha1 ($\alpha 1$) and alpha2 ($\alpha 2$), with the $\alpha 1$ subunit appearing to be more integrated in regulating protein synthesis and muscle growth [Mounier, 2009]. This work was conducted using an OVLD model to induce skeletal muscle hypertrophy of the plantaris in AMPK $\alpha 1$ $-/-$ mice. The findings by Mounier *et al* showed that mTOR activity was significantly upregulated in the AMPK $\alpha 1$ $-/-$ mice via increased phosphorylation of S6K, S6, and 4EBP1. McGee *et al* conducted similar research utilizing a plantaris OVLD model within an LKB1-KO mouse specifically in striated muscle, testis, kidney, and lung. This LKB1-KO mouse showed trace amounts of $\alpha 2$ activity and a 60% reduction in $\alpha 1$ activity in control plantaris muscles when compared to their wild type littermates. Despite the reduction in $\alpha 1$ activity in both control and OVLD muscles, McGee *et al* further reported that LKB1-KO did not impact the phosphorylation of S6K, S6, or 4EBP1 in response to OVLD [McGee, 2008]. To further complicate matters, Mounier *et al* later showed that the diameter of myotubes isolated from AMPK $\alpha 2$ -deficient mice were 25% smaller than control myotubes and that their ability to respond to a hypertrophic stimuli was impaired [Mounier, 2011]. It is clear that more experiments are needed to know how exclusively each alpha subunit functions with regards to mTOR activation.

Caffeine

Caffeine is arguably the most consumed drug today. It is found in a variety of products with a wide range of physiological and psychological effects. The health effects of caffeine can be positive including a temporary increase in attention, alertness, and metabolic rate while the side effects consist of anxiety, addiction, tolerance, increased blood pressure, and increased urination [Winston, 2005]. Caffeine can also impact cellular processes such as the cell cycle, apoptosis, increased calcium release from the sarcoplasmic reticulum within skeletal muscles, and enhanced central nervous system activity [Bode, 2007; Warren, 2010]. For reference, it is estimated that the average consumption of caffeine in the United States is about 2 cups of coffee (~150-250 mg) with 10% of the population ingesting over 1000 mg/d [Graham, 2001].

Caffeine is believed to have an ergogenic effect upon endurance exercise although the mechanisms are varied and complex [Graham, 2001, Warren, 2010; Gliottoni 2009]. Specifically, caffeine has been found to block adenosine receptors within tissues. Such receptors are found in most tissues including the brain, three different muscle types, and adipocytes. Due to this fact, the impact of caffeine upon the body can be the culmination of several specific tissue responses. At concentrations that are physiologically relevant to humans, caffeine has a pronounced effect upon the central nervous system as well as other brain centers and thus impacts various behavioral characteristics including learning, memory, performance, coordination, arousal, vigilance, and fatigue to name some of the more studied ones [Graham, 2001; Nehlig, 1992].

Because of these side effects, caffeine is widely used among the general population but recent literature might point to a negative impact upon skeletal muscle. Caffeine treatment at mM concentrations has been shown to block the activity of Akt and subsequently inhibit mTOR

potentially attenuating protein synthesis [Kolnes, 2010; Egawa, 2011]. Furthermore, *in vitro* studies involving incubation with caffeine also show increased activation of AMPK in rat epitrochlearis and soleus muscles [Lally, 2012]. In addition, Egawa *et al* found that caffeine activated both AMPK subunits at 3 mM, but Abbott *et al* reported that caffeine increased AMPK α 2 activity only at a similar concentration [Abbott, 2009]. McConell *et al* further found that at 5 mM, caffeine did not increase pAMPK in L6 myotubes [McConell, 2010]. Nevertheless, such levels are supraphysiological as 2 – 5 cups of coffee can result in blood concentrations in the 15 – 80 μ M range depending upon the individual [Birkett, 1991]. Therefore, it is not yet known whether caffeine at physiological concentrations has an impact upon skeletal muscle anabolic signaling and hypertrophy.

Specific Aims

To summarize, skeletal muscle is able to respond to increased loading by increasing muscle fiber size. This hypertrophy is dependent upon the mTOR signaling pathway. mTOR is regulated by a number of different pathways and compounds one of which is LKB1/AMPK, although the interaction is complex and varied. Caffeine is also believed to impair mTOR signaling by inhibiting Akt, an upstream molecule involved in its activation. However, the effect of chronic caffeine treatment upon skeletal muscle growth is unknown although literature searches suggest a potential deleterious effect upon hypertrophy.

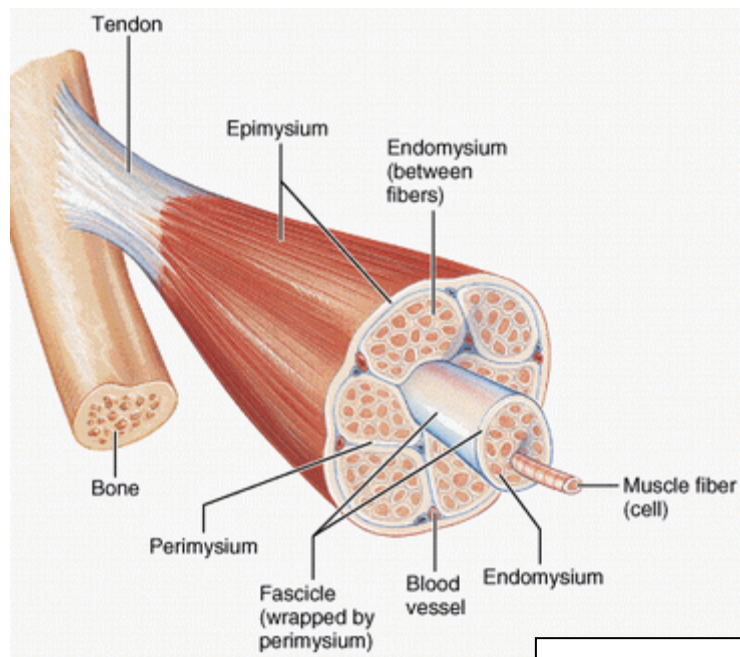
Specific Aim #1: Determine How Oral Caffeine Administration Affects Skeletal Muscle Hypertrophy and mTOR Signaling in Overloaded Rat Muscle.

Three month old rats were given tap water or tap + water plus caffeine (1 g/L) after which unilateral tenotomy of the gastrocnemius and soleus muscles to induce hypertrophy of the

plantaris was commenced. I hypothesized that caffeine treated animals would have decreased hypertrophy of the tenotomized limb at both time points.

Specific Aim #2: Determine the Role of LKB1 with Regards to Caffeine's Impairment of mTOR Activation after an Individual Bout of Resisted Muscle Contractions.

Three month old conditional or skeletal muscle specific LKB1 KO mice were given an intraperitoneal injection of saline or saline + caffeine after which high-frequency electrical stimulation of the sciatic nerve resulting in contraction of the tibialis anterior and extensor digitorum longus was commenced. I hypothesized that caffeine would attenuate anabolic signaling through activation of AMPK and that LKB1 KO mice would not experience this inhibition.



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Figure 1.1: A Simplified Cross Section of a Skeletal Muscle.

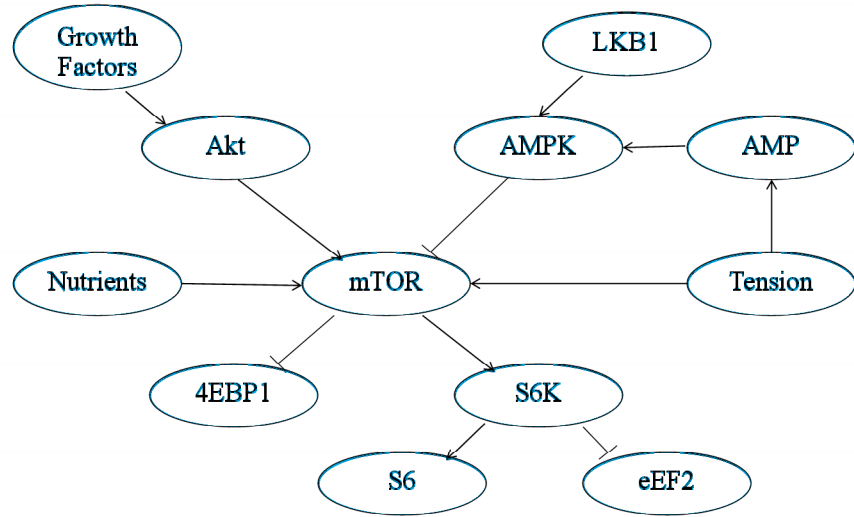


Figure 1.2: A Simplified Diagram Displaying Akt and AMPK as Regulators of the mTOR Pathway.

CHAPTER 2: Thesis

Introduction

Skeletal muscle has the ability to grow (hypertrophy) due to increased loading. Hypertrophy in response to training is marked by an overall increase in the size of the skeletal muscle fibers (myofibers) instead of amplification of the overall fiber number [Glass, 2010]. For myofibers to undergo hypertrophy, the rate of protein synthesis must be greater than the rate of protein degradation. Thus, there is a pivotal role for protein synthesis and other related pathways that either promote the formation of proteins or impede their degradation.

The mammalian target of rapamycin complex 1 (mTORC1) and its associated pathway has been shown to be a regulator of the increase in myofiber size with increased workload [Bodine, 2001]. Activation of mTOR within mTORC1 increases protein synthesis by subsequent phosphorylation of the serine/threonine kinase p70 S6 Kinase (S6K) which further targets and phosphorylates ribosomal protein S6 and eukaryotic elongation factor 2 kinase [Baar, 1999]. mTORC1 has additional targets including eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) and the downstream target eukaryotic elongation factor 2 (eEF2) just to name a few [Kubica, 2005].

Tenotomy of the gastrocnemius muscle of the lower hind limb in the rat effectively overloads (OVL) the synergistic plantaris and soleus muscles and results in a 20 and 40% increase in their weights respectively over a 14-day period [Goldberg, 1967]. Such hypertrophy is accompanied by increased phosphorylation of anabolic signaling intermediates including mTOR and S6 [Bodine, 2001; Thomson, 2006; Chale-Rush, 2009]. Additionally, high-frequency electrical stimulation (HFES) of the sciatic nerve has been shown to activate mTOR

and result in skeletal muscle hypertrophy in the tibialis anterior (TA) and extensor digitorum longus (EDL) muscles after a single bout [Baar 1999, Thomson, 2008].

The robust activation of the mTORC1 pathway can be accomplished by a number of different methods including various nutrients such as amino acids or muscle growth factor (MGFs) such as insulin-like growth factor-1 (IGF-1) [Rennie, 2003; Hornberger 2006]. One regulator of mTORC1 is the molecule protein kinase B, also known as Akt. Akt is a signal intermediate downstream of the insulin receptor and other growth receptors, which acts to increase protein synthesis through mTORC1 during times of higher nutrient availability [Suryawan, 2008].

An additional regulator is 5' adenosine monophosphate-activated protein kinase (AMPK). AMPK is activated by interaction with liver kinase B1 (LKB1) complexed with STE-related adaptor alpha (STRAD α) and mouse protein 25 alpha (MO25 α) [Hawley, 2003; Suter, 2006; Smith, 2011]. LKB1 is typically nuclear bound and must complex with STRAD α and MO25 α before it is exported to the cytosol. As metabolic activity increases within a cell and the ratio of AMP:ATP begins to increase, AMPK becomes activated [Shackelford, 2009]. This activation leads to the inhibition of the mTORC1 pathway in skeletal muscles which prevents anabolic signaling [Bolster, 2002; Thomson, 2008; Mounier, 2011].

Recent studies have shown that caffeine treatment at mM concentrations attenuates the phosphorylation of Akt [Kolnes, 2010], and reduces the insulin-stimulated activity of mTOR in incubated rat epitrochlearis muscle [Egawa, 2011]. Furthermore, multiple studies have shown an increase in the phosphorylation of threonine 172 on AMPK α with caffeine treatment [Abbott, 2009; Lally, 2012]. This suggests that protein synthesis might be impacted by both regulatory kinases. However, using caffeine concentrations of 3 – 10 mM are supraphysiological. Caffeine

administration of 150 mg every eight hours resulted in steady state plasma concentrations of 1 – 14 uM [Birkett, 1991] while larger doses (3 – 6 mg/kg) result in 15 – 80 uM plasma concentrations [Graham, 2001]. For reference, the average 8 oz cup of coffee usually contains around 75 - 150 mg of caffeine depending upon the brand and method of brewing.

We sought to determine if physiologically relevant ingestion of caffeine could inhibit anabolic signaling and ultimately attenuate skeletal muscle hypertrophy *in vivo*. We utilized tenotomized OVLD rat and HFES of a skeletal muscle specific LKB1 knockout (skmLKB1-KO) mouse models in these experiments. We hypothesized that OVLD induced hypertrophy of the plantaris muscle within the rat will be diminished with caffeine treatment and that our skmLKB1-KO mouse model will show that the effects of caffeine will be at least partially dependent upon LKB1.

Materials and Methods

Rat OVLD Model

All proposed experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of Brigham Young University.

Approximately 2 month old male Sprague Dawley rats were obtained from Charles River Laboratories and housed in cages maintained in a 12-h light/dark, temperature controlled (20-25°C) room with *ad libitum* food and water access. Rats were stratified based on weight into tap water or tap water + caffeine (1 g/L) groups. Two d post treatment initiation, all rats underwent unilateral tenotomy of the gastrocnemius and soleus muscles of the right hindlimb to induce hypertrophy of the synergistic plantaris muscle. Rats were anesthetized using isoflurane (2-3%) dissolved in oxygen and injected with the analgesic buprenorphine intraperitoneally at a

concentration of 0.015 mg/ml and a dosage of 0.075 mg/kg BW using a 26 gauge needle. Ophthalmic ointment was also applied to the eyes prior to surgery. Surgery was performed on a water-circulating heat pad set to 37°C. The hindlimbs were shaved and scrubbed with povidone-iodine solution. Using aseptic technique and materials, a 10-20 mm incision was made along the posterior aspect of the lower hindlimb. The gastrocnemius tendon of the right hindlimb was cut and sutured back onto the body of the muscle using 6-0 silk sutures to prevent reattachment of the tendon. The contralateral hindlimb also underwent a similar incision, but without the tenotomy. Both incisions were closed using sterile surgical staples and the rats were returned to the animal quarters. One or two wks post surgery, as indicated, tissues were harvested, quickly weighed, and then flash frozen in liquid nitrogen for protein analysis.

Mouse HFES Model

All mice were housed and bred in cages maintained in a 12-h light/dark temperature sensitive (20-25 °C) room with no restriction to food and water availability. The skeletal muscle specific LKB1 knockout (skmLKB1-KO) mouse was generated by crossing a transgenic mouse having an LKB1 gene flanked by LoxP sites, provided by R. DePinho and N. Bardeesy (Dana-Farber Cancer Institute in Boston, MA), with a Myf6-Cre transgenic mouse that heterozygously expressed Cre recombinase specifically in skeletal muscle under the Myf6 (MRF4) promoter [38]. The Myf6-Cre transgenic mouse was provided by M.R. Capecchi from the University of Utah in Salt Lake City, Utah. Mice genotypes were tested via polymerase chain reaction using primers specific for Cre and floxed LKB1 as described previously [Tanner, 2013]. Verification by western blotting for LKB1 protein content was also used.

At approximately 12 wks of age, the above described mice were weighed and stratified into groups according to weight. Mice received either an injection of saline or saline + caffeine

intraperitoneally via a 26 gauge needle at a concentration of 1 g/L and a dosage of 20 mg/kg BW. Sixty min post injection; mice were anesthetized with isoflurane (2-3%) in supplemental oxygen, injected with buprenorphine as an analgesic, and underwent a single bout of HFES. Surgery was performed using sterile technique on a water-circulating heat pad set to 37°C. The animals' left upper hindlimb was shaved on the lateral side and scrubbed with povidone-iodine solution. Ophthalmic ointment was applied to the eyes and the left sciatic nerve was exposed through an incision along the lateral aspect of the upper hindlimb. Electrodes were placed on the sciatic nerve and muscle contractions were stimulated in 3 sec durations with 10-12 V at 100 Hz. Six sets of ten contractions (3 sec per contraction) were performed. Ten sec of rest were allowed between contractions and 60 sec of rest between sets. Animals in the 0 h time point group had their lower hindlimb muscles and blood removed immediately post HFES while the 3 and 8 hr time points had the incision closed with wound clips. The 3 hr group remained under anesthesia until harvest while the 8 hr group was allowed to awaken and return back to the animal quarters. Tissues and blood were treated as described above.

Measurement of Protein Synthesis via Puromycin

Puromycin (Thermo Scientific, Waltham, MA, USA), was injected intraperitoneally into 1 wk treated rats and to mice in the 8 h group at a concentration of 5 mg/ml and a dosage of 22 mg/kg BW using a 26 gauge needle. At low concentrations, this antibiotic is able to mark newly synthesized proteins and is thus a reliable indicator of protein synthesis [Goodman, 2011].

Puromycin was detected via western blotting as described below.

Tissue Homogenization

Tissues were ground using a pestle and mortar to sufficiently distribute the tissue. They were then homogenized using a Bullet Blender from Next Advance Laboratories (Averill Park,

NY, USA) in an appropriate volume/kg (1/19) of tissue solution of homogenization buffer (50 mM Tris-HCl, 250 mM Mannitol, 50 mM NaF, 5 mM Sodium Pyrophosphate, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 50 mM β -Glycerol Phosphate, pH 7.5, 1 M DTT, 1 M Benzamidine, 5 μ g/uL STI, 200 mM Na_3VO_4 , and 200 mM PMSF). Homogenized tissues were then sonicated using EpiShear Probe Sonicator (Active Motif, Carlsbad, CA, USA) at 30% amplitude for 10 sec on ice after which a small aliquot was removed to determine the total protein content of the muscle and frozen at -90°C . The remaining homogenate was vigorously vortexed and spun at 21,000 g for 10 min to separate proteins from cellular components. Supernatants were removed, flash frozen in liquid nitrogen, and stored at -90°C .

Protein Quantification and Western Blotting

Muscle homogenates were analyzed for protein concentration using BioTek® Synergy HT multimode microplate reader and Bio-Rad DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA) according to manufacturer's protocols. Loading samples were then made in appropriate amounts of homogenate and loading buffer. Samples then underwent western blotting procedure on Criterion Precast Tris-HCl Bio-Rad gel. Gels underwent electrophoresis before being transferred onto activated PVDF membranes. Concluding the transfer, membranes were placed in Ponceaus-stain to ensure equal levels of protein per lane. PVDF membranes then underwent standard immunodetection protocol for horseradish peroxidase conjugated antibodies. The primary antibodies used were: pAMPK α (Cell Signaling 2535), AMPK α (Cell Signaling 2532), LKB1 (Millipore 07-694), pS6K (Cell Signaling 9206), S6K (Cell Signaling 2708), p4EBP1 (Cell Signaling 2855), 4EBP1 (Cell Signaling 9644), p $\text{eEF}2$ (Cell Signaling 2331), $\text{eEF}2$ (Cell Signaling 2332), pAkt (Cell Signaling 4060), Akt (Cell Signaling 9272), pS6 (Cell Signaling 4858), S6 (Cell Signaling 2217), Puromycin (Santa Cruz). Membranes were exposed

to autoradiographic film and resulting band intensities were determined using AlphaEase FC Software version 3.1.2 (Alpha Innotech Corporation, Santa Clara, California, USA).

Statistical Analysis

Figure composition and statistical analysis was performed using GraphPad Prism software (Graphpad Software, Inc.). All data collected is expressed as mean \pm standard error (SE). Each group was compared using repeated measures analysis of variance, Fishers least significant difference post-hoc analysis, or Student's t-test where appropriate to identify significant differences.

Results

Animal Parameters Following OVLD and Caffeine

The initial and final body weights along with the group size, body weight gain, heart and retroperitoneal fat pad weight, and food consumed of all rats used in the OVLD experiments are outlined in Table 2.1. Caffeine treatment resulted in decreased body weight gains and final weights in 1 wk treated rats. However, weights began to normalize after two wks which showed no difference between tap water and tap water + caffeine treatment. Heart weight remained unchanged over the course of the experiment while caffeine treatment resulted in smaller retroperitoneal fat pads in both 1 and 2 wk treated rats. Food consumption of standard chow for both 1 and 2 wk treated rats is shown as total amount consumed for the duration of the particular treatment period. Caffeine treatment resulted in decreased food consumption in both 1 and 2 wk treated rats.

The hypertrophy of the plantaris was calculated as the percent increase in wet weight of the OVLD vs. SHAM plantaris muscles (Figure 2.1A). Tap water treated rats resulted in significant hypertrophy of the OVLD plantaris after 1 wk. Caffeine treatment attenuated this

hypertrophy in 1 wk treated rats significantly. However, this diminishing effect was not seen in the 2 wk treated rats where tap water and tap water + caffeine treated rats hypertrophied at similar rates. Notably, OVLD tap water treated plantaris muscles did not show a significant increase from 1 to 2 wks consistent with previous reports [Goldberg, 1967]. The total protein content of plantaris muscles was calculated as mg of protein per plantaris muscle. Total protein content data was consistent with the above stated plantaris hypertrophy data showing an increase in protein accretion with OVLD after 1 wk (Figure 2.2A). This increase was diminished with the addition of caffeine. However, after 2 wks of treatment, caffeine did not abate the increase in total protein levels as tap water and tap water + caffeine showed significant amplification with OVLD (Figure 2.2B).

The atrophy of the tenotomized gastrocnemius muscle was also calculated as percent decrease in wet weight of the tenotomized vs. SHAM operated gastrocnemius (Figure 2.1B). Tap water treated rats showed higher rates of gastrocnemius atrophy when compared to those treated with caffeine after 1 wk. Nevertheless, this was absent after 2 wks of treatment which showed similar rates of atrophy in tap water and tap water + caffeine treated rats.

Effect of OVLD and Caffeine on Regulators of Protein Synthesis

Western blotting of the plantaris muscle from 1 wk treated rats showed an increase in the phosphorylation of Akt (pAkt) with OVLD which was attenuated with the addition of caffeine (Figure 2.3A). After 2 wks of OVLD, pAkt was not upregulated in tap water or tap water + caffeine treated rats (Figure 2.3B). The phosphorylation of AMPK (pAMPK) decreased in the 1 wk caffeine SHAM group when compared to the corresponding tap water treated group (Figure 2.3C). pAMPK was not upregulated after 1 wk of OVLD in tap water treated rats. However, OVLD resulted in upregulation of pAMPK to tap water treated levels with caffeine treatment

after 1 wk. pAMPK levels in 2 wk treated rats tended to increase with OVLD in both treatment groups (Figure 2.3D). There were no changes in the total protein content for Akt or AMPK in either 1 or 2 wk treated rats (data not shown).

Effect of OVLD and Caffeine on Markers of Protein Synthesis

Western blotting for markers of protein synthesis including S6K, S6, 4EBP1, and eEF2 was undertaken on the plantaris muscle from both 1 and 2 wk treated rats. The phosphorylation of S6K (pS6K) increased in all OVLD muscle in both 1 and 2 wk rats with no differences observed with caffeine treatment (Figure 2.4A). A similar result was observed in phosphorylation of S6 (pS6) where again caffeine treatment had no impact (Figure 2.4B). The phosphorylation of 4EBP1 (p4EBP1) tended to increase with OVLD although the effect was only significant in tap water treated 1 wk rats (Figure 2.4C). The phosphorylation of eEF2 (peEF2) did not show an increase with OVLD in any treatment group although it tended to increase in tap water and tap water + caffeine treated 1 wk rats (Figure 2.4D). There were no changes in the total protein content for S6K, S6, 4EBP1, or eEF2 in any 1 or 2 wk treated rats (data not shown). Puromycin levels increased with OVLD in 1 wk tap water treated rats and was not impacted by caffeine treatment (Figure 2.4E).

Effect of HFES and Caffeine on Regulators of Protein Synthesis

Animal parameters for all mice used in the HFES experiments are shown in table 2.2. Western blotting of the combined TA and EDL for regulators of protein synthesis, AMPK and Akt, was undertaken in the 0, 3, and 8 h conditional (COND) or skmLKB1-KO mice. In the 0 h group, pAkt did not change with HFES in saline treated muscles (Figure 2.5A). However, pAkt increased at 0 h with HFES in skmLKB1-KO saline treated mice while those treated with caffeine had no response to HFES. The 3 and 8 h mice showed little changes in pAkt although it

is interesting to note a potent response in the 8 h skmLKB1-KO mice when compared to their COND mice littermates (Figure 2.5B & C). There were no changes observed in the total protein content of Akt in any of the 0, 3, or 8 h groups (Data not shown). pAMPK significantly increased with HFES in 0 h COND mice; an event that was completely abolished in the skmLKB1-KO mice as expected (Figure 2.5D). The 3 and 8 h groups showed unchanging levels of pAMPK with HFES in either genotypes but the COND mice had pAMPK levels that were higher than their skmLKB1-KO littermates (Figure 2.5E & F). In all time groups measured, the total protein content of AMPK was significantly higher in the skmLKB1-KO mice when compared to COND mice (data not shown).

Effect of HFES and Caffeine on Markers of Protein Synthesis

Western blotting of the combined TA and EDL for markers of protein synthesis including S6K, S6, 4EBP1, and eEF2 was undertaken in the 0, 3, and 8 h COND or skmLKB1-KO mice. pS6K increased with HFES at all time points which was only attenuated with caffeine in skmLKB1-KO mice at 3 h (Figure 2.6A-C). Interestingly, the phosphorylation state of S6K showed a main effect increase in the skmLKB1-KO mice for all time points, an observation also seen in the total levels of S6K (data not shown). These results were also noticed in the S6K target S6 where the phosphorylation increased with HFES and skmLKB1-KO (Figure 2.6D-F). Total S6 protein content showed similar main effect increases with skmLKB1-KO (data not shown). The phosphorylation levels of eEF2 were lower in muscles from skmLKB1-KO mice at all time points and tended to decrease with HFES (Figures 2.6G-I). Total eEF2 protein content showed similar main effect increases with skmLKB1-KO (data not shown). Additionally, our KO mouse continued to show increased band shifting, indicative of increased phosphorylation, in 4EBP1 along with HFES in 0, 3, and 8 h mice (Figure 2.6J-L). HFES resulted in increased band

shifting of 4EBP1 in 0 h saline treated COND mice as well as both saline and saline + caffeine treated 3 h COND mice. Puromycin increased with HFES after 8 h in COND saline treated mice as well as with skmLKB1-KO, consistent with previous findings (Figure 2.7A).

Discussions

Caffeine is arguably the most consumed compound today and it is also routinely found in those supplements that are intended to aide in skeletal muscle hypertrophy. Recent scientific literature has suggested that caffeine could potentially have deleterious effects upon hypertrophy essentially rendering the nutritional supplements they are commonly found in less effective [Kolnes, 2010; Egawa, 2011]. However, to our knowledge, no studies to date have analyzed the impact of caffeine treatment upon anabolic signaling and skeletal muscle hypertrophy *in vivo*.

Our findings indicate that caffeine at physiological levels does not induce sustained abatement of anabolic signaling or skeletal muscle hypertrophy. Previous work in cell culture showed that caffeine at 3 – 5 mM levels effectively attenuated the insulin induced increases in pS6K and pAkt [Egawa, 2011]. Initial studies conducted by our group repeated these results in L6 myotubes at concentrations as low as 0.625 mM but not lower (unpublished data). These concentrations show that caffeine is effective, however, at dosage levels that are not physiologically relevant and suggests that caffeine at physiological levels may not have any effect. Experimentally safe caffeine concentrations in the blood are 40 – 80 uM, still about 10 times below the lowest dose that we could elicit a response with the LD50 in humans around 40 – 80 mM depending upon the individual [Graham, 2001]. The dosage used here results in plasma levels of 20 – 50 uM, equivalent to consumption of 2 – 5 cups of coffee in humans [Egawa, 2011].

While caffeine did not attenuate anabolic signaling in our study, hypertrophy and total protein content were diminished by caffeine in 1 wk treated rats. By two wks this effect was no longer seen. This raises several interesting questions as to how caffeine is inhibiting the growth of the plantaris after 1 wk but not after 2 wks of treatment. One possible explanation could be a tolerance effect that rats gained towards constant caffeine administration after 1 wk due to a potential upregulation of adenosine receptors, consistent with previous findings [Fredholm, 1982; Zhang, 1990]. A sec explanation is that caffeine is a diuretic and could be dehydrating the muscles after 1 wk impairing the rate of protein synthesis [Maughan, 2003]. 1 wk treated rats did tend to drink less water although the difference was not significant ($p=0.13$, data not shown) and was nearly equally after 2 wks. This could explain the “lighter” plantaris muscles being shown as decreased hypertrophy with caffeine treatment. It is also curious to note the difference in hypertrophy in 1 and 2 wk treated rats. Tap water only treated rats did not show significant growth differences from 1 to 2 wks of OVLD implying a ceiling effect for this model, consistent with previous findings [Goldberg, 1967]. Caffeine treated rats were able to grow at an increased rate and essentially catch up to their tap water treated counterparts again perhaps suggesting a tolerance to caffeine was obtained. Decreased food consumption does not adequately explain the diminished plantaris hypertrophy or total protein levels in 1 wk treated animals as food consumption was also lower in 2 wk treated rats. Additionally, the argument cannot be made that if food consumption was depressed after 1 wk, it would stay lower even after 2 wks. This is because the food consumption difference between treatment groups in 1 wk treated rats was less than the difference after 2 wks suggesting caffeine sustained its ability to impact eating habits.

We also observed in caffeine treated rats after 1 wk of OVLD a larger gastrocnemius within the tenotomized limb when compared to their tap water only counterparts. This suggests

caffeine resulted in a decrease in the rate of skeletal muscle atrophy. Atrophy of the tenotomized gastrocnemius is an expected result due to decreased load [Goldspink 1983]. The OVLD experiment and tenotomy surgery is taxing upon the hindlimb of the animal resulting in high levels of local inflammation, swelling, and scarring during the first wk but which typically subsides by 2 wks. Our observation would be consistent with the idea that higher levels of inflammatory cytokines contribute to skeletal muscle atrophy and that caffeine suppress the inflammatory response as shown by others [Miwa, 2012; Wüst, 2007; Barcelos, 2014]. The larger caffeine treated tenotomized gastrocnemius was not observed in 2 wk treated rats suggesting that the major inflammatory events have subsided by that time or that a tolerance to caffeine had been obtained. It would be interesting to analyze the impact of caffeine upon such inflammatory markers within the 1 wk treated animals.

Additionally, in both 1 and 2 wk treated rats, the size of the retroperitoneal fat pad was reduced. This suggests that caffeine has the ability to mobilizing fat stores. However, both 1 and 2 wk treated rats consumed less chow which could explain the diminished fat pads, a result that may or may not be consistent with previous findings as current research is highly conflicting [Sugiura, 2012; Gavrieli, 2013; Jessen, 2005; Carter, 2012]. Sugiura *et al* utilized 10 wk old female mice and a similar caffeine concentration which showed no change in total food intake after 4 wks but caffeine treated mice did tend to have lower body weights. However, studies by Gavrieli *et al*, Jessen *et al*, and Carter *et al* in humans all suggest that caffeine has the ability to decrease appetite. Further studies are warranted concerning this matter. Pair feeding of the rats would have been undertaken had previous literature in rats suggested differing consumption rates.

Rather unexpectedly, we also observed that by knocking out the gene that encodes for LKB1, an upstream regulator of AMPK, markers of anabolic signaling were enhanced. We initially hypothesized that caffeine would down regulate mTOR activity through increased phosphorylation of AMPK. Thus, we utilized a skmLKB1-KO mouse in the sec experiment to help elucidate the mechanism with which caffeine could potentially impair anabolic signaling. However, caffeine did not negatively impact anabolic signaling which was further proven by our HFES results. Nevertheless, we did unexpectedly observe differences between genotypes where the total protein and phosphorylation states of nearly all markers of anabolic signaling measured including S6K, S6, 4EBP1, eEF2, and Puromycin were elevated in skmLKB1-KO muscles. Because total protein levels of targets measured were also higher in the 0 h group, it can be reasoned that skmLKB1-KO mice chronically have modest, but statistically heightened levels of anabolic signaling. This result is consistent with some [Thomson, 2008; Ikeda, 2009; Jessen, 2010] but not all published literature [McGee, 2008]. Thomson *et al*, Ikeda *et al*, and Jessen *et al* utilized LKB1-KO mice that included cardiac muscle with all groups indicated a heightened anabolic signaling response. However, McGee *et al* utilized a skeletal muscle specific LKB1-KO mouse which reported no differences in muscle size but a trend for an increase in anabolic signaling after 7 d of OVLD. McGee *et al* did not report the age of mice used and only reports a sample size of 3 which could explain why no significant differences are seen. It is also worth noting that multiple studies utilizing various cancer models indicate heightened mTOR signaling in response to aberrant LKB1 activity. While it is tempting to say all of this is caused by diminished AMPK activity, LKB1 has a variety of other targets within skeletal muscle which could also play a role in anabolic signaling. It would be interesting to determine the impact that other LKB1 targets have upon anabolic signaling within skeletal muscle.

We further observed that the marked upregulation of anabolic signaling occurs independent of Akt phosphorylation in response to HFES. This suggests that contraction or some other mechanism is activating mTOR targets. We also observed that this upregulation is evident immediately after HFES but continues to amplify at least up to 8 h later. Both observations are consistent with previous findings [Sherwood, 1999; Parkington, 2003; Thomson, 2008]. Furthermore, in our skmLKB1-KO mice, there was an increase in Akt phosphorylation 8 h after stimulation suggesting an ability of LKB1 to regulate Akt, in harmony with published data [Koh, 2006]. It is interesting to note that this increase is in both stimulated and unstimulated muscles at 8 h but is not observed 0 or 3 h post stimulation perhaps because mice were kept under anesthesia for their respective durations and did not feed.

In conclusion, we observed that caffeine at physiological concentrations did not induce a sustained impact upon anabolic signaling or skeletal muscle hypertrophy after OVLD or HFES in rats and mice respectively. In addition, deletion of LKB1 within skeletal muscle was enough to result in small, but sustained increases in markers of protein synthesis. Further work should be commenced to determine if these effects are through interaction with AMPK or another of the LKB1 family members.

	1 Week Water (n=9)	1 Week Caffeine (n=9)	2 Week Water (n=10)	2 Week Caffeine (n=10)
Initial BW (g)	199.9 ± 4.6	197.8 ± 4.2	202.8 ± 3.2	205.1 ± 4.0
Final BW (g)	273.8 ± 3.9	250.1 ± 5.5 §	305.5 ± 8.0	295.1 ± 8.2
Body Weight Gain (%)	37 ± 1.7	26 ± 2.5 §	51 ± 3.8	44 ± 3.7
Food Consumed (g)	195 ± 8	167 ± 8 #	373 ± 9	335 ± 14 #
Heart Weight (% of BW)	0.317 ± 0.004	0.328 ± 0.014	0.300 ± 0.005	0.297 ± 0.006
Adipose Weight (% of BW)	1.89 ± 0.17	1.49 ± 0.08 #	2.80 ± 0.28	1.76 ± 0.17 §

Table 2.1: Animal Measurements in 1 and 2 Wk Treated Rats. The initial and final body weight (grams) along with the group size (n), body weight gain (% of initial BW), food consumed (grams), heart weight (% of BW), and retroperitoneal fat pad weight (% of BW) of rats used in the OVLD experiments. All values are mean ± SE. # Significantly different from corresponding water treated animal (P < 0.05). § Significantly different from corresponding water treated animal (P < 0.01).

		Cond Saline	Cond Caffeine	KO Saline	KO Caffeine
0 Hour (n = 8)	Weight (g)	23.6 ± 0.8	24.0 ± 0.5	23.4 ± 0.7	23.2 ± 0.8
	Age (days)	102 ± 2	104 ± 2	102 ± 3	98 ± 2
3 Hour (n = 6-10)	Weight (g)	23.2 ± 0.6	22.8 ± 0.9	21.6 ± 1.4	23.1 ± 0.8
	Age (days)	98 ± 4	96 ± 5	89 ± 2	95 ± 3
8 Hour (n = 7-9)	Weight (g)	24.1 ± 1.1	24.1 ± 1.0	22.7 ± 1.8	22.7 ± 0.6
	Age (days)	96 ± 2	97 ± 2	92 ± 3	96 ± 3

Table 2.2: Animal Group Parameters in the 0, 3, and 8 H Treated Mice. The age, weights, and group size of all mice used in the HFES experiments. All values are mean ± SE.

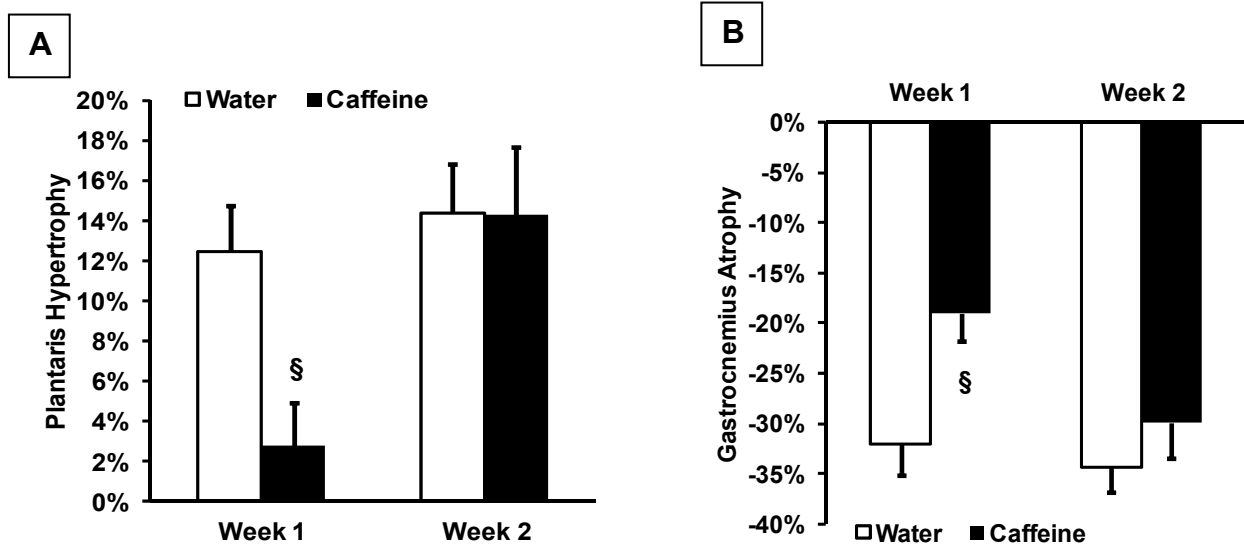


Figure 2.1: Plantaris Hypertrophy and Gastrocnemius Atrophy in 1 and 2 Wk Treated OVLD Rats. Plantaris hypertrophy for both 1 and 2 wk treated rats (A). Gastrocnemius atrophy for both 1 and 2 wk treated rats (B). Calculated as percent hypertrophy or atrophy from the SHAM operated plantaris using the “wet” weights of the OVLD and SHAM plantaris muscles. All values are mean \pm SE (n=9-10). § Significantly different from corresponding water treated muscle ($P < 0.01$).

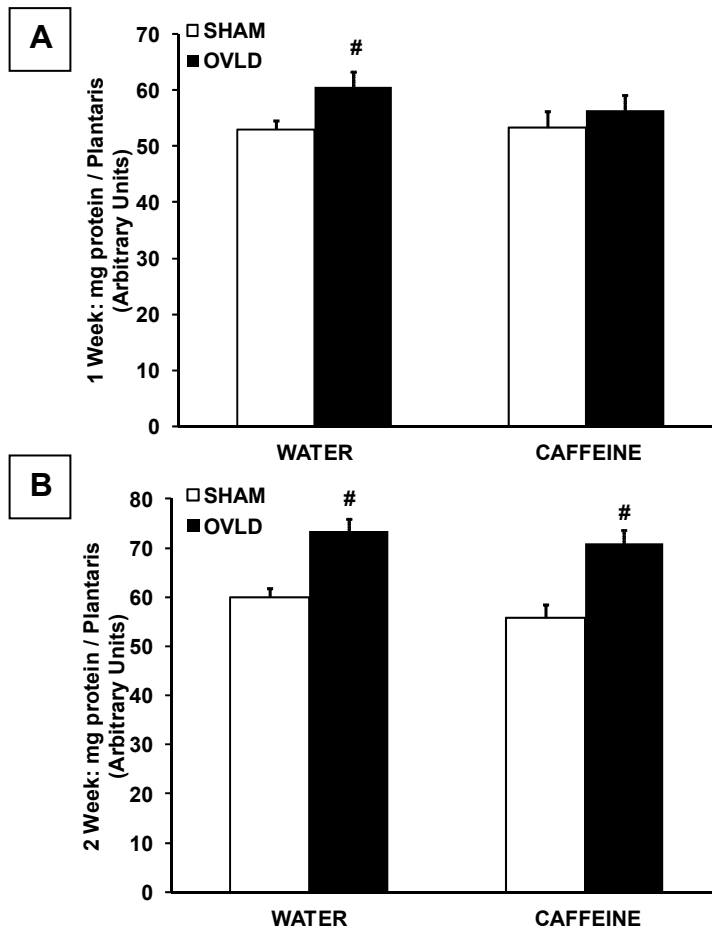


Figure 2.2: Total Protein Content of the Plantaris in 1 and 2 Wk Treated OVLD Rats. Displayed as mg protein / plantaris. All values are mean \pm SE (n=7-10). # Significantly different from corresponding SHAM muscle ($P < 0.05$).

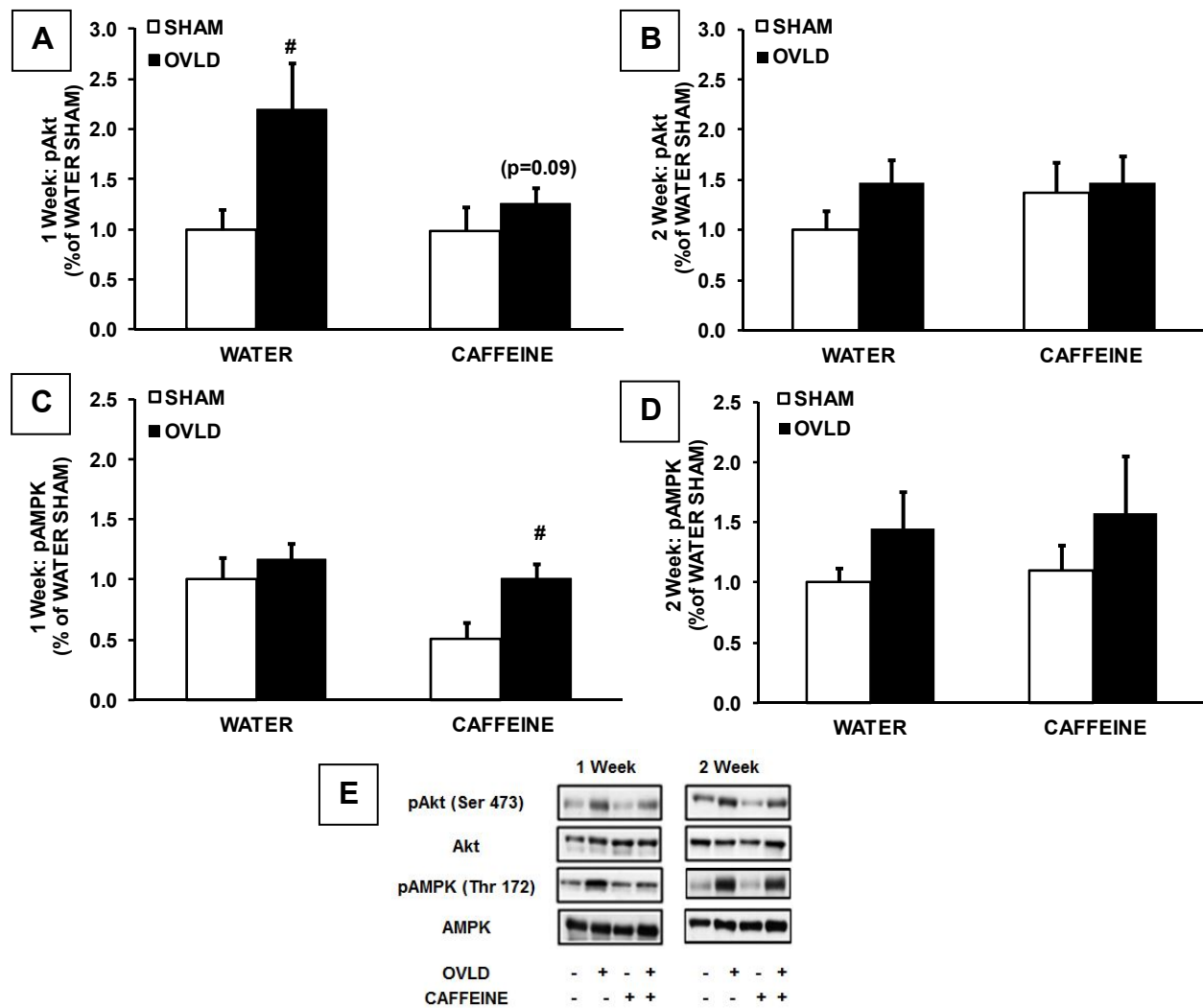


Figure 2.3: Western Blots of pAkt and pAMPK in 1 and 2 Wk Treated OVLD Rats. Phosphorylation states of Akt at Ser 473 and AMPK Thr 172 (A-D). Representative images from immunoblots (E). All values are mean \pm SE (n=8-10). # Significantly different from corresponding SHAM muscle ($P < 0.05$). Θ Significantly different from corresponding water treated muscle ($P < 0.05$). \ddagger Significant main effect vs. water treated muscles ($P < 0.05$). $P = 0.09$ from corresponding water treated muscles.

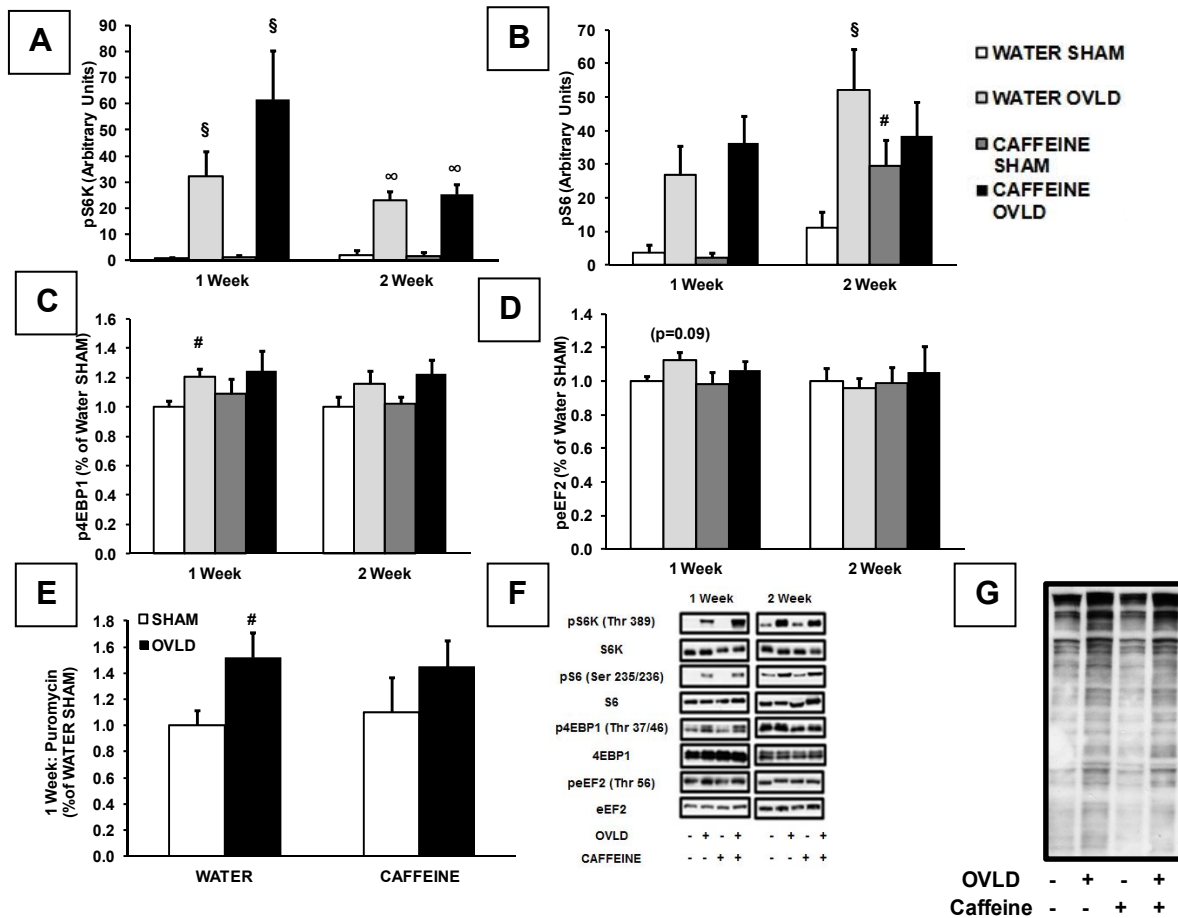


Figure 2.4: Western Blots of mTOR Targets S6K, S6, eEF2, and 4EBP1 along with Puromycin in 1 and 2 Wk Treated OVLD Rats. Phosphorylation states of S6K at Thr 389, S6 at Ser 235/236, 4EBP1 at Thr 37/46, and eEF2 at Thr 56 (A-D). Representative images from immunoblots (F). Puromycin injected into 1 wk rats was detected via western blotting of the plantaris muscle, showed main effect OVLD > SHAM ($p=0.02$) (E). Representative images from immunoblot (G). All values are mean \pm SE ($n=5-10$). # Significantly different from corresponding SHAM or water treated muscle ($P < 0.05$). § Significantly different from corresponding SHAM muscle ($P < 0.01$). ∞ Significantly different from corresponding SHAM muscle ($p < 0.001$). $P = 0.09$ from corresponding SHAM treated muscles.

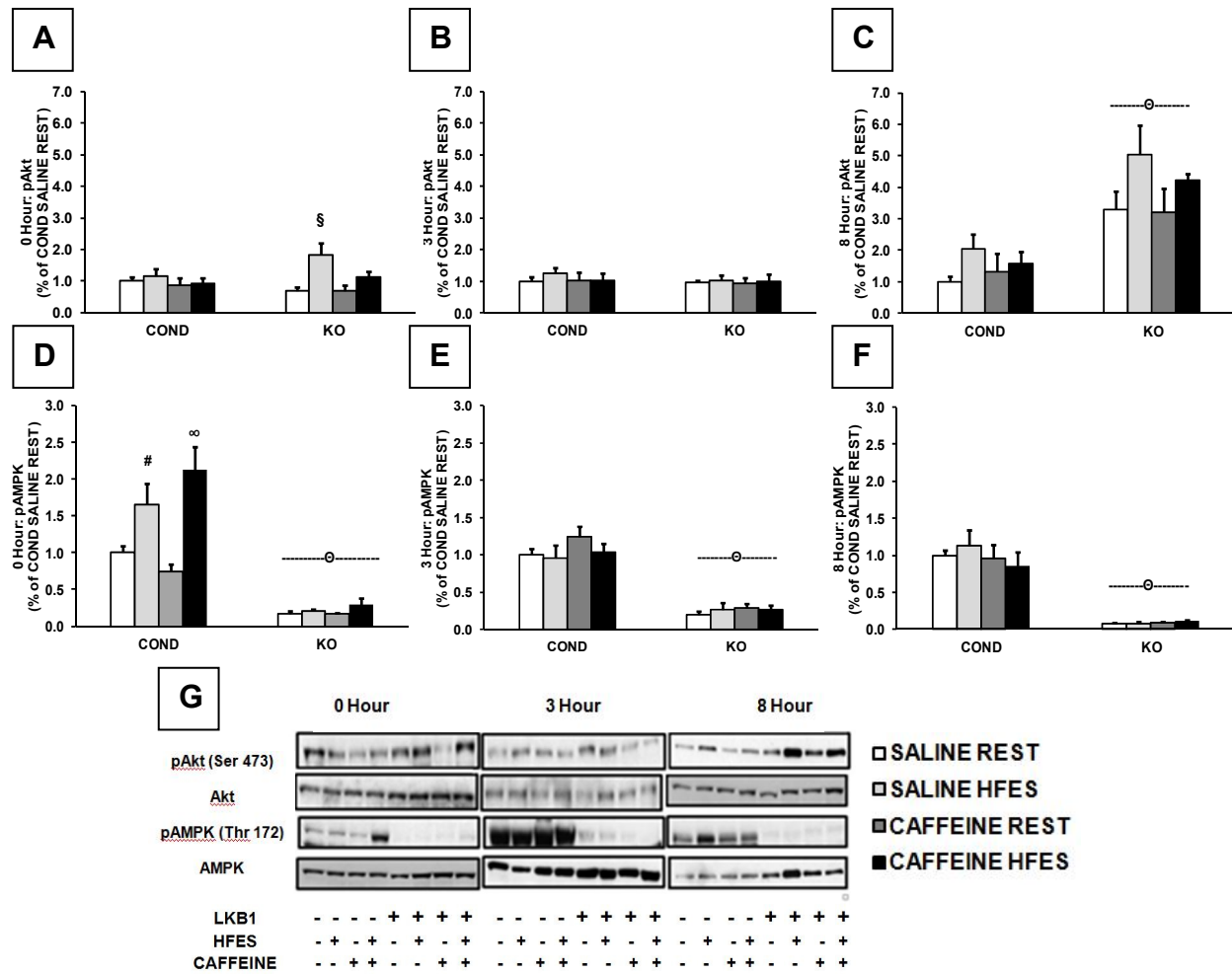


Figure 2.5: Western Blots of pAkt and pAMPK in 0, 3, and 8 H HFES Mice. Western blots are of the TA/EDL for time groups and are assessing the phosphorylation states of Akt at Ser 473 (A-C) and AMPK Thr 172 (D-F). Representative images from immunoblots (G). All values are mean \pm SE (n=6-10). # Significantly different from corresponding Rest muscle (P < 0.05). § Significantly different from corresponding Rest muscle (P < 0.01). ⊙ Significant difference main effect from COND muscles (P < 0.05).

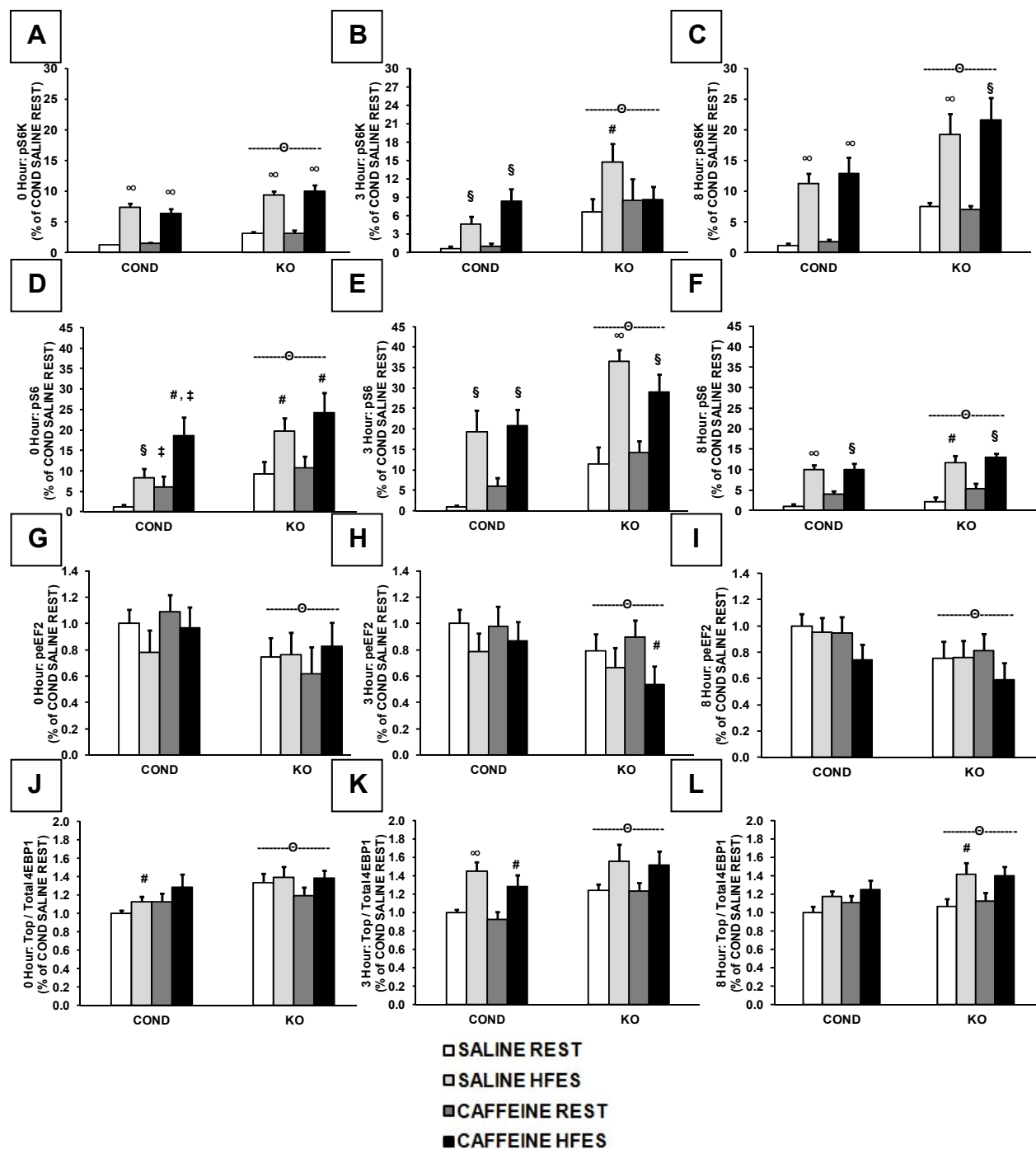


Figure 2.6: Western Blots of mTOR Targets S6K, S6, eEF2, and 4EBP1 in 0, 3, and 8 H HFES Mice. Phosphorylation states of S6K at Thr 389, S6 at Ser 235/236 and eEF2 at Thr 56 with 4EBP1 represented by phosphorylated band shifting (A-L). All values are mean \pm SE (n=6-10). # Significantly different from corresponding resting muscle ($P < 0.05$). § Significantly different from corresponding resting muscle ($P < 0.01$). ∞ Significantly different from corresponding resting muscle ($P < 0.001$). ‡ Significantly different from corresponding saline treated muscle ($P < 0.05$). \oplus Significant difference main effect from COND muscles ($P < 0.05$).

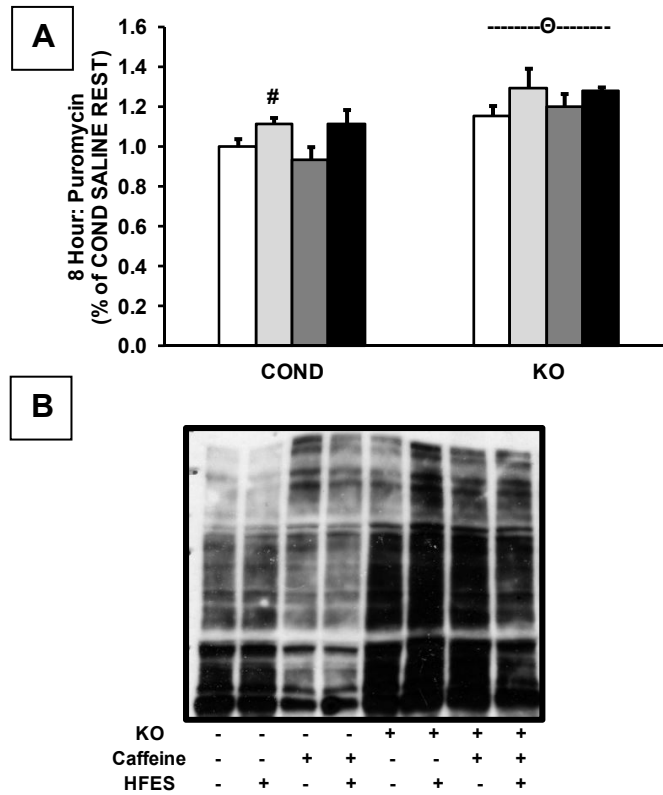


Figure 2.7: Western Blots of Puromycin in 8 H HFES Mice. Puromycin injected into 8 h mice was detected via western blotting of the plantaris and TA/EDL (A). Representative images from immunoblots (B). All values are mean \pm SE (n=4-8). # Significantly different from corresponding SHAM muscle ($P < 0.05$). \oplus Significant difference main effect from COND muscles ($P < 0.05$).

REFERENCES

- Abbott MJ, Edelman AM & Turcotte LP. (2009). CaMKK is an upstream signal of AMP-activated protein kinase in regulation of substrate metabolism in contracting skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* **297**, R1724-1732.
- Adams GR & Haddad F. (1996). The relationships among IGF-1, DNA content, and protein accumulation during skeletal muscle hypertrophy. *J Appl Physiol (1985)* **81**, 2509-2516.
- Adams J, Chen ZP, Van Denderen BJ, Morton CJ, Parker MW, Witters LA, Stapleton D & Kemp BE. (2004). Intrasteric control of AMPK via the gamma1 subunit AMP allosteric regulatory site. *Protein Sci* **13**, 155-165.
- Armstrong RB, Marum P, Tullson P & Saubert CW. (1979). Acute hypertrophic response of skeletal muscle to removal of synergists. *J Appl Physiol Respir Environ Exerc Physiol* **46**, 835-842.
- Baar K & Esser K. (1999). Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. *Am J Physiol* **276**, C120-127.
- Baldwin KM, Valdez V, Herrick RE, MacIntosh AM & Roy RR. (1982). Biochemical properties of overloaded fast-twitch skeletal muscle. *J Appl Physiol Respir Environ Exerc Physiol* **52**, 467-472.
- Barcelos RP, Souza MA, Amaral GP, Stefanello ST, Bresciani G, Figuera MR, Soares FA & de Vargas Barbosa N. (2014). Caffeine intake may modulate inflammation markers in trained rats. *Nutrients* **6**, 1678-1690.
- Birkett DJ & Miners JO. (1991). Caffeine renal clearance and urine caffeine concentrations during steady state dosing. Implications for monitoring caffeine intake during sports events. *Br J Clin Pharmacol* **31**, 405-408.
- Bode AM & Dong Z. (2007). The enigmatic effects of caffeine in cell cycle and cancer. *Cancer Lett* **247**, 26-39.
- Bodine SC. (2006). mTOR signaling and the molecular adaptation to resistance exercise. *Med Sci Sports Exerc* **38**, 1950-1957.
- Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, Zlotchenko E, Scrimgeour A, Lawrence JC, Glass DJ & Yancopoulos GD. (2001). Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol* **3**, 1014-1019.

- Bolster DR, Crozier SJ, Kimball SR & Jefferson LS. (2002). AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. *J Biol Chem* **277**, 23977-23980.
- Carter BE & Drewnowski A. (2012). Beverages containing soluble fiber, caffeine, and green tea catechins suppress hunger and lead to less energy consumption at the next meal. *Appetite* **59**, 755-761.
- Chalé-Rush A, Morris EP, Kendall TL, Brooks NE & Fielding RA. (2009). Effects of chronic overload on muscle hypertrophy and mTOR signaling in young adult and aged rats. *J Gerontol A Biol Sci Med Sci* **64**, 1232-1239.
- Corradetti MN, Inoki K, Bardeesy N, DePinho RA & Guan KL. (2004). Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome. *Genes Dev* **18**, 1533-1538.
- Degens H, Meessen NE, Wirtz P & Binkhorst RA. (1995). The development of compensatory hypertrophy in the plantaris muscle of the rat. *Ann Anat* **177**, 285-289.
- Dorfman J & Macara IG. (2008). STRADalpha regulates LKB1 localization by blocking access to importin-alpha, and by association with Crm1 and exportin-7. *Mol Biol Cell* **19**, 1614-1626.
- Egawa T, Tsuda S, Ma X, Hamada T & Hayashi T. (2011). Caffeine modulates phosphorylation of insulin receptor substrate-1 and impairs insulin signal transduction in rat skeletal muscle. *J Appl Physiol (1985)* **111**, 1629-1636.
- Fluckey JD, Kraemer WJ & Farrell PA. (1995). Pancreatic islet insulin secretion is increased after resistance exercise in rats. *J Appl Physiol (1985)* **79**, 1100-1105.
- Fredholm BB. (1982). Adenosine actions and adenosine receptors after 1 wk treatment with caffeine. *Acta Physiol Scand* **115**, 283-286.
- Gavrieli A, Karfopoulou E, Kardatou E, Spyrelli E, Fragopoulou E, Mantzoros CS & Yannakoulia M. (2013). Effect of different amounts of coffee on dietary intake and appetite of normal-weight and overweight/obese individuals. *Obesity (Silver Spring)* **21**, 1127-1132.
- Glass DJ. (2010). PI3 kinase regulation of skeletal muscle hypertrophy and atrophy. *Curr Top Microbiol Immunol* **346**, 267-278.
- Gliottoni RC, Meyers JR, Arngrimsson SA, Broglio SP & Motl RW. (2009). Effect of caffeine on quadriceps muscle pain during acute cycling exercise in low versus high caffeine consumers. *Int J Sport Nutr Exerc Metab* **19**, 150-161.

- Goldberg AL. (1967). Work-induced growth of skeletal muscle in normal and hypophysectomized rats. *Am J Physiol* **213**, 1193-1198.
- Goldspink DF, Garlick PJ & McNurlan MA. (1983). Protein turnover measured in vivo and in vitro in muscles undergoing compensatory growth and subsequent denervation atrophy. *Biochem J* **210**, 89-98.
- Goodman CA, Mabrey DM, Frey JW, Miu MH, Schmidt EK, Pierre P & Hornberger TA. (2011). Novel insights into the regulation of skeletal muscle protein synthesis as revealed by a new nonradioactive in vivo technique. *FASEB J* **25**, 1028-1039.
- Graham TE. (2001). Caffeine and exercise: metabolism, endurance and performance. *Sports Med* **31**, 785-807.
- Guarino MP, Ribeiro MJ, Sacramento JF & Conde SV. (2013). Chronic caffeine intake reverses age-induced insulin resistance in the rat: effect on skeletal muscle Glut4 transporters and AMPK activity. *Age (Dordr)* **35**, 1755-1765.
- Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE & Shaw RJ. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* **30**, 214-226.
- Hawley SA, Boudeau J, Reid JL, Mustard KJ, Udd L, Mäkelä TP, Alessi DR & Hardie DG. (2003). Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. *J Biol* **2**, 28.
- Ho KW, Roy RR, Tweedle CD, Heusner WW, Van Huss WD & Carrow RE. (1980). Skeletal muscle fiber splitting with weight-lifting exercise in rats. *Am J Anat* **157**, 433-440.
- Hornberger TA & Chien S. (2006). Mechanical stimuli and nutrients regulate rapamycin-sensitive signaling through distinct mechanisms in skeletal muscle. *J Cell Biochem* **97**, 1207-1216.
- Ikeda Y, Sato K, Pimentel DR, Sam F, Shaw RJ, Dyck JR & Walsh K. (2009). Cardiac-specific deletion of LKB1 leads to hypertrophy and dysfunction. *J Biol Chem* **284**, 35839-35849.
- Inoki K, Zhu T & Guan KL. (2003). TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **115**, 577-590.
- Janssen I, Heymsfield SB, Wang ZM & Ross R. (2000). Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. *J Appl Physiol (1985)* **89**, 81-88.
- Jessen A, Buemann B, Toubro S, Skovgaard IM & Astrup A. (2005). The appetite-suppressant effect of nicotine is enhanced by caffeine. *Diabetes Obes Metab* **7**, 327-333.

- Jessen N, Koh HJ, Folmes CD, Wagg C, Fujii N, Løfgren B, Wolf CM, Berul CI, Hirshman MF, Lopaschuk GD & Goodyear LJ. (2010). Ablation of LKB1 in the heart leads to energy deprivation and impaired cardiac function. *Biochim Biophys Acta* **1802**, 593-600.
- Koh HJ, Arnolds DE, Fujii N, Tran TT, Rogers MJ, Jessen N, Li Y, Liew CW, Ho RC, Hirshman MF, Kulkarni RN, Kahn CR & Goodyear LJ. (2006). Skeletal muscle-selective knockout of LKB1 increases insulin sensitivity, improves glucose homeostasis, and decreases TRB3. *Mol Cell Biol* **26**, 8217-8227.
- Kolnes AJ, Ingvaldsen A, Bolling A, Stuenkel JT, Kreft M, Zorec R, Shepherd PR & Jensen J. (2010). Caffeine and theophylline block insulin-stimulated glucose uptake and PKB phosphorylation in rat skeletal muscles. *Acta Physiol (Oxf)* **200**, 65-74.
- Krakstad C & Chekenya M. (2010). Survival signalling and apoptosis resistance in glioblastomas: opportunities for targeted therapeutics. *Mol Cancer* **9**, 135.
- Kubica N, Bolster DR, Farrell PA, Kimball SR & Jefferson LS. (2005). Resistance exercise increases muscle protein synthesis and translation of eukaryotic initiation factor 2Bepsilon mRNA in a mammalian target of rapamycin-dependent manner. *J Biol Chem* **280**, 7570-7580.
- Lally JS, Jain SS, Han XX, Snook LA, Glatz JF, Luiken JJ, McFarlan J, Holloway GP & Bonen A. (2012). Caffeine-stimulated fatty acid oxidation is blunted in CD36 null mice. *Acta Physiol (Oxf)* **205**, 71-81.
- Lowe DA & Alway SE. (2002). Animal models for inducing muscle hypertrophy: are they relevant for clinical applications in humans? *J Orthop Sports Phys Ther* **32**, 36-43.
- Maughan RJ & Griffin J. (2003). Caffeine ingestion and fluid balance: a review. *J Hum Nutr Diet* **16**, 411-420.
- McConnell GK, Ng GP, Phillips M, Ruan Z, Macaulay SL & Wadley GD. (2010). Central role of nitric oxide synthase in AICAR and caffeine-induced mitochondrial biogenesis in L6 myocytes. *J Appl Physiol (1985)* **108**, 589-595.
- McGee SL, Mustard KJ, Hardie DG & Baar K. (2008). Normal hypertrophy accompanied by phosphorylation and activation of AMP-activated protein kinase alpha1 following overload in LKB1 knockout mice. *J Physiol* **586**, 1731-1741.
- Miwa S, Sugimoto N, Yamamoto N, Shirai T, Nishida H, Hayashi K, Kimura H, Takeuchi A, Igarashi K, Yachie A & Tsuchiya H. (2012). Caffeine induces apoptosis of osteosarcoma cells by inhibiting AKT/mTOR/S6K, NF-κB and MAPK pathways. *Anticancer Res* **32**, 3643-3649.
- Mounier R, Lantier L, Leclerc J, Sotiropoulos A, Foretz M & Viollet B. (2011). Antagonistic control of muscle cell size by AMPK and mTORC1. *Cell Cycle* **10**, 2640-2646.

- Mounier R, Lantier L, Leclerc J, Sotiropoulos A, Pende M, Daegelen D, Sakamoto K, Foretz M & Viollet B. (2009). Important role for AMPK α 1 in limiting skeletal muscle cell hypertrophy. *FASEB J* **23**, 2264-2273.
- Nehlig A, Daval JL & Debry G. (1992). Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Res Brain Res Rev* **17**, 139-170.
- Parkington JD, Siebert AP, LeBrasseur NK & Fielding RA. (2003). Differential activation of mTOR signaling by contractile activity in skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* **285**, R1086-1090.
- Rennie MJ & Wackerhage H. (2003). Connecting the dots for mechanochemical transduction in muscle. *J Physiol* **553**, 1.
- Reynolds TH, Bodine SC & Lawrence JC. (2002). Control of Ser2448 phosphorylation in the mammalian target of rapamycin by insulin and skeletal muscle load. *J Biol Chem* **277**, 17657-17662.
- Scott W, Stevens J & Binder-Macleod SA. (2001). Human skeletal muscle fiber type classifications. *Phys Ther* **81**, 1810-1816.
- Shackelford DB & Shaw RJ. (2009). The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. *Nat Rev Cancer* **9**, 563-575.
- Sherwood DJ, Dufresne SD, Markuns JF, Cheatham B, Moller DE, Aronson D & Goodyear LJ. (1999). Differential regulation of MAP kinase, p70(S6K), and Akt by contraction and insulin in rat skeletal muscle. *Am J Physiol* **276**, E870-878.
- Smith CD, Compton RA, Bowler JS, Kemp JT, Sudwks SN, Thomson DM & Winder WW. (2011). Characterization of the liver kinase B1-mouse protein-25 -Ste-20-related adaptor protein complex in adult mouse skeletal muscle. *J Appl Physiol (1985)* **111**, 1622-1628.
- Steinberg GR & Kemp BE. (2009). AMPK in Health and Disease. *Physiol Rev* **89**, 1025-1078.
- Sugiura C, Nishimatsu S, Moriyama T, Ozasa S, Kawada T & Sayama K. (2012). Catechins and Caffeine Inhibit Fat Accumulation in Mice through the Improvement of Hepatic Lipid Metabolism. *J Obes* **2012**, 520510.
- Suryawan A, Jeyapalan AS, Orellana RA, Wilson FA, Nguyen HV & Davis TA. (2008). Leucine stimulates protein synthesis in skeletal muscle of neonatal pigs by enhancing mTORC1 activation. *Am J Physiol Endocrinol Metab* **295**, E868-875.
- Suter M, Riek U, Tuerk R, Schlattner U, Wallimann T & Neumann D. (2006). Dissecting the role of 5'-AMP for allosteric stimulation, activation, and deactivation of AMP-activated protein kinase. *J Biol Chem* **281**, 32207-32216.

- Tanner CB, Madsen SR, Hallowell DM, Goring DM, Moore TM, Hardman SE, Heninger MR, Atwood DR & Thomson DM. (2013). Mitochondrial and performance adaptations to exercise training in mice lacking skeletal muscle LKB1. *Am J Physiol Endocrinol Metab* **305**, E1018-1029.
- Thomson DM, Fick CA & Gordon SE. (2008). AMPK activation attenuates S6K1, 4E-BP1, and eEF2 signaling responses to high-frequency electrically stimulated skeletal muscle contractions. *J Appl Physiol (1985)* **104**, 625-632.
- Thomson DM & Gordon SE. (2005). Diminished overload-induced hypertrophy in aged fast-twitch skeletal muscle is associated with AMPK hyperphosphorylation. *J Appl Physiol (1985)* **98**, 557-564.
- Thomson DM & Gordon SE. (2006). Impaired overload-induced muscle growth is associated with diminished translational signalling in aged rat fast-twitch skeletal muscle. *J Physiol* **574**, 291-305.
- Warren GL, Park ND, Maresca RD, McKibans KI & Millard-Stafford ML. (2010). Effect of caffeine ingestion on muscular strength and endurance: a meta-analysis. *Med Sci Sports Exerc* **42**, 1375-1387.
- Winder WW & Hardie DG. (1999). AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. *Am J Physiol* **277**, E1-10.
- Wullschleger S, Loewith R & Hall MN. (2006). TOR signaling in growth and metabolism. *Cell* **124**, 471-484.
- Wüst RC & Degens H. (2007). Factors contributing to muscle wasting and dysfunction in COPD patients. *Int J Chron Obstruct Pulmon Dis* **2**, 289-300.
- Yang ZZ, Tschopp O, Baudry A, Dümmler B, Hynx D & Hemmings BA. (2004). Physiological functions of protein kinase B/Akt. *Biochem Soc Trans* **32**, 350-354.

CURRICULUM VITAE

Education

- University of Southern California, *Los Angeles, CA* *Fall 2014 - Present*
PhD in Integrative Biology Anticipated Spring 2019
GPA: --/4.00
Awards: College Fellowship, Full Tuition Scholarship
- Brigham Young University, *Provo, UT* *Summer 2012 – Spring 2014*
Masters in Physiology and Developmental Biology Anticipated Summer 2014
Thesis: Examination of Muscle Growth Post Caffeine Treatment in Overloaded Rat Hindlimb and High-Frequency Electrically Stimulated Mice Lacking Liver Kinase B1
GPA: 3.69/4.00
Awards: Research Assistantship, Teaching Assistantship, Half Tuition Scholarship
- Colorado Mesa University, *Grand Junction, CO* *Fall 2009 – Spring 2012*
Bachelor of Science Cum Laude
GPA: 3.5/4.00. Major in Biology. Minor in Chemistry
Awards: Deans List, Biology Merit Based Scholarship, Athletic Scholarship Recipient
Activities: Medical Science Club (Member 2009-2011), Graduate Education and Medical Sciences Club (President 2011-Present), Division II Baseball (Pitcher)
- Salt Lake Community College, *Taylorsville, UT* *Fall 2008 – Spring 2009*
Activities: National Junior College Athletic Association Baseball (Pitcher)
- University of Hawaii, *Manoa, HI* *Fall 2007 – Spring 2008*
Awards: Athletic Scholarship Recipient
Activities: Division I Baseball (Pitcher)
- International Baccalaureate Graduate, *Cherokee Trail High School, Aurora, CO* *Fall 2005 – Spring 2007*

Research Experience

- Turcotte Lab, University of Southern California, *Los Angeles, CA* *Fall 2014 – Present*
PhD Dissertation Research Project with Dr. Lorraine Turcotte, Principal Investigator
- Thomson Lab, Brigham Young University, *Provo, UT* *Summer 2012 – Spring 2014*
Master's Thesis Research Project with Dr. Dave Thomson, Principal Investigator
Two main projects: 1) Investigating the impact of caffeine on mTOR pathway as a potential inhibitor of skeletal muscle hypertrophy. 2) Investigating the impact of LKB1 knockout in skeletal muscle and its role in metabolism.
- McQuade Lab, Colorado Mesa University, *Grand Junction, CO* *Fall 2011 – Spring 2012*
Structured Research Project with Dr. Kyle McQuade, Principal Investigator
Conducting research using the model organism *Dictyostelium discoideum* and the effect that certain natural compounds with chemo preventative functions have on this amoeba.
- Reyland Lab, Colorado University at Denver, *Denver, CO* *Summer 2011*
Summer Research Internship with Dr. Mary Reyland, Principal Investigator
Conducted self led research regarding the phosphorylation of PKC δ by the Src family of tyrosine kinases on various knockout models of Mouse Embryonic Fibroblast cells. Learned how to perform Western blots analysis, plate cells, grow cells, harvest cells, immunoprecipitation, protein purification, protein assays, antibody preparation and other basic science techniques involved in the immunoblotting process.

Developmental Cognitive Neuroscience Lab, University of Denver, *Denver, CO* *Summer 2010*
Summer Research Internship with Dr. Kelly Snyder, Principal Investigator

Directly involved in two different studies regarding the effects of early experiences on brain development, learning, and memory. Main responsibilities include: assisting in data collection of infants in eye tracking paradigms, data analysis, data entry, childcare for siblings of participants and recruitment/reminder calls.

Field Biology, *Grand Junction area, CO* *Fall 2009*
Dr. Thomas Walla, Principle Investigator

Helped conduct field research observing animal patterns and plant growth tendencies in several different habitats and biomes from deserts (Colorado National Monument) to alpine (San Juan Mountains) to tundra (Grand Mesa) to wetlands (Colorado River) over a wk's worth of trips.

Publications

Colby B. Tanner, Steven R. Madsen, David M. Hollowell, Darren M.J. Goring, Timothy M. Moore, Shalene E. Hardman, Megan R. Heninger, Daniel R. Atwood, David M. Thomson. Mitochondrial and performance adaptations to exercise training in mice lacking skeletal muscle LKB1. *American Journal of Physiology - Endocrinology and Metabolism*. Published 27 August 2013 Vol. no. DOI: 10.1152/ajpendo.00227.2013

Oral Presentations

Graduate Education for Multicultural Students (GEMS), *University of Colorado, Denver* *2011*
End of summer power point presentation/ competition concerning work completed over the course of the 10 wk internship.

Abstracts and Poster Presentations

Grigg, Carlie A; Moore, T.M.; Moretnsen, X.M.; Thomson, David M. Effect of Caffeine on NF-kB Phosphorylation In Hypertrophying Skeletal Muscle. [abstract]. In: 2013 ACSM Annual Regional Meeting; 2013 October 18-19; Newport Beach, CA: SWACSM; 2013.

McVey, Natalie L; Chen, Ting; Moore, Tim M.; Madsen, Steven R; Hollowell, David M.; Thomson, David M. LKB1 Regulates Skeletal Muscle Inflammation After Metabolism. [abstract]. In: 2013 ACSM Annual Regional Meeting; 2013 October 18-19; Newport Beach, CA: SWACSM; 2013.

Moore, Tim M.; Mortensen, Xavier M.; Harris, Alex M.; Thomson, David M. Impact of Caffeine On Functional Overload-Induced Muscle Growth. [abstract]. In: 2013 ACSM Annual Regional Meeting; 2013 October 18-19; Newport Beach, CA: SWACSM; 2013.

Matthew B. Jacobs, Shalene E. Hardman, Timothy M. Moore, Jessica J. Lew, Paul R. Reynolds, David M. Thomson. RAGE and STAT3 Signaling in Chronic AICAR-Treated Young Adult and Old Skeletal Muscle [abstract]. In: 2012 APS Intersociety Meeting: Integrative Biology of Exercise; 2012 October 10 – 13; Denver, CO: APS; 2012.

Steven R. Madsen, Carlie, A. Rindlisbach, Jared M. Bradshaw, Tim M. Moore, David M. Hollowell, David M. Thomson. Contraction induced gene expression changes in skeletal muscle-specific LKB1 knockout mice [abstract]. In: 2012 APS Intersociety Meeting: Integrative Biology of Exercise; 2012 October 10 – 13; Denver, CO: APS; 2012.

Teaching Experience

- Life Sciences Learning Center, *Brigham Young University* *Winter 2014 – Spring 2014*
Responsible for tutoring students in all core courses within the PDBIO and MMBIO departments.
- PDBIO 120: Systems Biology, *Brigham Young University* *Winter 2014*
Responsibilities including teaching one of three lecture sections per wk, grading of assignments, assigning grades, developing assignments/homeworks, and holding review sessions.
- PDBIO 120: Systems Biology, *Brigham Young University* *Fall 2013*
Responsibilities including grading of assignments, assigning grades, developing assignments/homeworks and holding wkly review sessions for two lecture sections.
- PDBIO 561: Physiology of Drug Mechanisms, *Brigham Young University* *Fall 2013*
Responsibilities including grading of assignments and holding wkly review sessions for one lecture section.
- PDBIO 363: Advanced Physiology Lab, *Brigham Young University* *Fall 2012 - Winter 2013*
Organized and oversaw two lab sections per wk. Responsible for introducing the lab, lab prep, overseeing students throughout the lab, troubleshooting, grading papers, and assigning grades.

Honors and Special Recognitions

- Dana and David Dornsife University Fellow, *University of Southern California* *Fall 2014 – Spring 2019*
- Graduate Education and Medical Sciences Club President, *Colorado Mesa University* *Fall 2011 – Spring 2012*
- Graduate Education for Multicultural Students Summer Internship, *UC - Denver* *Summer 2011*
- Co-Captain Baseball Team, *Colorado Mesa University* *Fall 2011 – Spring 2012*
- Co-Captain Baseball Team, *Salt Lake Community College* *Fall 2008 – Spring 2009*

Volunteer Medical Experience

- Community Hospital, *Grand Junction, Colorado* *Spring 2010*
Emergency Department Assistant
Assisted ER doctors and nurses in basic patient care. Observed procedures (for example: intubation of an overdose patient, treatment of a jaundiced patient, and setting/casting of a broken arm), organized/input patient information into the patient online database and cleaned/restocked ER rooms.
- St. Mary's Hospital, *Grand Junction, Colorado* *Fall 2009*
Rehabilitation Department Assistant
Interacted with patients during therapy sessions helping to provide motivation while furthering their rehab. Assisted Physical Therapists in the transport of patients or the preparation of the rehab sessions.
- Adam's Camp, *Rocky Mountain National Park, Colorado* *Winter 2006*
Therapy Assistant
Assisted Physical, Occupational, and Speech Therapists in providing intensive, personalized, and integrated therapeutic programs and activities for children with special needs as well as their families.